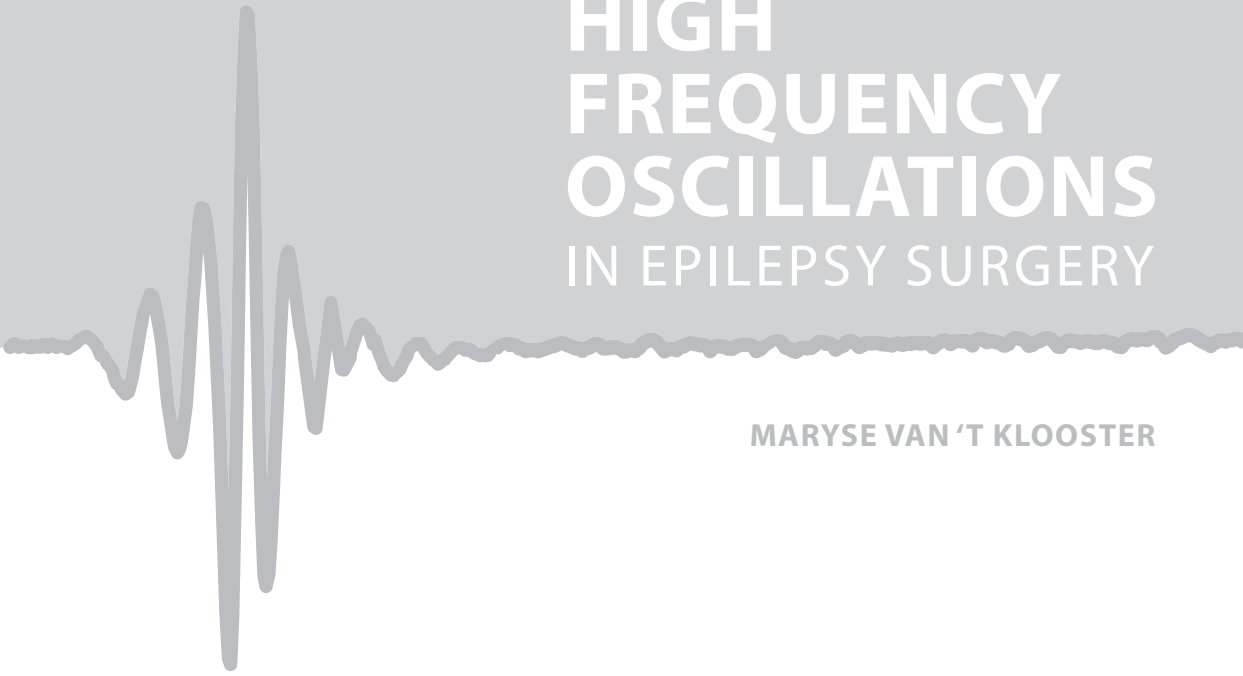

HIGH FREQUENCY OSCILLATIONS
IN EPILEPSY SURGERY

MARYSE VAN 'T KLOOSTER

HIGH FREQUENCY OSCILLATIONS IN EPILEPSY SURGERY

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ISBN: 978-90-393-6645-5
Design: Wendy Schoneveld | wenz iD.nl
Printed by: Uitgeverij Boxpress B.V. , 's-Hertogenbosch || Proefschriftmaken.nl

The research in this thesis was financially supported by the Dutch Epilepsy Foundation.

The author gratefully acknowledges the financial support for the reproduction of this thesis by the UMC Utrecht Brain Center Rudolf Magnus, KMI, ANT Neuro B.V. / ANT Benelux B.V., ChipSoft B.V. and UCB Pharma B.V.

HIGH FREQUENCY OSCILLATIONS
IN EPILEPSY SURGERY

HOOG-FREQUENTE OSCILLATIES
TEN BATE VAN EPILEPSIE CHIRURGIE
(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan,
ingevolge het besluit van het college voor promoties in het openbaar te verdedigen
op donderdag 20 oktober 2016 des middags te 4.15 uur

door

MARYSE ANTONET VAN 'T KLOOSTER
geboren op 11 april 1985 te Nijkerk

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CHAPTER 1

GENERAL INTRODUCTION AND THESIS OUTLINE

INTRODUCTION

EPILEPSY SURGERY

Epilepsy is one of the most common neurological disorders, and occurs in 0.7% of the population[4, 5]. The highest incidence of epilepsy is in the pediatric and adolescent population with about 70 new cases per 100.000 persons per year [4, 6]. Epilepsy is usually treated with, often multiple, anti-epileptic drugs. In 30% of cases the patient's seizures are not sufficiently controlled. In focal epilepsy, where the epilepsy originates from a single source or epileptogenic lesion in the brain, surgery can provide a cure[1, 7, 8]. Complete removal of the brain region responsible for the generation of epileptic seizures, the hypothesized epileptogenic zone, is needed in order to become seizure free after surgery. The success rate of epilepsy surgery in focal refractory epilepsy patients varies between 36 and 84%[9]. In recent years it has been recognized that the gain of epilepsy surgery may not only be complete seizure freedom, but also improved cognitive and social development, especially in children, resulting in an overall increase of quality of life[10, 11]. The chances of success are determined by how well the borders of the epileptic zone can be defined in order to assure complete resection of the diseased brain tissue. Despite all efforts with non-invasive pre-operative diagnostic tools, such as MRI, scalp EEG, PET and MEG, the location and extent of the epileptogenic tissue cannot be determined with 100% certainty.

THE INVASIVE EEG

One way to obtain more information about the exact location and margins of the epileptogenic tissue is to perform invasive EEG. During invasive EEG, electrodes are placed directly into (depth-EEG) or on the cortex (grid electrodes). Long-term intracranial grid monitoring with simultaneous video registration is usually done in case no clear epileptogenic lesion on MRI is found or when eloquent regions near the presumed lesion need to be located as well.

ZONES INVOLVED IN EPILEPSY SURGERY[1]

Epileptogenic lesion = Abnormal brain tissue visible on the MRI or during pathological examination, that has a relation to the epilepsy.

Seizure onset zone = The area where the seizures starts.

Epileptogenic zone = Hypothetical region of the brain that is responsible for generating epileptic seizures, that needs to be removed in order to become seizure-free after surgery.

Irritative zone = The region with inter-ictal spikes.

Eloquent region = A region of the brain that is responsible for a specific function, for example language, movement, or vision.

Resection area = The area of the brain that is resected during surgery, and is considered the gold standard for the epileptogenic zone in case of post-surgical seizure freedom.

The goal is to capture the patient's seizures and to pinpoint their origin, the so called seizure onset zone. Drawback of such a monitoring period of 5-7 days is the burden it puts on the patient, and increasing duration of the implanted electrodes is associated with higher infection risks[12]. Alternatively, intra-operative electrocorticography (ECoG) during surgery can be performed; repeated ECoG recordings before- and after each surgical resection of brain tissue. The goal of this so called tailoring is to assist the surgeon in the resection by delineating the borders of the tissue that shows epileptic activity[13] (figure 1.1).

INTER-ICTAL EPILEPTIC ACTIVITY

Whereas the point of onset of the patient's seizures is considered a good reflection of the epileptogenic zone, waiting for seizures during surgery, in order to localize the epileptogenic source with intra-operative ECoG is virtually impossible. The inter-ictal period (in-between seizures) can show epileptiform discharges or spikes[14]. Drawback of these spikes is that they seem to represent a larger area, the irritative zone, rather than the epileptogenic zone[1]. Currently, inter-ictal spikes are considered the gold standard biomarker in the intra-operative ECoG to guide the surgeon's resection. Removal of areas with inter-ictal spikes has been associated with seizure freedom[14, 15]. The value of spikes for tailoring is increasingly debated as surgical manipulation can cause new spikes to arise in the resection margin and 'chasing' spikes unrelated to the epileptogenic focus is undesirable[16]. The popularity of intra-operative ECoG - also requiring an expert clinical neurophysiologist to be involved during surgery - has therefore decreased.

About 15 years ago a new and more precise biomarker in the ECoG was discovered, when studying the signal in frequency ranges higher than the usual maximum of 70 Hz. Diseased epileptogenic tissue produces high frequency oscillations (HFOs, >80-500 Hz), that can be subdivided into ripples (80-250 Hz) and fast ripples (250-500 Hz)[17, 18]. Retrospective research in pre-surgical long-term depth-EEG and grid monitoring suggests that inter-ictal HFOs, especially fast ripples, overlap with but occur in a smaller area than spikes and are a better representative of the seizure onset zone than inter-ictal spikes[18]. Complete removal of tissue with fast ripples recorded during pre-surgical long-term invasive EEG is related to seizure freedom after surgery[19, 20]. Tailoring based on fast ripples in the intra-operative ECoG has the potential to outperform the current spike-based approach, especially if HFOs proof to be insensitive to surgical manipulation and may lead to a higher success rate of epilepsy surgery (figure 1.1).

HFO ANALYSIS

With time being always a limiting factor for ECoG tailored surgery, because of costs as well as infection risks, there is a need for innovative techniques to optimize the yield of HFO detection procedures. Especially, since the visual analysis of spontaneous HFOs is very time consuming and requires expertise, worldwide acceptance of HFOs based tailoring will only be possible when it can be easily implemented in clinical practice. This requires smart solutions for reliable and real-time automated analysis of intra-operative ECoG recordings.

If one considers that epileptic seizures are thought to arise in diseased neuronal tissue patches with a mismatch in cortical excitability and inhibition mechanisms[5], an answer might for example lie in electrical stimulation to evoke epileptic activity instead of waiting for spontaneous epileptic

activity. Single pulse electrical stimulation, based on series of brief single electrical pulses of 1 ms, evokes time-locked responses similar to inter-ictal spikes, that are good indicators of the seizure onset zone[21-24]. Since HFOs often co-occur with spikes, we may find evoked HFOs as well when looking at the higher frequency ranges in the signal. Second, the characteristics of HFOs seem to lend themselves better for automatic detection algorithms and other innovations of signal analysis than those of inter-ictal spikes[25]. These algorithms need to be specifically (re-) designed for use in the surgical theater, which is an artifact-inducing environment.

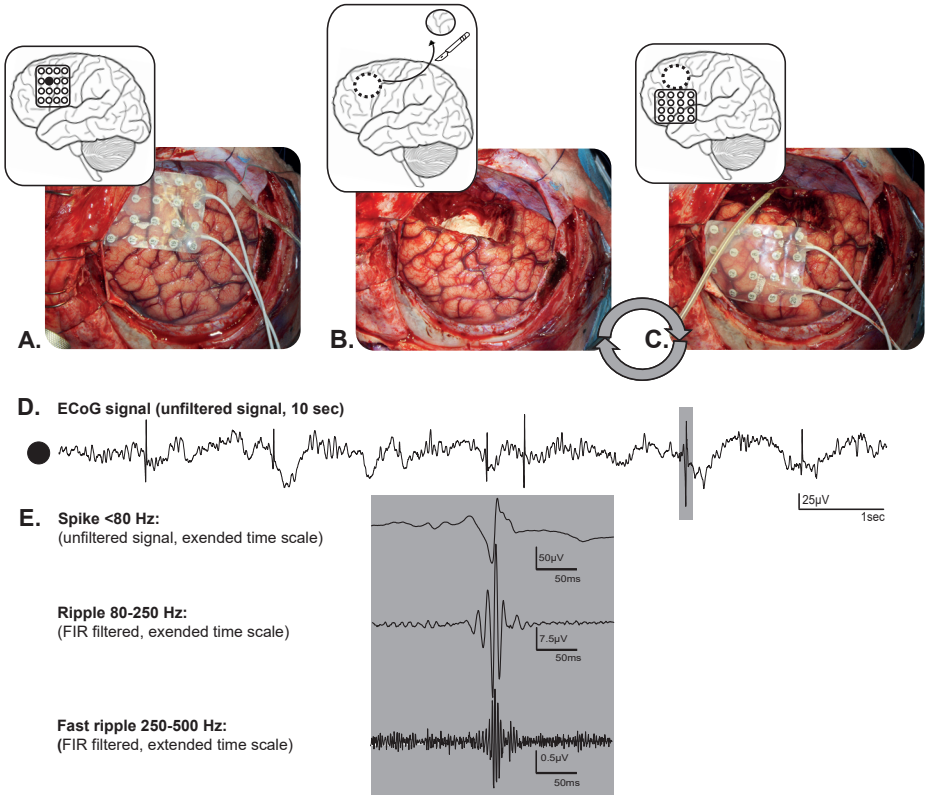

FIGURE 1.1
A schematic representation of the tailoring procedure and recorded intra-operative ECoG signals. A.) Pre-resection ECoG recording, the electrode grid is placed over the presumed epileptogenic zone. B.) Based on the non-invasive pre-surgical findings, anatomy and pre-resection ECoG recording the extent of the resection is determined. C.) After the resection ECoG is again recorded to warrant that all epileptic activity is completely removed. If epileptic activity is still present, the resection could be extended if not limited by functional eloquent regions. Ideally steps B and C are repeated until the ECoG is without epileptic activity. In this thesis, the post-resection ECoG is defined as the last recording after completion of the resection. D.) A representative inter-ictal spike signal. E.) HFOs often co-occur with spikes, but not all spikes have HFOs. HFOs become visible after filtering the data so that only high frequencies in the signal remain (finite impulse response filter, FIR), so >80 Hz for ripples and >250 Hz for fast ripples and after extending the time scale. An HFO, ripple or fast ripple, is defined as at least four oscillations that clearly stand out of the background activity of the ECoG signal.

OUTLINE OF THE THESIS

The objective of this thesis is to lay a foundation for the clinical use of HFOs during epilepsy surgery to increase the chance of seizure freedom after surgery. Aims of this thesis are threefold: to evaluate the predictive value of HFOs in the intra-operative ECoG for post-surgical outcome, to collect evidence on the performance and feasibility of actual clinical tailoring based on HFOs in comparison to spikes, and to investigate advanced signal analysis methods to improve the usability of HFOs during neurosurgery.

PART I – INTERPRETATION OF HFOS

The first part of this thesis describes how to interpret the characteristics of HFOs in comparison to spikes and post-surgical outcome in the intra-operative ECoG in a retrospectively collected dataset. First, the differences in occurrence of HFOs and spikes in recordings before and after the surgical resection of the presumed epileptogenic focus are studied (**chapter 2**). Then, the predictive value of remaining HFOs after resection in the intra-operative ECoG for surgical outcome is investigated (**chapter 3**). In the last study we explore the potential to tailor surgery based on spikes and fast ripples in the intra-operative ECoG (**chapter 4**).

PART II – INTERVENTION WITH HFOS

The second part of the thesis describes the protocol for a multi-center randomized clinical trial to determine prospectively if intra-operative ECoG tailored surgery using HFOs, instead of inter-ictal spikes, is feasible and will lead to an equal or better post-operative seizure outcome (**chapter 5**). This unique clinical trial was approved in 2014, and the first patient was enrolled in November that same year. The results of this interventional trial are expected in 2018.

PART III – INNOVATION DRIVEN BY HFOS

The final part of this thesis describes innovative ways to make clinical implementation of HFOs for tailored surgery feasible in the international epilepsy surgery community. This is done by looking for means to optimize the yield of inter-ictal HFOs by evoking them with single pulse electrical stimulation (SPES) (**chapter 6**), increase the yield of SPES by taking not only pathological but also physiological time-locked responses into account (**chapter 7**) and validate the results of evoked HFOs against spontaneous inter-ictal HFOs (**chapter 8**). In **chapter 9**, a new approach for automated analysis of the intra-operative ECoG is proposed, that captures HFO information without counting exact numbers of HFOs.

In **chapter 10** the results of this thesis will be summarized and discussed, leading up to recommendations for future research.

POST-SURGICAL OUTCOME CLASSIFICATION

The Engel classification of post-operative outcome related to seizure freedom and seizure recurrence since surgery is the most widely used classification of seizure outcome. It was established and approved by the International League against Epilepsy in 1999 [2]. In short, seizure outcome is divided into four classes:

Class I = Free of disabling seizures, which is further subdivided into
- IA: Completely seizure free since surgery,
- IB: Only non-disabling partial seizures, called auras,
- IC: Some disabling seizures after surgery, but free of disabling seizures for at least 2 years, and
- ID: Only seizures after anti-epileptic medication discontinuation

Class II = Almost seizure free, having rare disabling seizures or only seizures during sleep
Class III =Worthwhile improvement, meaning a big reduction in seizure load
Class IV = No worthwhile improvement, no appreciable change or worsening of the seizures

Traditionally, post-surgical outcome is evaluated for the first time twelve months after surgery, and re-evaluated every year. In the Netherlands, adult patients typically still use the same anti-epileptic medication as before their surgery up to the first evaluation after one year, before withdrawal is considered when patients remain seizure-fee. In children withdrawal and discontinuation of medication is often started earlier [3].

PART I

INTERPRETATION OF HFOS

CHAPTER 2

HIGH FREQUENCY OSCILLATIONS IN INTRA-OPERATIVE ELECTROCORTICOGRAPHY BEFORE AND AFTER EPILEPSY SURGERY

Clin Neurophysiol 2014;125:2212-219.

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ABSTRACT

INTRODUCTION

Removal of brain tissue showing high frequency oscillations (HFOs; ripples: 80-250 Hz and fast ripples: 250-500 Hz) in pre-resection electrocorticography (pre-ECoG) in epilepsy patients seems a predictor of good surgical outcome. We analyzed occurrence and localization of HFOs in intra-operative pre-ECoG and post-resection electrocorticography (post-ECoG).

METHODS

HFOs were automatically detected in one-minute epochs of intra-operative ECoG sampled at 2048 Hz of fourteen patients. Ripple, fast ripple, spike, ripples on a spike (RoS) and not on a spike (RnoS) rates were analyzed in pre- and post-ECoG for resected and non-resected electrodes.

RESULTS

Ripple, spike and fast ripple rates decreased after resection. RnoS decreased less than RoS (74% vs. 83%; p=0.01). Most fast ripples in pre-ECoG were located in resected tissue. Post-ECoG fast ripples occurred in one patient with poor outcome. Patients with good outcome had relatively high post-ECoG RnoS rates, specifically in the sensorimotor cortex.

CONCLUSION

Our observations show that fast ripples in intra-operative ECoG, compared to ripples, may be a better biomarker for epileptogenicity. Further studies have to determine the relation between resection of epileptogenic tissue and physiological ripples generated by the sensorimotor cortex. Fast ripples in intra-operative ECoG can help identify the epileptogenic zone, while ripples might also be physiological.

INTRODUCTION

Epilepsy surgery aims at the removal of epileptic brain tissue to achieve seizure freedom. In 60-70% of the patients who undergo epilepsy surgery, long-term seizure freedom is achieved[26, 27]. Successful surgery may lead to improved cognitive outcome and better cognitive and social development in children[11]. Intra-operative electrocorticography (ECoG) can be used during surgery to delineate the epileptogenic area. Removal of areas showing inter-ictal epileptiform discharges, especially spikes, in the pre-resection ECoG (pre-ECoG) has been associated with increased seizure freedom[14, 28]. After the resection, ECoG can be performed again (post-ECoG) to check for the presence of remaining spikes. The use of intra-operative ECoG to tailor surgery is disputed. Some studies showed that the presence of remaining spikes was correlated with seizure recurrence and removal led to improved outcome[28, 29] while others showed no correlation[30, 31]. Post-ECoG spikes might arise from surgical manipulation of the neocortex, especially at the edge of the resection[16].

Spontaneous high frequency oscillations (HFOs: ripples 80-250 Hz and fast ripples 250-500 Hz) are proposed as new biomarkers for the epileptogenic zone. HFOs, especially fast ripples, have shown to be more correlated with the seizure onset zone (SOZ) than inter-ictal epileptiform discharges such as spikes[17-19, 32, 33]. Most previous studies concentrated on HFOs in ECoG and depth EEG recordings obtained before resection, showing increased seizure freedom when more electrodes with ictal HFOs[34] or inter-ictal HFOs were removed[20, 35, 36]. One study in intra-operative pre-ECoG found a correlation between removal of areas showing fast ripples and seizure freedom[19].

Ripples are not necessarily pathological, as physiological ripples occur in the mesiotemporal, occipital, and sensorimotor area[37] and have been related to cognitive functions like memory[38]. It seems difficult to distinguish physiological from pathological ripples[39], but the co-occurrence with spikes might be an indicator of pathology[40]. Fast ripples in healthy tissue were only found after stimulation; spontaneous fast ripples have not been described[41, 42].

Remaining HFOs in intra-operative post-ECoG have not been studied before and the influence of resection on HFO occurrence is unknown. This information can establish the clinical usefulness of HFOs during surgery and improve the understanding of their pathophysiology. We focused on intra-operative ripples, fast ripples and spikes in pre-ECoG, their difference in rate between pre- and post-ECoG and their relation to surgical outcome. Further, we studied occurrence of HFOs and spikes at the edge of the resected area.

METHODS

PATIENTS

Patients with refractory epilepsy who underwent tailored surgery with intra-operative ECoG between 2008 and 2012 at our center and for whom one year post-surgical outcome was available were retrospectively selected. Only spikes were used in clinical decision making. Patients were excluded if they had recurrent tumor growth, had disconnection surgery, had chronic ECoG

registration before surgery, did not have both pre- or post-ECoG, or if their data had been used in optimizing the automatic HFO detector, which will be discussed later. Recordings with numerous artifacts or a burst suppression pattern were excluded. Post-surgical outcome was classified using the Engel classification, dichotomized into good (Engel 1) and poor (Engel ≥2) outcome. Patients who met the inclusion criteria were divided into four groups: (1) patients with temporal lobe epilepsy (TLE) and good outcome, (2) patients with TLE and poor outcome, (3) patients with extra-temporal lobe epilepsy (ETLE) and good outcome and (4) patients with ETLE and poor outcome. We randomly selected as many patients as possible from each group, while maintaining equal numbers in the groups (table 2.1).

INTRA-OPERATIVE ECOG

A 4x5 electrode grid and sometimes 1x8 electrode strips (Ad-Tech, Racine, WI, USA) were placed on the cortex during surgery. ECoG was registered with a 128-channel EEG system (MicroMed, Veneto, Italy), at 2048 Hz sampling rate with an anti-aliasing filter at 538 Hz. Electrode grids and strips were placed, and pre-ECoG was recorded for three to four minutes. Then the grid and strips were replaced into different positions, to make sure the suspected epileptogenic cortex and surrounding areas were covered. After initial resection, electrode grids and strips were again placed on the cortex, around the resected area, and ECoG was again measured. If needed, resection was extended and ECoG recording repeated. The last recorded ECoG was defined as the post-ECoG.

The grid and strips were placed in at least one and maximum five positions in pre- and post-ECoG. ECoG during propofol anesthesia shows a burst suppression pattern, which is not appropriate for clinical decision making. Propofol was therefore interrupted typically 5 to 10 minutes until the signal showed a continuous background pattern with conventional EEG settings (1.6-70 Hz, 10 s/page, 100 μV/mm) in which spikes can be found[43]. Patients did not wake up.

DATA SELECTION

ECoG was visually assessed in a bipolar montage along the length of the electrode grid or strip. One-minute epochs of ECoG were selected from each electrode position pre- and post-ECoG, near the end of each recording.

DETECTION OF HFOS

Ripples and fast ripples were detected retrospectively by an automatic detection algorithm, adapted from the Montreal Neurological Institute (MNI) HFO detector of Zelmann et al. (2010)[44]. Identified ripples and fast ripples were visually checked using Stellate Harmonie Reviewer (Montreal, QC, Canada), and artifacts identified as HFOs were removed.

The MNI detector was designed to identify HFOs in depth EEG recordings and was adapted and optimized for intra-operative ECoG recordings. Optimization of the detection parameters was performed on 103 pre- and post-ECoG channels from recordings from six patients, randomly selected from our database, different from the patients selected for this study (table 2.2). Ripples and fast ripples in one minute epochs were visually scored in each bipolar channel by two reviewers (NvK, MvtK or MZ) in Stellate Harmonie Reviewer. Inter-rater variability was determined

TABLE 2.1
Patient characteristics

#	Gender	Age	Location	Side	Outcome	Pathology	#ELs	#EL$_{overlap}$	FRs
1	F	15	Mesiotemporal	R	1A	MTS	38	7	Pre
2	F	12	Frontal	L	1A	FCD	46	12	Pre
3	F	5	Frontal	R	1A	Cortical tuber	47	0	None
4	M	29	Temporal	R	2A	Cavernoma	44	12	Pre
5	M	12	Frontal	R	1A	DNET	22	12	None
6	F	24	Mesiotemporal	R	4B	DNET	47	14	None
7	M	11	Central	R	3A	MCD	64	13	Pre
8	F	37	Mesiotemporal	R	1A	Cavernous angioma	29	12	None
9	M	25	Frontal	R	4B	Venous angioma	43	11	Pre
10	M	13	Mesiotemporal	R	1A	MTS	46	9	Pre
11	F	23	Mesiotemporal	L	2A	MTS	36	4	Pre
12	M	34	Mesiotemporal	L	2A	Cavernoma	35	12	Pre
13	F	3	Temporal	L	1A	Ganglioma	50	13	None
14	M	13	Parietal	L	3A	Cortical tuber + FCD	48	2	Pre +Post

Post-surgical outcome in Engel classification. F = female, M = male, L = left, R = right, MTS = mesiotemporal sclerosis, FCD = focal cortical dysplasia, DNET = dysembryoplastic neuroepithelial tumor, MCD = malformation of cortical development, #ELs = number of electrode locations, #EL$_{overlap}$ = number of electrode locations with both pre- and post-ECoG data, FRs = occurrence of fast ripples, Pre = pre-resection, Post = post-resection

and channels were discussed until kappa was at least 0.5[45]. Visual analysis was performed as described by Zelmann et al. (2010)[45], but amplitude sensitivity for ripples was set at 5 μV/mm because intra-operative ECoG baseline amplitude was too high for evaluation at 1 μV/mm. HFOs in consensus of both reviewers were used as gold standard reference for optimization of the parameters. Two new steps were added to the detection algorithm to limit the number of falsely detected events. These were restrictions on the amplitude and on the number of cycles of each HFO. The optimized detector was validated on 377 other pre- and post-ECoG channels of the same six patients. Comparing HFOs identified by reviewers and by the detector resulted in a sensitivity of 93.6% and specificity of 84.6% for ripples and 94.2% sensitivity and 93.4% specificity for fast ripples. Sensitivity and specificity were defined asin Zelmann et al. (2010)[44]. Visual post-processing of the automatically detected HFOs was still required.

IDENTIFICATION OF SPIKES

Spikes were marked visually in Stellate Harmonie Reviewer in bipolar montage with regular EEG settings of 0.5-80 Hz, 10 s/page, and amplitude 100 μV/mm by MvtK and MZ. A spike was defined as a sharp transient, standing out above baseline and lasting 80 ms maximum. Sharp waves co-occurring with spikes on another channel were also marked as spikes.

TABLE 2.2
Characteristics of patients used for optimization of the HFO detector

#	Gender	Age	Location	Side	Pathology
A	F	43	Mesiotemporal	L	No diagnosis
B	M	47	Mesiotemporal	L	Astrocytoma (III)
C	M	7	Temporal	R	Encephalopatic cyst
D	M	19	Parietal	L	Astrocytoma (I)
E	F	1	Temporo-occipital	L	Glioma (II)
F	F	13	Frontal	R	Ganglioglioma (I)

F = female, M = male, L = left, R = right, (I) = WHO grade 1, (II) = WHO grade 2, (III) = WHO grade 3

ELECTRODE POSITIONS

Electrode positions on the cerebral cortex pre- and post-resection were schematized by using photographs taken during surgery. Photographs were merged based on anatomical structures to match electrode positions and to construct a scheme in Photoshop CS5 (Adobe Systems Inc., San Jose, CA, USA; figure 2.1). Each square in the scheme was called an electrode location (EL) and represented a cortical surface area of 1 cm^2 with the bipolar electrode traces that were located in that area. Each EL had pre- or post-ECoG data. Electrode locations sampled during both pre- and post-ECoG recordings were defined as 'overlapping electrode locations' ($ELs_{overlap}$). ELs were divided in resected (ELs_{res}) and non-resected ELs (ELs_{nres}). An EL was considered resected if more than 50% of the EL was removed.

ANALYSIS

Event rates

We analyzed the amount of ripples, fast ripples and spikes in pre- and post-ECoG. The number of events in each EL was called the event rate (events / EL / minute). The same was done for ripples on a spike (RoS) and ripples not on a spike (RnoS).

Distribution over resected and non-resected areas

The rate for ELs_{res} and ELs_{nres} was calculated as the average number of events in resected and non-resected ELs respectively. We analyzed the difference in event rate in pre-ECoG between ELs_{res} and ELs_{nres}, to investigate the distribution of events before resection.

Difference between pre- and post-ECoG

Second, we were interested in the difference between pre- and post-ECoG event rates. We used $ELs_{overlap}$ to compare pre- and post-ECoG rates. We defined 'de novo' events as an increase in rate between pre- and post-ECoG with more than 10 events per minute. Since the number of $ELs_{overlap}$ could be small in some patients, we also evaluated the difference between pre- and post-ECoG ELs_{nres} rates.

Relation to outcome

Third, we related pre- and post-ECoG events to post-surgical outcome. Pre-ECoG events were related to outcome by calculating the ratio of the events in ELs_{res} and ELs_{nres} [20]:

$$Ratio_{[EV]} = \frac{Rate\ EV_{res} - Rate\ EV_{nres}}{Rate\ EV_{res} + Rate\ EV_{nres}} \quad \text{(Eq 2.1)}$$

where EV is the event type, EV_{res} the rate of events in ELs_{res}, and EV_{nres} the events in ELs_{nres}. A ratio of +1 identifies patients in whom all event locations were resected, whereas a ratio of -1 indicates that all event locations remained in place. This ratio was compared to outcome.
Post-ECoG events could not be related to outcome by a ratio in ELs_{res} and ELs_{nres}. Therefore we looked at the event rate of all post-ECoG ELs and related this to outcome.

Events at the edge of resection

Furthermore, we wanted to investigate the behavior of events at the edge of the resected area. We defined edge ELs (ELs_{edge}), adjacent to the resected area, and distant ELs (ELs_{dist}), at more than one EL from the resected area. We analyzed event rates in those areas in pre- and post-ECoG.
Event rates were analyzed by a Wilcoxon Signed Rank test, paired per patient. Statistical analysis was performed in IBM SPSS Statistics 20 (IBM Corp., Armonk, NY, USA). A p-value below 0.05 was considered significant.

RESULTS

PATIENTS AND RECORDINGS

Eighty-two patients with documented one year follow up underwent epilepsy surgery with intra-operative ECoG between 2008 and 2012. Forty-six patients were excluded for various reasons: 29 patients had incomplete data, six had been used for optimizing the HFO detector, five had preceding chronic ECoG registrations, four had recurrent tumor growth, and two had a disconnection surgery. From the remaining patients, three patients with ETLE and poor outcome met the inclusion criteria. To maintain variety in the study population we randomly selected four patients with TLE and good outcome, four with TLE and poor outcome, three with ETLE and good outcome and three patients with ELTE and poor outcome. This resulted in seven patients with good and seven with poor outcome (table 2.1). The selected one-minute epochs were on average 7:46 minutes after propofol interruption in pre-ECoG and 9:01 minutes after propofol interruption in post-ECoG (no significant difference). Schematization of the electrode positions resulted in 596 ELs for analysis; on average 42.5 (SD 10) ELs per patient. Of those, 171 ELs were ELs_{res}; on average 12.2 (SD 5.0) per patient. Thirteen patients had $ELs_{overlap}$, with a total of 133 $ELs_{overlap}$.

EVENT RATES

Ripples were found in pre-ECoG in all patients, and in post-ECoG in thirteen patients. Spikes could be identified in all patients in pre- and post-ECoG. Fast ripples were found in pre-ECoG in nine patients, and in post-ECoG in one patient. In total 5692 ripples, 7288 spikes, and 805 fast ripples were identified.

DISTRIBUTION OVER RESECTED AND NON-RESECTED AREAS

The rates of ripples and spikes were not significantly different in pre-ECoG ELs_{res} and ELs_{nres} ($p = 0.47$ and $p = 0.16$). The rate of fast ripples in ELs_{res} was higher than in ELs_{nres} ($p = 0.008$, table 2.3A).

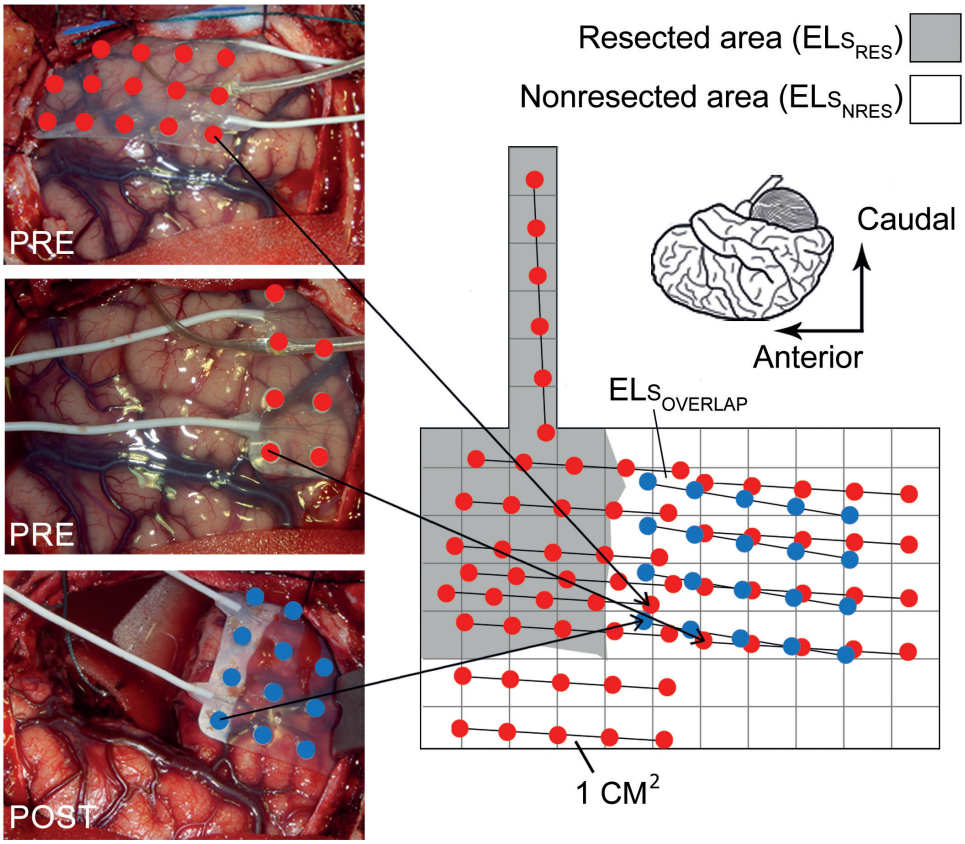

FIGURE 2.1

An example of how electrode grids and resected area (grey) of a patient during temporal lobe surgery were schematized into electrode locations (ELs) of 1cm^2, representing one or more bipolar electrode channels. Left: Examples of photos of grid electrode positions Right: Four grid electrode positions, three pre-ECoG (red) and one post-ECoG (blue), are combined into a scheme. Bipolar montages were used along the length of the electrode grid. Note that some ELs contain only pre-ECoG data, some contain only post-ECoG data and some contain both (overlapping EL, $ELs_{overlap}$). ELs were considered resected (ELs_{res}) when more than 50% of the EL was in the resected area; otherwise the EL was non-resected (ELs_{nres}).

TABLE 2.3
Median event rate per EL per minute

	A. Pre-ECoG ELs	B. Pre-ECoG vs. Post-ECoG		C. $ELs_{overlap}$	
	Pre-ECoG ELs_{res}	**Pre-ECoG ELs_{nres}**	**Post-ECoG ELs_{nres}**	**Pre-ECoG**	**Post-ECoG**
Ripples	6.2	5.6	1.3	7.1	0.9
Range	(0.0 – 42.2)	(1.3 – 35.3)	(0.0 – 16.9)	(0.9 – 36.7)	(0.0 – 18.1)
p-value	0.47		0.01*		0.007*
Fast Ripples	0.4	0.0	0.0	0.0	0.0
Range	(0.0 – 12.5)	(0.0 – 9.9)	(0.0 – 5.4)	(0.0 – 8.6)	(0.0 – 0.0)
p-value	0.008#		0.25		0.03*
Spikes	9.4	4.3	1.0	5.3	0.6
Range	(0.2 – 60.6)	(0.3 – 57.1)	(0.1 – 13.5)	(0.0 – 62.7)	(0.0 – 12.1)
p-value	0.16		0.002*		0.005*
RoS	2.6	0.6	0.1	1.0	0.0
Range	(0.0 – 37.7)	(0.0 – 40.9)	(0.0 – 7.1)	(0.0 – 42.5)	(0.0 – 1.3)
p-value	0.07		0.009*		0.01*
RnoS	2.5	4.2	1.1	5.3	0.9
Range	(0.1 – 9.5)	(0.6 – 12.0)	(0.0 – 16.9)	(0.6 – 15.0)	(0.0 – 18.1)
p-value	0.26		0.02*		0.01*

RoS = ripple on a spike, RnoS = ripple without a spike. Median event rate per EL per minute is shown for pre- and post-ECoG recordings in resected and non-resected tissue (EL_{res} and EL_{nres}). The range reflects the patient with lowest average rate and patient with highest average rate. P-values are given for the comparison between A.) Pre-ECoG ELs_{res} and ELs_{nres}, B.) Pre-ECoG and post-ECoG ELs_{nres}, and C.) Pre-ECoG and post-ECoG ELs with both pre- and post-ECoG data ($ELs_{overlap}$). *Rate in post-ECoG is lower than pre-ECoG ($p < 0.05$), #Rate in resected area is higher than in non-resected area ($p < 0.05$). Note that fast ripples in post-ECoG occurred in only one patient.

DIFFERENCE BETWEEN PRE- AND POST-ECOG

When considering $ELs_{overlap}$, event rates were higher pre-ECoG compared to post-ECoG (table 2.3C). Ripples decreased by 87% ($p = 0.007$) and spikes decreased by 88% ($p = 0.005$) after resection. Two patients showed de novo ripples (patient 2 and 5), which were all RnoS. No patient showed de novo spikes. Fast ripples did not occur in $ELs_{overlap}$.

Due to the small number of $ELs_{overlap}$ in some patients (table 2.1), we looked at all pre- and post-ECoG ELs_{nres} as well (table 2.3B). This showed similar results: event rates had decreased in post-ECoG. Both ripple and spike rates decreased by 77% ($p = 0.01$ and $p = 0.002$). In post-ECoG less ripples were RoS compared to pre-ECoG (22% vs. 72%, $p = 0.002$). The decrease of RoS after resection was higher than the decrease of RnoS (RoS: 83%, RnoS: 74%; $p = 0.01$). Fast ripples in post-ECoG were found in one patient, who had a higher fast ripple rate post-ECoG than pre-ECoG, and poor outcome.

On individual patient level, the rate of ripples in ELs_{nres} decreased in all but two patients (patient 5 and 6; figure 2.2). In both these patients rates of RoS decreased and RnoS increased. Patient 5 had good outcome. The rate of spikes decreased in all but two patients (patient 3 and 13), who showed a small increase.

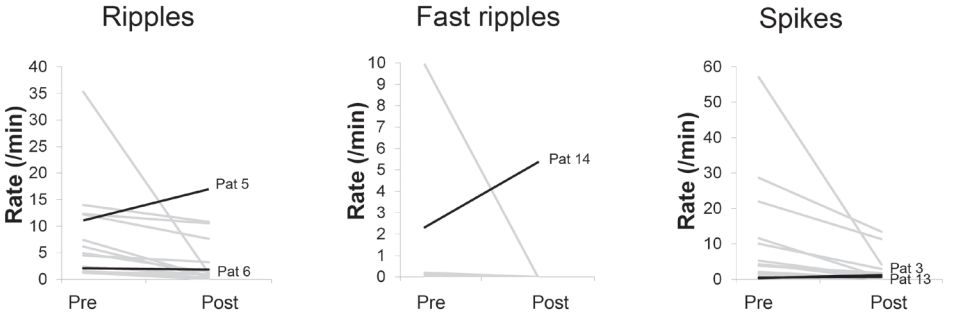

FIGURE 2.2
Average event rate per EL per minute of ripples, fast ripples, and spikes in pre-ECoG ELs_{nres} and post-ECoG ELs in all individual patients. Most rates decreased post-ECoG. In two patients (patient 5 and 6) ripple rate increased after resection, in one other patient (patient 14) fast ripple rate increased after resection and in two patients (patient 3 and 13) spike rate increased after resection.

RELATION TO OUTCOME

Pre-ECoG and outcome

The ratio between ELs_{res} and ELs_{nres} for ripples, spikes and RoS was similar in patients with good and poor outcome (figure 2.3). The ratio for fast ripples seemed higher in patients with good outcome. All patients with pre-ECoG fast ripples and good outcome had more than twice as many fast ripples in ELs_{res} compared to ELs_{nres}.

Post-ECoG and outcome

The one patient without ripples in post-ECoG had good outcome. The two patients with de novo ripples in the non-resected sensorimotor area after resection had good outcome. Strikingly, the rates of ripples in post-ECoG seemed higher in patients with good than with poor outcome (medians: 3.3/min vs. 1.3/min; figure 2.4A), which was especially true for the rate of RnoS (3.2/min vs. 1.0/min), while RoS rates seemed to show no difference (0.1/min vs. 0.2/min). The rate of spikes in post-ECoG was similar in patients with good and poor outcome. Post-ECoG fast ripples were found in one patient, who had a poor outcome.

EVENTS AT THE EDGE OF RESECTION

No patient showed an increase in ripples or spikes in ELs_{edge}, and no patient showed de novo events in ELs_{edge}. After resection the spike rate in ELs_{edge} was not different from rates in ELs_{dist} (ELs_{edge}: 0.4/min, ELs_{dist}: 1.1/min, $p = 0.13$). The one patient with fast ripples in showed them in four ELs, two ELs_{edge} and two ELs_{dist}.

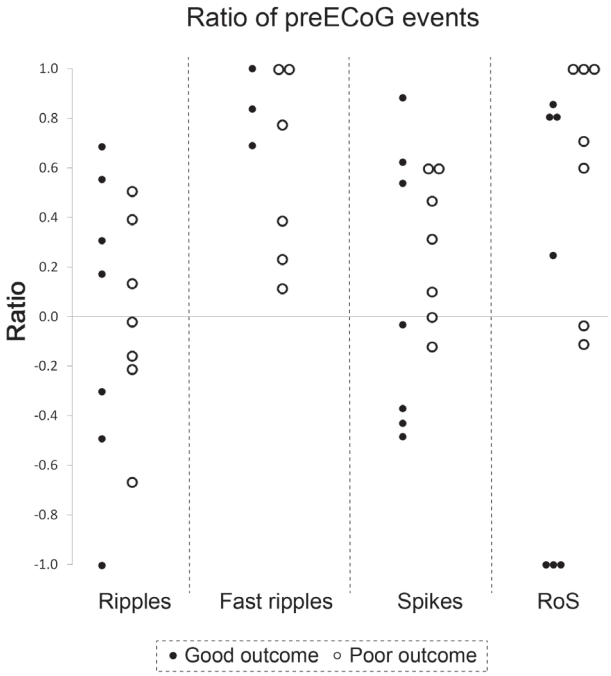

FIGURE 2.3
Ratio of pre-ECoG events over ELs_{res} and ELs_{nres} in patients with good (dots) and poor (circles) outcome. Each dot/circle represents one patient. A ratio > 0 indicates that the majority of the events was resected during surgery. Five patients did not have fast ripples in pre-ECoG. Ripples, spikes and RoS seem distributed over ELs_{res} and ELs_{nres} for both patients with good and poor outcome. Fast ripples are more prominent in ELs_{res}, especially in patients with good outcome.

EVENTS IN SENSORIMOTOR CORTEX

We noticed an increased rate of ripples in the sensorimotor area. We therefore specifically analyzed RnoS in this area. Sensorimotor and non-sensorimotor ELs (ELs_{sm} and ELs_{nsm}) were identified by an electrical stimulation protocol performed during intra-operative ECoG, photographs and clinical reports. Event rates in the ELs_{sm} were analyzed with a Mann Whitney U test, considering each EL independently.

We compared the higher post-ECoG ripple rates in good outcome patients with poor outcome patients, and found high ripple rates especially in the sensorimotor area of good outcome patients. Four patients had electrodes covering the sensorimotor area, in three patients in pre- and post-ECoG (patient 2, 3 and 5), and in one patient only in pre-ECoG (patient 7). For pre-ECOG (4 patients) in total 18 ELs_{sm} with average rate of 8.4/min and 71 ELs_{nsm} with average rate of 6.4/min existed. For post-ECoG in total 24 ELs_{sm} with an average rate of 14.8/min and 63 ELs_{nsm} with average rate of 3.9/min existed. Overall, RnoS rates in ELs_{sm} were higher than in ELs_{nsm} in post-ECoG but not in pre-ECoG ($p = 0.27$ and $p < 0.001$).

We found a higher rate of RnoS in ELs_{sm} compared to ELs_{nsm} in post-ECoG in the two patients with good outcome (Pat 2 and 5, p = 0.02 and p = 0.04, figure 2.4B). They also showed an increase in RnoS in ELs_{sm} and de novo RnoS in ELs_{sm} after resection. No difference was found in pre-ECoG nor in the patients with poor outcome.

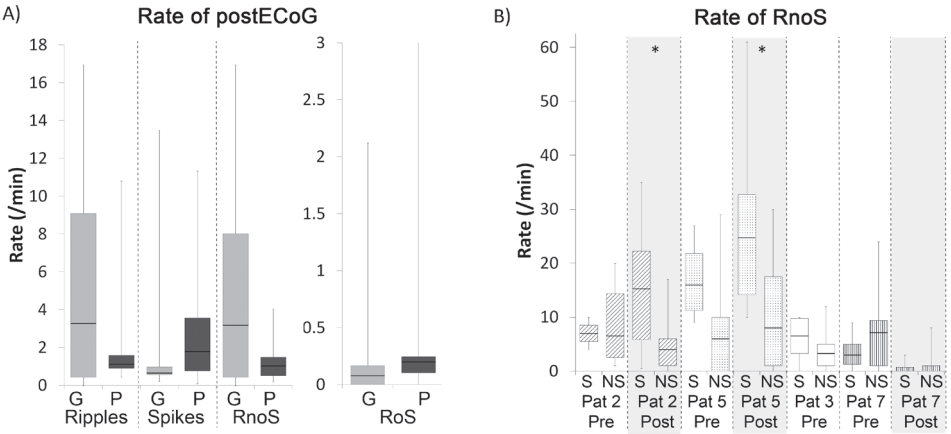

FIGURE 2.4
A.) Rate per EL of post-ECoG events in patients with good (G, grey) and poor (P, black) outcome. Fast ripples in post-ECoG occurred in only one patient and are therefore not depicted. B.) Ripple without spike (RnoS) rate ELs_{sm} (S) and ELs_{nsm} (NS) in four patients. In patient 2, 5 and 7 ELs_{sm} were recorded in both pre- and post-ECoG and in patient 3 only in pre-ECoG. Patient 2 and 5 had good outcome. *RnoS rates in the ELs_{sm} were higher than in ELs_{nsm} in patient 2 and 5 ($p < 0.05$).

DISCUSSION

We investigated the effect of surgical resection on ripples, fast ripples and spikes in intra-operative ECoG. Event rates decreased after resection in most non-resected brain areas. The resection did not seem to cause new events on the edge of the resection. Removal of fast ripples before and after resection seemed related to surgical outcome, while this was not true for spikes and ripples. The rate of RnoS decreased less than RoS and remarkably we found de novo RnoS in the sensorimotor cortex after successful surgery, suggesting that removing the epileptogenic focus leads to an increase in physiological ripples.

Fast ripples in pre-ECoG are presumably highly epileptogenic and removal of fast ripple generating tissue is correlated with good surgical outcome in our study as it has been in previous studies[18, 19, 34-36]. Five out of the 14 patients we studied, however, did not show fast ripples. Only one patient showed fast ripples in post-ECoG and did not become seizure free. This patient had tuberous sclerosis and showed fast ripples near a second tuber that was not resected. During surgery some

doubt existed whether to resect this second tuber, as spikes were also found adjacent to the tuber. Knowledge of these post-resection fast ripples during surgery might have led to a different decision. Fast ripples seem to give useful information during surgery if present. One might want to increase the analysis epoch, use smaller electrodes or wait for fast ripples to occur, to profit from fast ripples in tailoring.

Ripples occurred in all patients. Most chronic ECoG and depth EEG studies showed ripples correlated with the seizure onset zone[18, 20, 33, 34, 46]. In our intra-operative study ripple rates in resected tissue were not higher than in non-resected tissue, like some others found[35, 36]. More insight into the differences between intra- and extra-operative ECoG is necessary to determine the nature of the different behavior of ripples.

Tailoring based on ripples is complicated by the fact that not all ripples are pathological[37, 38, 47]. The prevailing hypothesis is that ripples co-occuring with a spike (RoS) are more epileptogenic[40]. In pre-ECoG 72% of all ripples were RoS, while in post-ECoG this was only 22%. The rate of RnoS in post-ECoG seemed higher in patients with good outcome and two patients showed more RnoS in the sensorimotor area compared to other areas, which also increased after resection. We hypothesize that post-ECoG ripples are mainly physiological and that resection of the epileptogenic zone alters the brain network in such a way that physiological ripples remain or even increase. This increase might be a good sign, as physiological ripples might be related to cognitive function[11]. The difference between physiological and pathological ripples needs further investigation to determine if pathological ripples can be used to delineate epileptogenic tissue. Perhaps 'red' and 'green' ripples exist, like 'red' and 'green' spikes[28].

Spike rates in the resected area seemed unrelated to surgical outcome, which is in accordance with some previous studies[30, 31], but not with others[18, 28, 48]. Clinical experience taught us to be cautious with spikes at the edge of the resection because they can be caused by the procedure[16]. We, however, did not find increased spike, nor ripple or fast ripple rates at the edge of the resection, so we could not objectify this phenomenon.

Note that we visually marked all spikes per channel, which is different from how spikes were used during surgery: the neurophysiologist may differentiate primary from propagation spikes and weighs primary spikes more heavily[28, 49]. Brain tissue generating those primary spikes was resected while some propagation spikes were considered less important and sites showing them were not always included in the resection. Due to the difference in interpretation, spikes in pre-ECoG in our results could be equally distributed over resected and non-resected areas. Whether a similar propagation also exists for HFOs is not known. A study comparing these aspects of spikes to the occurrence of HFOs is needed to see if this refines the value of HFOs as biomarker for surgery.

The decrease in event rates in post-ECoG could be explained by the hypothesis that HFOs are linked to an epileptogenic network[50-52]. Spikes are generated by a neural network and can spread over the cortex. Complete removal of the spiking zone is therefore not always necessary to achieve seizure freedom[14, 28, 49]. The same might hold for pathological ripples. A striking example is patient 10 who showed some fast ripples in pre-ECoG at approximately four centimeter from the resection site, which were not found in post-ECoG, and seizure freedom was achieved. Analysis of the brain network based on HFOs can help gain insight in the relation between HFOs in- and outside the resected area and alterations in the network during surgery.

A practical necessity for the use of HFOs for tailoring during surgery is a fast detection algorithm that reduces analysis time and facilitates onsite interpretation. We optimized and validated an existing detector[44], which required a visual check after running the detector. The combination of the slow algorithm and over detection renders this algorithm not yet ready for onsite use during surgery; most proposed detection algorithms encounter these problems[53, 54]. A recently published algorithm based on sample-to-sample analysis is faster and might be accurate for onsite detection, however validation on visually identified HFOs is necessary[55].

METHODOLOGICAL ASPECTS

This study recorded ECoG during surgery. General anesthesia was induced using a combination of propofol and a synthetic opioid and maintained using a target-controlled propofol infusion pump. Propofol is known to have anti-epileptic properties and decreases the number of spikes and HFOs in intra-operative ECoG[43, 56]. Accumulation of propofol during surgery could have influenced the relatively low HFO rate we found, although epochs were selected when the ECoG background pattern was continuous and appeared the same in pre- and post-ECoG. HFO analysis in chronic ECoG or depth EEG recordings is not hampered by anesthetic agents, which makes comparison between HFO rates in extra- and intra-operative recordings unrepresentative.

The fact that ECoG was measured intra-operatively meant we could use only one minute for analysis. Intra-operative recordings were usually 3-4 minutes, of which the first minutes often contaminated by burst suppression. These short epochs could have influenced our findings, as longer epochs might give a better representation of the data[45].

Ideally one would only compare event rates in ELs with both pre- and post-ECoG data. Unfortunately the same sites were not always covered in pre- and post-ECoG. Prospective gathering of data with overlapping pre-ECoG and post-ECoG electrode positions is necessary to verify our findings in a larger patient group.

Awareness of artifacts of electrical devices and electrode movements is important in intra-operative recordings. The energy of short (< 50 ms) high amplitude (> 100 μV) transients can spread over the entire frequency range. When passed through a filter these events result in a signal close to the impulse response of the filter, which looks like an HFO[33, 57]. Artifacts were differentiated from genuine HFOs during visual inspection based on their morphology and appearance in unfiltered ECoG. We took a conservative approach and discarded all HFOs that could have been caused by an artifact.

We related ECoG findings with the one-year post-surgical outcome, which is short to measure seizure freedom[58]. The study did not include patients with seizures after ceasing drugs, but some good outcome patients were still on anti-epileptic drugs. This, combined with the small population, makes drawing conclusions based on outcome difficult. We have, however, shown a trend that should be confirmed in a larger study, with a focus on seizure-free and drug-free outcome.

CONCLUSION AND FUTURE DIRECTIONS

The most important question for the future is: can we use intra-operative HFOs to delineate the resection in epilepsy surgery? We investigated the epileptogenic value of ripples, fast ripples and spikes in intra-operative ECoG in patients with various underlying pathologies, pre- and post-resection. Fast ripples seem a reliable biomarker, but are absent in some patients and are therefore not applicable as stand-alone biomarker. Ripples in intra-operative ECoG seem less related to epileptogenic tissue than ripples in chronic ECoG or depth EEG and are therefore hard to use as biomarker. The influence of delineation of the resection based on HFOs can be directly compared with outcome in a prospective study, but the presence of physiological ripples, especially in post-ECoG complicates in decision making based on ripples. We need more information on occurrence and behavior of pathological and physiological ripples was well as on differences between intra- and extra-operative ECoG before delineation of the resection based on HFOs can be recommended. Interestingly, we found de novo, presumably physiological, ripples in the sensorimotor cortex after resection which could even to be related to a good outcome. Physiological ripples should ideally be studied in healthy subjects, which is not possible with invasive recordings. HFO analysis in scalp EEG or MEG[59] allows the study of healthy subjects to gain knowledge in physiological ripple behavior.

CHAPTER 3

RESIDUAL FAST RIPPLES IN THE INTRA-OPERATIVE CORTICOGRAM PREDICT EPILEPSY SURGERY OUTCOME

Neurology 2015;85:120-128.

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ABSTRACT

INTRODUCTION

We studied if residual high frequency oscillations (HFOs, 80-500 Hz; ripples 80-250 Hz), especially fast ripples (FRs, 250-500 Hz), in post-resection intra-operative electrocorticography (ECoG) predicted seizure recurrence in comparison to residual inter-ictal spikes and ictiform spike patterns.

METHODS

We studied, retrospectively, ECoG recorded at 2048 Hz after resection in a cohort of patients with refractory focal epilepsy. We analyzed occurrence and number of residual FRs, ripples, inter-ictal spikes and ictiform spike patterns within the last minute of each recording and compared these to seizure recurrence.

RESULTS

We included 54 patients (median age 15.5y) with 25 months median follow-up. 24 patients had recurrent seizures. We found residual FRs, ripples, spikes and ictiform spike patterns in 12, 51, 38 and 9 patients. Nine out of 12 patients with residual FRs had recurrent seizures (p=0.016, positive predictive value=75%). Other ECoG events did not predict seizure recurrence. Patients with seizures had higher FR rates than seizure-free patients (p=0.022). FRs near the resection and in distant pathological areas could have changed the resection in eight patients without harming functionally eloquent areas. One seizure-free patient had FRs in distant functionally eloquent areas.

CONCLUSION

Residual FRs in post-resection ECoG are prognostic markers for seizure recurrence, especially if their number is high. Tailoring could rely on FRs, but requires careful assessment of the ECoG, as FRs in functionally eloquent areas might not be pathological.

INTRODUCTION

The current success rate of epilepsy surgery in patients with refractory focal epilepsy lies between 36 and 84% after one year [9]. Intra-operative electrocorticography (ECoG), can be performed during surgery to optimize delineation of the epileptogenic area by taking into account inter-ictal spikes or spike patterns. This so-called tailoring affects surgical decision making[13].

Removal of areas with inter-ictal spikes in the pre-resection ECoG has been associated with seizure freedom[14, 28, 29, 48, 60]. Especially ictiform spike patterns, consisting of recruiting, repetitive bursting and continuous rhythmic spiking patterns are considered markers of epileptic brain tissue, and it is advocated that those areas should be removed to achieve seizure freedom[61, 62].

Residual inter-ictal spikes in the post-resection ECoG (post-ECoG) might carry prognostic value[14, 29, 63, 64], but some studies contradict this[15, 30, 31, 65]. Residual spikes in post-ECoG may arise from surgical manipulation of the neocortex at the resection margin. This poses the question whether spike hunting is worthwhile[16]. Discriminating between epileptic 'red' and meaningless 'green' spikes is difficult[28].

High frequency oscillations (HFOs; ripples 80-250 Hz; fast ripples (FRs) 250-500 Hz) have been proposed as better biomarkers for the epileptogenic zone than spikes[18, 20, 36, 66]. Research on intra-operative HFOs has focused on FRs in pre-resection ECoG, and concluded that removal of tissue with FRs is linked to seizure freedom[19]. Currently, there is no evidence on how HFOs affect tailoring when the planned resection, based on anatomical pathology and spikes, is accomplished. Studying the ECoG for FRs and ripples after resection and comparison with residual spikes is needed to establish the value of HFOs for surgical tailoring. We hypothesize that residual FRs are the best predictor for seizure recurrence. We investigated the prognostic value of residual FRs in post-ECoG for seizure recurrence compared to ripples, spikes and ictiform spike patterns.

METHODS

COHORT

We included patients of all ages with refractory epilepsy who underwent surgery with intra-operative ECoG tailoring at the University Medical Center in Utrecht, The Netherlands, between 2008 and 2012. At our center intra-operative ECoG is performed when it is expected to affect surgical decisions. We included patients if: i.) the ECoG was recorded at a 2048 Hz sampling frequency, ii.) post-resection ECoG recordings were performed and iii.) post-surgical seizure outcome after ≥1 year was available. We excluded patients if they underwent chronic subdural ECoG monitoring, if ECoG was solely intended for functional mapping, if patients had recurrent tumor growth or radiotherapy after surgery or when a functional disconnection was performed.

STANDARD PROTOCOL APPROVALS, REGISTRATIONS AND PATIENTS CONSENTS

The institutional ethical committee approved the study and waived the need for written informed consent because of the retrospective character, provided that we coded the data and handled them anonymously.

INTRA-OPERATIVE ECOG

We recorded ECoG using 4x5 or 4x8 electrode grids and 1x6 or 1x8 electrode strips (Ad-Tech, Racine, WI, USA) placed directly on the cortex. Platinum electrodes with 4.2 mm^2 contact surface, embedded in silicone, and 1 cm inter-electrode distances were used. Recordings were made with a 64-channel EEG system (MicroMed, Veneto, Italy) at 2048 Hz sampling rate using an anti-aliasing filter at 538 Hz. Grids and electrode strips were placed in multiple configurations before and after resection

General anesthesia was induced with propofol, a synthetic opioid and atracurium, and maintained using a propofol infusion pump. Propofol was stopped during ECoG registration until a continuous ECoG background pattern was achieved. Resection was based on pre-surgical video-EEG, MRI and other diagnostics together with tailoring based on inter-ictal spikes and ictifom spike patterns, using conventional EEG settings (70 Hz low-pass filter, 10 s/page) in a common average reference montage. HFOs were never analyzed during surgery. After resection, the grids and strips were placed around the resected area and propofol was again discontinued. The resection was extended when deemed necessary and the ECoG repeated. We designated the recordings after the final resection as 'post-ECoG'. We selected the last minute of an ECoG recording to minimize propofol effects.

We collected information about surgical procedures and positioning of ECoG electrodes from clinical reports. We schematized electrode positions using photos taken during surgery. Different post-ECoG recordings could overlap. Electrode pairs from a bipolar montage were classified as being near the resection (≤ 1cm from the resection margin) or in extra-marginal tissue (> 1cm from the resection margin) (figure 3.1).

HFOS

We analyzed HFOs using an automated, Matlab based, detector adapted from the MNI detector[44] for the use on our intra-operative ECoG[67]. The detector first detects baseline segments without oscillatory activity. HFOs are detected if the energy of the signal is larger than baseline during a certain period with a minimum of four oscillations. We used a high pass finite impulse response (FIR) filter >80Hz for ripples and >250 Hz for FRs. All detected HFOs were visually post-processed in Stellate Harmonie Reviewer (v7.0, Montreal, QC, Canada) by one observer (MvtK/NvK), then checked and discussed to reach consensus by a second observer (MZ) to correct for artifacts[43], falsely identified and missed HFOs. Filter settings for visual analyses were as mentioned above. We used a split screen to visualize ripples (gain 5 μV/mm) and FRs (gain 1 μV/mm) simultaneously at an elongated time interval of 0.4 s/page[18].

SPIKES AND ICTIFORM PATTERNS

Spikes in the post-ECoG were visually marked retrospectively by MvtK in Stellate Harmonie Reviewer in a bi-polar montage, using conventional filter settings 0.5-70 Hz (IIR-filter) at a gain of 75-200 μV/mm and 10 sec/page. Marked spikes were checked, also against the common average reference as routinely used in the OR, by a neurophysiologist (FL). We defined spikes as paroxysmal sharp transients, with a maximum duration of 80 ms[49] and minimal amplitude of twice the baseline. Very sharp waves occurring at the same time on other bipolar channel traces

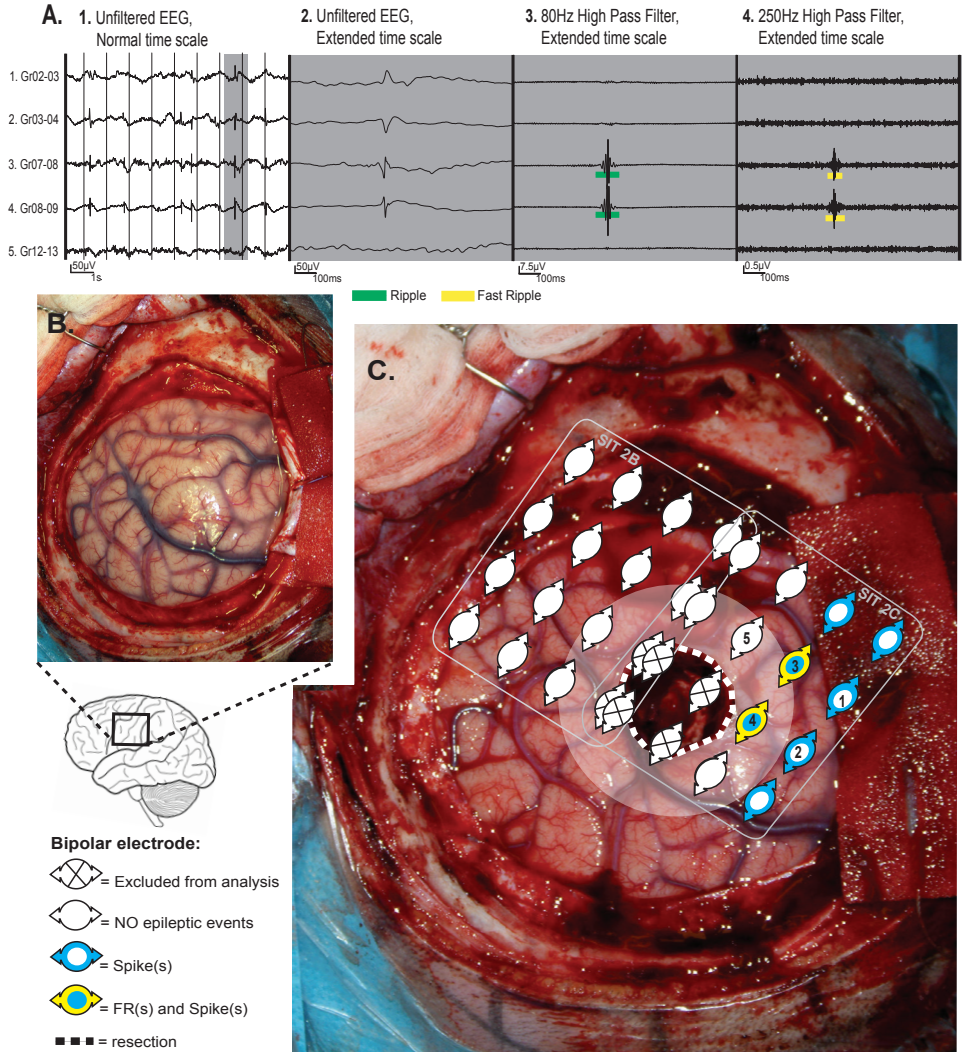


FIGURE 3.1
Patient example. Patient (pt 26) with a left central lesion, with FRs in post-ECoG and recurrent seizures (auras) after surgery. A.) Spike and HFO events in a selection of bipolar channels (indicated as 1-5 in part C). B.) Pre-resection photo. C.) Post-ECoG. The resected area is delineated by a dotted white line. The area being near the resection (≤1 cm, resection margin) is marked transparent white. Two ECoG recordings post-resection were performed. We represented the location of the bipolar channels analyzed. Note that FRs (rate: 25/min/electrode) (yellow) were present in the margin of the resected lesion (DNET), in a larger region with spikes (range rate: 4-41/min/electrode) (blue). Almost all electrodes showed ripples and are therefore not depicted. Based on the FRs present in the resection margin a different surgical decision could have been made if FRs would not have been that close to eloquent central cortex. See also table 3.3 for details on patient characteristics and post-ECoG findings.

were also marked as spikes. All channels were marked for ictiform spike patterns, consisting of continuous spiking, bursts and recruiting patterns (supplementary figure 3.1), by MvtK and FL jointly. We marked spikes and ictiform spike patterns independently and blinded for HFOs and vice versa. We marked all events blinded for outcome.

POST-SURGICAL ASSESSMENTS

We collected pathology, post-surgical outcome and use of AEDs after surgery from the clinical reports. We divided the pathology results in four subgroups, i) mesial temporal sclerosis (MTS), ii.) glioneural tumors including gangliogliomas and dysembryoplastic neuroepithelial tumors (DNET), iii) malformations of cortical development, including focal cortical dysplasia (FCD) and tuberous sclerosis, and iv.) others, such as cavernomas and gliosis.

Post-surgical outcome was classified by the Engel classification, which we dichotomized into complete seizure freedom (Engel IA) and recurrent seizures (Engel 1B–4)[5]. We determined the outcome for the most recent follow-up with a minimum of one year. We notated if seizure-free patients continued their pre-surgical AEDs, started withdrawal or stopped AED use.

DATA ANALYSIS

We analyzed the following residual post-ECoG events: number of FRs, ripples, spikes and ictiform spike patterns. We further divided the HFOs into FRs coinciding with spikes (FRonS), FRs without spikes (FRnonS), ripples coinciding with spikes (RonS) and ripples without spikes (RnonS).

We calculated for each patient: the total number of events on all channels (# events), the number of channels with events (# channels with events), the mean event rate per minute over all channels with events (mean # events/minute/channels with events) and the presence of events in- and outside the resection margin.

We tested for demographic differences between the recurrent seizure group and the seizure free group. We determined the prognostic value of occurrence of residual events for outcome by calculating its association, the positive (PPV) and negative predictive value (NPV). The PPV was defined as the percentage of patient with recurrent seizures and residual ECoG events out of all patients with residual events. The NPV was defined as the percentage of patients with seizure freedom and no residual ECoG events out of all patients without residual events. We tested for differences in mean event rates of FRs, ripples and spikes with regard to outcome. We report median and interquartile range (IQR) and used Mann-Whitney test for continuous variables which were not normally distributed (Kolmogorov–Smirnov test). Categorical variables were compared using the X^2-test or Fisher exact test, where appropriate. Statistical analysis was performed in IBM SPSS Statistics 21 (IBM Corp, Armonk, NY, USA). We considered p-values ≤0.05 significant.

RESULTS

COHORT

Between 2008 and 2012, 101 Patients with focal refractory epilepsy underwent surgery with intra-operative ECoG tailoring at 2048 Hz sampling rate. Fifty-four patients (29 male) met the inclusion criteria (table 3.1).We excluded 47 patients: 19 had incomplete data, 13 underwent preceding chronic ECoG monitoring, nine had recurrent tumor growth or radiotherapy, and six had a disconnection surgery.

SURGERY

Thirty patients underwent right-sided surgery. The resected area was temporal in 33 patients, frontal in 12 patients, peri-central in seven patients, parietal and occipital both in one patient. Pathology revealed MTS in eight patients, tumors with glial components in 23 patients, malformations of cortical development in 15 patients, and other pathologies in eight patients (table 3.1).

POSTSURGICAL OUTCOME

The median (IQR) post-operative follow-up period was 25.0 (17.0-35.8) months. Twenty-four of the 54 patients continued having seizures or relapsed (44%). Worthwhile improvement (Engel 1b-3C) was achieved in 80% of these patients. Seventeen of the 30 patients with seizure freedom had all AEDs withdrawn during follow-up, eight started withdrawal, and five had an unaltered regimen. The seizure free group had a younger age at seizure onset and showed a trend towards a younger age at surgery (table 3.1).

POST-RESECTION ECOG

We included 1325 bipolar channels in the analysis, with a median (IQR) of 19.5 (15.0-29.0) channels per patient. We excluded 339 bipolar channels because of artifacts, a median (IQR) of 4.0 (2.0-9.3) channels per patient. The selected one-minute epochs were on average 10:13 minutes after propofol was stopped. After final resection, we found residual FRs in 12 (22%), ripples in 51 (94%), spikes in 38 (70%) and ictiform spike patterns in nine patients (17%) (table 3.2). FRonS, FRnonS, RonS and RnonS yielded similar results (table 3.2). All patients with FRs had also spikes and ripples. Two patients showed no other events than spikes, 15 showed only ripples. All patients with ictiform spike patterns had spikes and ripples, five had FRs. One patient (seizure-free) had no post-resection events at all.Out of the nine patients with ictiform spike patterns, one patient showed a recruiting pattern, five patients continuous spiking, two patients both bursts and a recruiting or continuous pattern and one showed all three patterns. All patients with ictiform patterns had individual spikes and ripples, five had FRs. Pathology revealed malformations of cortical development in eight of these nine patients.

TABLE 3.1
Demographic characteristics

		Seizure freedom (Engel 1A)	Recurrent seizures (Engel 1B-4)	p-value (test)
No.	54	30	24	
Gender (No. M/F)	29/25	15/15	14/10	0.54[a]
Age at surgery in years (median(IQR))	15.5 (12.0-30.5)	13.5 (11.8-21.0)	24.5 (13.3-33.3)	0.06[b,c]
Age at seizure onset in years (median (IQR))	9.5 (0.0-21.5)	7.0 (1.0-12.5)	16.0 (3.3 -24.5)	0.05[b,c]
Duration of epilepsy in years (median (IQR))	6.5 (2.3-11.8)	8.0 (2.8-11.3)	4.5 (2.0-12)	0.68[b]
Follow-up period in months (median(IQR))	25.0 (17.0-35.8)	24.0 (14.0-36.5)	28.0 (17.5-35.8)	0.40[b]
TLE vs extra-TLE	33/21	17/13	16/8	0.45[a]
AED use (--/ +-/++)	17/8/5	17/8/5		
RESECTED REGION (%, NO.):				0.14[a]
- Temporal	61.1%	17	16	
- Frontal	22.2%	10	2	
- Central	13.0%	3	4	
- Parietal	1.9%	0	1	
- Occipital	1.9%	0	1	
PATHOLOGY (%, NO.):				0.25[a]
- MTS	14.8%	4	4	
- Tumor with glial component	42.6%	15	8	
- Malformation of cortical development	27.8%	9	6	
- Other	14.8%	2	6	

Abbreviations: (--)= stopped anti-epileptic drug use, (+-)= anti-epileptic drugs withdrawal, (++)= pre-operative anti-epileptic drug dose, AED=anti-epileptic drugs, extra-TLE= epilepsy outside the temporal lobe, F=female, IQR= interquartile range, M=male, MTS=mesial temporal sclerosis, TLE=temporal lobe epilepsy,
Overview of demographic characteristics of all patients. The last column shows the p-value for the difference between the seizure free group and the recurrent seizures group. The seizure free group has a younger age at seizure onset and a corresponding trend towards a younger age at surgery. There was no difference between the two groups for the other clinical characteristics. No correction for multiple comparisons was performed. [a]=Pearson X^2-test, [b]=Mann-Whitney U-test, [c]= significant ($p \leq 0.05$).

POST-ECOG EVENTS AND OUTCOME

Fast ripples

Nine out of 24 patients with recurrent seizures had residual FRs. Residual FRs were associated with seizure recurrence (p=0.022, Pearson X^2) with a PPV of 75.0% and NPV of 64.3% (table 3.2). Patients with recurrent seizures had a higher median event rate of FRs than patients with good outcome (p=0.016, Mann Whitney U) (figure 3.2). Residual FRs occurred in the resection margin in six patients (table 3.2), of whom five had recurrent seizures.

Three patients with post-resection FRs were seizure free (table 3.3; one tumor, two with FCD). One patient still used his pre-surgical AEDs doses. Another patient had a frontal lesion and showed epileptic spikes and HFOs remote from the resected area, in Broca's and peri-central areas. The third patient showed a single FR in the extra-marginal tissue in an area with residual spikes.

Ripples, spikes & ictiform spike patterns

All 24 patients with recurrent seizures had residual ripples, versus 27 out of 30 patients who were seizure-free. Eighteen patients with recurrent seizures showed spikes, versus 20 seizure-free patients. Four patients with recurrent seizures showed ictiform spike patterns, versus five patients with seizure freedom. Neither presence of ripples, spikes, nor ictiform spike patterns in post-ECoG were associated with seizure recurrence (table 3.2). Nor did we find a difference in the mean ripple and spike rate between patients with poor and good outcome (table 3.2 and figure 3.2). Thirty-seven patients showed residual ripples and 24 showed residual spikes in the resection margin (table 3.2), about half of them (17 and 15) having seizure recurrence.

Fast Ripples and clinical decisions

We assessed whether resections could have been different when the residual FRs would have been taken into account instead of spikes.

In five out of six patients with FRs within and adjacent to the resection margin (pt 23, 51, 15, 48 and 38) the resection might have been extended without compromising eloquent regions. Additionally, in three out of the six patients with FR in the extra-marginal tissue (pt 16, 43 and 54) removal of the tissue with FRs was deemed possible. In summary, in eight patients, more tissue could have been removed without further deficit; the influence on post-surgical outcome of this extension is unclear. One of these patients (patient 15) was already initially seizure-free but has not yet attempted to reduce AEDs.

TABLE 3.2
Events post-resection ECoG and association with post-surgical outcome

	Events in post-ECoG					Post-surgical outcome					
	No. patients (total=54)	No. Events	No. bipolar channels (total =1333)	% Events in resection margin	Mean (range) event rate, (ev/min/channels with events)	Re-current seizures (total=24)	Seizure freedom (total=30)	PPV,%	NPV,%	p-value	Test
FRs	12	407	53	40.50%	8.4 (1-98)	9	3	75.00	64.30	0.016[C]	a
Ripples	51	11236	801	13.90%	9.9 (1-132)	24	27	47.10	100	0.25	b
Spikes	38	11635	514	14.00%	13.7 (1-128)	18	20	47.40	62.50	0.51	a
FRonS	11	375	51	40.20%	8.4 (1-89)	8	3	72.30	62.80	0.05[C]	b
FRnonS	8	34	13	41.20%	2.3 (1-13)	6	2	75.00	60.90	0.12	b
RonS	32	3389	312	15.50%	6.5 (1-131)	17	15	53.10	68.20	0.12	a
RnonS	51	8045	762	13.10%	7.7 (1-100)	24	27	47.10	100	0.25	b
Ictiform spike patterns	9	-	-	-	-	4	5	44.40	55.60	1	b

Abbreviations: ECoG=electrocorticography, FRs=Fast ripples, FRonS=Fast ripple on spike, FRnonS=Fast ripple without spike, RnonS=Ripple without spike, RonS=Ripple coinciding with spike.
Overview of the number of patients that showed residual events and its characteristics (per event type). We tested per event type if the presence of residuals was related to seizure recurrence, using PPV, NPV and association. Residual FRs were the only prognostic marker for seizure recurrence (p=0.016, PPV=75%, NPV=64.3%). Association between events and outcome was tested with a Pearson X^2 (a) or Fishers exact test (b) where appropriate. No correction for multiple comparisons was performed. [c] p-value≤0.05.

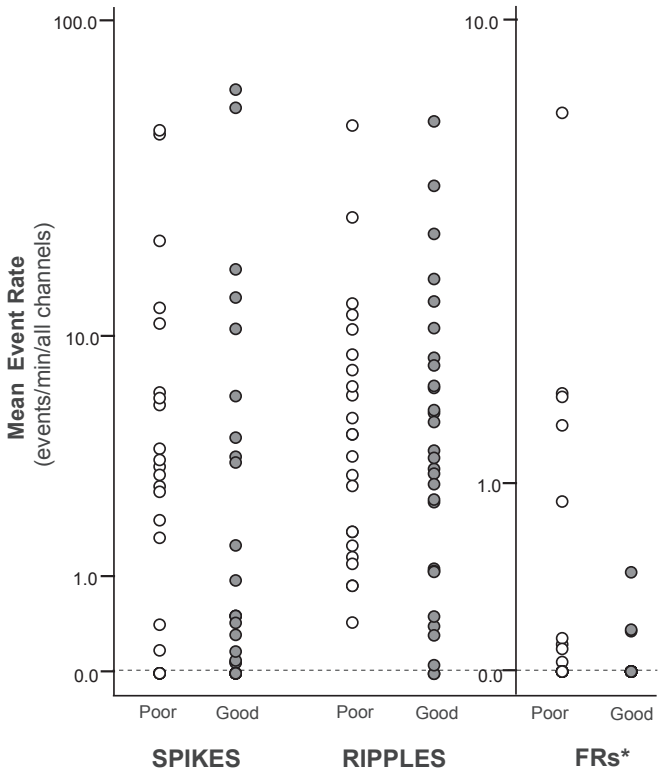


FIGURE 3.2
Distribution mean event rate. Distribution of the mean event rate (events/min/ all channels) per patient for residual spikes, ripples, and fast ripples (FRs). Note that patients with recurrent seizures had a higher median event rate of FRs than patients with good outcome (p=0.016, Mann Whitney U). This analysis was not significant when leaving out the patients without events. No difference was found for spikes and ripples (p_{spike}=0.64, p_{ripple}=0.16,Mann-Whitney U). Outcome is grouped as poor (Engel 1B-4, white dots, N=24)) versus good (Engel IA, grey dots, N=30). *significant (p≤0.05).

DISCUSSION

We found more residual FRs in patients with recurrent seizures than in patients who reached seizure freedom. Residual FRs were correlated with seizure recurrence with a PPV of 75.0%. Patients with recurrent seizures had a higher median event rate of FRs than patients with good outcome. Residual ripples, spikes or even ictiform spike patterns showed no relation with seizure recurrence. Residual FRs were the only prognostic marker for seizure recurrence. In eight patients these FRs could have resulted in a clinically feasible, different surgical strategy.

This study shows the prognostic value of residual FRs recorded *after* tailored surgery and the first comparison with ripples, inter-ictal spikes and ictiform spike patterns. These post-resection data enabled a direct comparison of events in remaining tissue with regard to outcome, while in pre-resection data one cannot know which events will be influenced by the removal of tissue.

TABLE 3.3
Characteristics of patients with residual FR in post-resection ECoG

										Findings post-ECoG						
Patient no.	Sex	Age at surgery, y	Epilepsy duration, y	Age at epilepsy onset, y	Epilepsy Side	Resected region	Pathology	Outcome (AED use)	FU mo	No. Total Bipolar channels	No. Bipolar channels analysed	Ictiform Spike Pattern	No.Spikes (no. channels)	No. Ripples (no. channels)	No. FRs (no. channels)
15[a]	M	15	9	6	Left	Temporal	Ganglioglioma (WHO gr 1-2)	1A (++)	45	28	25	-	86 (6)	137 (20)	4 (3)
16	M	31	9	22	Left	Temporal	MTS (Wyler gr 4)	2A	33	28	23	-	81 (19)	71 (20)	3 (2)
18[a]	F	13	13	0	Left	Frontal	FCD 2b	1A (--)	51	23	17	Continuous	882 (13)	422 (15)	3 (1)
21	M	12	1	11	Right	Central	DNET (WHO gr 1)	2A	40	32	28	-	45 (13)	213 (18)	1 (1)
23	M	44	19	25	Right	Central	TSC	4B	29	52	42	Continous, recruiting, bursts	1911 (36)	1025 (33)	62 (7)
25[a]	M	8	8	0	Left	Frontal	FCD 2b	1A (--)	31	69	68	Continuous	3639 (64)	3344 (63)	30 (11)
26	M	14	2	12	Left	Central	DNET (WHO gr 1)	1B	36	32	28	-	176 (7)	241 (23)	50 (2)
38	M	13	12	1	Left	Parietal	FCD 2b /TSC	3A	17	32	29	Continuous	588 (24)	304 (25)	198 (4)
43	F	6	5	1	Left	Temporal	Gliosis	1B	24	26	19	-	74 (10)	223 (18)	2 (2)
48[b]	F	3	3	0	Left	Temporal	FCD 1a	3A	11	32	24	Bursts, recruiting	136 (20)	107 (20)	42 (14)
51	M	28	6	22	Left	Temporal	Ganglioglioma (WHO gr 1)	2D	17	26	23	-	60 (16)	34 (12)	2 (2)
54	M	16	15	1	Right	Occipital	MCD + gliosis	2A	12	12	12	-	130 (9)	154 (8)	10 (4)

Abbreviations: (--)= stopped anti-epileptic drug use. (+-)= anti-epileptic drug withdrawal, (++)=pre-operative anti-epileptic drug use, AED=anti-epileptic drugs, DNET=dysembryoplastic neuroepithelial tumor, ECoG=electrocorticography, F=female, FCD= focal cortical dysplasia, FR= fast ripples, FU= follow-up, M=male, MCD= mild cortical dysplasia, MTS= mesial temporal sclerosis, TSC= tuberous sclerosis.
Overview of the twelve patients who showed residual FRs in their post-resection ECoG. Represented are the patients' characteristics and the post-ECoG findings regarding FRs, ripples, spikes and ictiform spike patterns. Nine patients showed post-resection ictiform spike patterns, but only five patients had residual FRs. Supplementary table 3.1 provides the comple patient characteristics.
[a] The three patients who were seizure free.
[b] The patient kept having seizures and underwent a second surgery (hemispherectomy) 11 months later.

A strength is that we included patients in whom the intra-operative ECoG was intended to affect surgical decisions. A weakness is that the post-surgical outcome is biased by the continued use of AEDs in many patients. Furthermore, post-resection data leaves less patients with events, especially FRs. We did not find FRs in 15 patients with poor outcome. This low sensitivity could be explained by insufficient spatial sampling of FRs, particularly in deeper structures[19]. Longer epochs for analysis could have revealed more HFOs. Propofol or AEDs can influence on the HFO rate[43, 66].

This type of data poses a statistical challenge to perform analysis on a group level. The number of recorded channels and findings differ per patient. Ideally one would normalize for the number of recorded channels or sampled cortex. On the other hand, not all channels are equally important, as we recorded with standard electrode grids and the number of recordings and thus electrodes merely results from pragmatic choices during surgery. We deliberately chose to focus on analysis of the presence or absence of residual events. Further, we used a commonly used method[18-20] of calculations based on het mean value of each patient for group analysis.

Our cohort contains a relatively large group of refractory patients with highly epileptogenic glioneuronal tumors. Nevertheless, we think findings can be generalized to the total population of epilepsy surgery candidates, as previous studies have shown HFOs in all types of pathology[68]. Previous studies have related the removal of the majority of HFOs in the *pre*-resection ECoG to good outcome, categorized as Engel score 1 and 2[18-20, 34-36, 67]. We found Engel score 3 and 4 mainly in patients with a high number and broad area with FRs, supporting the hypothesis that both the extent and amount of FRs relate to disease severity[46, 69-72].

We chose our primary outcome parameter to differentiate between absolute seizure freedom and seizure recurrence including auras, since auras are nothing less than simple partial seizures and thus an indicator that some epileptic tissue is still present[73]. We hypothesize that removing the majority of pre-resection FRs might be enough to achieve improvement in disease severity (i.e. Engel 1B to 2), but that complete removal of FRs is necessary to achieve true long term seizure freedom (Engel 1A). This is illustrated by two patients with residual FRs who were seizure free after one year, but relapsed (auras) when they started AED withdrawal.

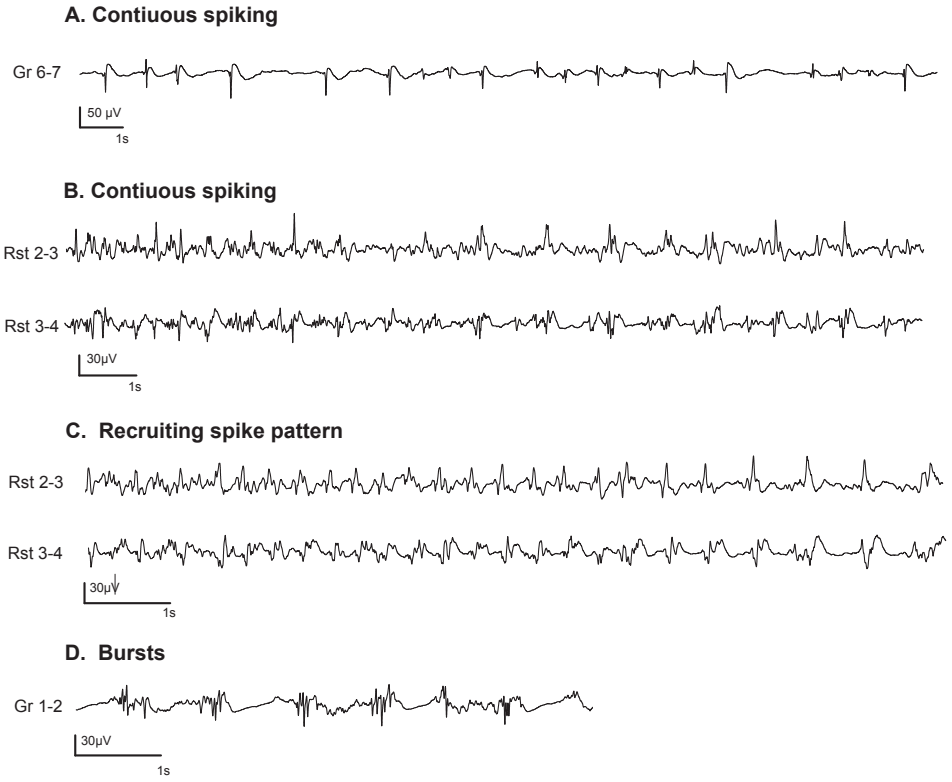
Contrary to the accumulating evidence for FRs, the value of ripples is debated. Several studies have shown that pre-surgical ripples also appear to identify the epileptogenic region[18, 20, 36, 66, 67]. We found, however, no evidence for an association between residual ripples and outcome, though RonS showed a relatively high association. Differentiation between pathological and physiological ripples is difficult[37, 38, 47]. Ripples associated with spikes are probably epileptogenic and those without spikes may be physiological[40]. Almost all patients showed ripples and it would be interesting to investigate which ripples are epileptogenic. We described in a previous study the occurrence of 'de novo' post-resection ripples without spikes in sensorimotor areas, which suggests that these are physiological[67]. We found residual FRs, with and without spikes, in functionally eloquent areas in two of the three patients with good outcome. This suggests some FRs might be physiological too[37, 74].

One might better rely on FRs for tailoring than on ripples, spikes or ictiform spikes patterns. Careful assessment of the intra-operative ECoG for potentially physiological HFOs in functionally eloquent areas is necessary. Similar to tailoring based on spikes, FR findings have to be put in the clinical

context. Not only the presence of FRs but also the amount, extent and location, in- or outside the resection margin, is important to consider. Increasing the analyzed epoch length or using additional depth or flexible electrodes may increase the sensitivity for FRs. Especially patients with FCDs and MCDs could benefit from FR-based tailoring. These pathologies are characterized by blurred or MRI negative lesions, and the evidence for a relation between HFO rate and disease severity is rising[36].

Ten years of retrospective research have gained us increasing evidence that FRs are more specific biomarkers than spikes. Future research should focus on collecting evidence of the actual clinical usefulness of HFOs.

SUPPLEMENTS



SUPPLEMENTARY FIGURE 3.1
Ictiform spike patterns. Example of the ictiform spike patterns, consisting of three types, as marked in this study. A and B.) Two types of continuous spiking patterns, C.) A recruiting spike pattern, D.) A burst pattern.

SUPPLEMENTARY TABLE 3.1
Patient characteristics of all patients

										Findings post-ECoG					
Patient no.	**Sex**	**Age at surgery, y**	**Epilepsy duration, y**	**Age at epilepsy onset, y**	**Epilepsy Side**	**Resected region**	**Pathology**	**Outcome (AED use)**	**FU mo**	**No. Total Bipolar channels**	**No. Bipolar channels analysed**	**Ictiform Spike Pattern**	**No.Spikes (no. channels)**	**No. Ripples (no. channels)**	**No. FRs (no. channels)**
1	F	12	2	10	Left	Frontal	FCD 2b	1A (--)	38	69	51	continuous	676 (48)	540 (44)	-
2	M	12	1	11	Right	Frontal	DNET (WHO gr 1)	1A (--)	49	46	29	-	-	447 (25)	-
3	F	24	2	22	Right	Temporal	DNET (WHO gr 1)	4B	43	40	34	-	6 (4)	59 (17)	-
4	M	11	4	7	Right	Central	MDC	4B	43	48	39	-	16 (8)	46 (15)	-
5	M	25	2	23	Right	Frontal	developmental venous anomalies+ reactive changes	4B	26	48	37	-	222 (21)	32 (9)	-
6	M	34	2	32	Left	Temporal	Cavernoma	2A	34	16	16	-	53 (9)	7 (4)	-
7	F	3	2	1	Left	Temporal	DNET (WHO gr 1)	1A (--)	30	42	28	-	459 (15)	235 (26)	-
8	F	46	10	36	Right	Temporal	Iatrogenic cortical leasion - Hippocampal damage	1A (+-)	47	16	16	recruiting	58 (7)	208 (12)	-
9	M	16	2	14	Left	Temporal	Ganglioglioma (WHO gr 2)	1A (++)	25	12	12	-	6 (2)	-	-
10	F	28	3	25	Right	Temporal	Ganglioglioma (WHO gr 2)	1D	27	21	20	-	245 (19)	124 (17)	-
11	M	20	1	19	Left	Temporal	Ganglioglioma (WHO gr 2)	2D	35	28	24	-	-	123 (17)	-
12	M	35	12	23	Left	Temporal	Suspected low-grade glioma	1A (++)	27	21	17	-	-	91 (13)	-
13	F	35	28	7	Left	Temporal	MTS (Wyler gr 4)	3B	43	16	14	-	-	39 (10)	-
14	F	46	26	20	Right	Temporal	Cavernoma +gliosis	1B	51	16	11	-	-	49 (10)	-

15	M	15	9	6	Left	Temporal	Ganglioglioma (WHO gr 1-2)	1A (++)	45	28	25	-	86 (6)	137 (20)	4 (3)
16	M	31	9	22	Left	Temporal	MTS (Wyler gr 4)	2A	33	28	23	-	81 (19)	71 (20)	3 (2)
17	M	14	11	3	Right	Temporal	MTS (Wyler gr 3)	1A (--)	43	32	22	-	-	85 (12)	-
18	F	13	13	0	Left	Frontal	FCD 2b	1A (--)	51	23	17	continuous	882 (13)	422 (15)	3 (1)
19	F	53	7	46	Right	Central	Adenocarcinoma	1A (--)	34	16	10	-	1 (1)	70 (2)	-
20	M	14	13	1	Right	Frontal	FCD 2b	1A (--)	25	84	48	-	15 (10)	379 (32)	-
21	M	12	1	11	Right	Central	DNET (WHO gr 1)	2A	40	32	28	-	45 (13)	213 (18)	1 (1)
22	M	2	1	1	Right	Frontal	Aspecific migratory disorder +reactive changes	2A	31	26	15	continous	663 (12)	716 (14)	-
23	M	44	19	25	Right	Central	TSC	4B	29	52	42	continous, recruiting, bursts	1911 (36)	1025 (33)	62 (7)
24	F	31	4	27	Right	Temporal	Ganglioglioma (WHO gr 1)	2A	29	21	18	-	-	8 (7)	-
25	M	8	8	0	Left	Frontal	FCD 2b	1A (--)	31	69	68	continuous	3639 (64)	3344 (63)	30 (11)
26	M	14	2	12	Left	Central	DNET (WHO gr 1)	1B	36	32	28	-	176 (7)	241 (23)	50 (2)
27	M	41	40	1	Right	Frontal	FCD 2b	1A (+-)	36	42	35	-	-	14 (4)	-
28	F	11	3	8	Left	Temporal	Ganglioglioma (WHO gr 1)	1A (++)	24	16	16	-	8 (5)	38 (9)	-
29	F	49	47	2	Left	Temporal	MTS (Wyler gr 4)	1D	22	38	36	-	99 (8)	132 (24)	-
30	F	15	6	9	Right	Temporal	MTS (Wyler gr 4)	1A (++)	12	16	14	-	13 (4)	7 (5)	-
31	F	5	3	2	Right	Frontal	TSC	1A (+-)	21	16	11	-	-	36 (6)	-
32	M	29	4	25	Right	Temporal	Cavernoma	2A	15	42	21	-	41 (11)	27 (10)	-
33	F	37	20	17	Right	Temporal	Cavernoma	1A (+-)	15	16	13	-	1 (1)	-	-
34	M	13	13	0	Right	Temporal	MTS (Wyler gr 4)	1A (--)	14	16	16	-	-	5 (2)	-
35	F	23	10	13	Left	Temporal	MTS (Wyler gr 4)	2B	17	28	15	-	46 (10)	13 (7)	-

SUPPLEMENTARY TABLE 3.1 CONTINUED
Patient Characteristics of all patients

										Findings post-ECoG					
Patient no.	**Sex**	**Age at surgery, y**	**Epilepsy duration, y**	**Age at epilepsy onset, y**	**Epilepsy Side**	**Resected region**	**Pathology**	**Outcome (AED use)**	**FU mo**	**No. Total Bipolar channels**	**No. Bipolar channels analysed**	**Ictiform Spike Pattern**	**No.Spikes (no. channels)**	**No. Ripples (no. channels)**	**No. FRs (no. channels)**
37	M	61	12	49	Left	Temporal	Aspecific migratory disorder	3A	19	16	15	-	-	100 (11)	-
38	M	13	12	1	Left	Parietal	FCD 2b /TSC	3A	17	32	29	continuous	588 (24)	304 (25)	198 (4)
39	F	14	8	6	Right	Frontal	DNET (WHO gr 1)	1A (+-)	12	16	14	-	6 (3)	15 (6)	-
40	F	16	9	7	Right	Temporal	Ganglioglioma (WHO gr 1)	4A	20	16	15	-	-	26 (8)	-
41	F	1	1	0	Left	Temporal	Difuse glioma (WHO gr 2)	1A (+-)	24	16	10	-	61 (10)	216 (10)	-
42	F	20	5	15	Right	Temporal	Pleiomorf xantho-astrocytoma (WHO gr 2)	1A (--)	15	16	14	-	-	-	-
43	F	6	5	1	Left	Temporal	Gliosis	1B	24	26	19	-	74 (10)	223 (18)	2 (2)
44	F	13	8	5	Left	Central	DNET	1A (--)	24	28	23	-	-	154 (13)	-
45	M	17	5	12	Right	Central	FCD 2b	1A (--)	12	80	69	continuous, bursts	719 (33)	216 (38)	-
46	M	6	5	1	Right	Temporal	Astrocytoma (WHO gr 2)	1A (--)	17	23	21	-	2 (2)	76 (15)	-
47	F	12	10	2	Right	Temporal	Gliosis	1A (+-)	14	16	16	-	-	39 (7)	-
48*	F	3	3	0	Left	Temporal	FCD 1a	3A	11	32	24	bursts, recruiting	136 (20)	107 (20)	42 (14)
49	M	12	2	10	Left	Temporal	DNET	1A (--)	17	21	18	-	3 (3)	51 (8)	-
50	M	24	16	8	Right	Temporal	Ganglioglioma (WHO gr 1)	1A (--)	13	21	17	-	73 (7)	18 (4)	-

51	M	28	6	22	Left	Temporal	Ganglioglioma (WHO gr 1)	2D	17	26	23	-	60 (16)	34 (12)	2 (2)
52	M	13	2	11	Right	Frontal	FCD 2b	1A (--)	14	23	16	-	-	1 (1)	-
53	M	9	9	0	Right	Frontal	FCD 2b /TSC	1A (+-)	12	84	68	-	100 (20)	337 (38)	-
54	M	16	15	1	Right	Occipital	MCD + gliosis	2A	12	12	12	-	130 (9)	154 (8)	10 (4)

Abbreviations: (--)= stopped anti-epileptic drug use. (+-)= anti-epileptic drug withdrawal, (++)=pre-operative anti-epileptic drug use, AED=anti-epileptic drugs, DNET=dysembryoplastic neuroepithelial tumor, ECoG=electrocorticography, F=female, FCD= focal cortical dysplasia, FR= fast ripples, FU= follow-up, M=male, MCD= mild cortical dysplasia, MTS= mesial temporal sclerosis, TSC= Tuberous sclerosis. Highlighted in grey are the patients with residual FRs (dark gray= seizure recurrence, light grey=seizure free). * Patient 48 kept having seizures and underwent a second surgery (hemispherectomy) 11 months later.

CHAPTER 4

TAILORING EPILEPSY SURGERY WITH FAST RIPPLES IN THE INTRA-OPERATIVE ELECTROCORTICOGRAM

Under revision, Ann Neurol.

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ABSTRACT

INTRODUCTION

Intra-operative electrocorticography (ECoG) can be used to delineate the resection area in epilepsy surgery. High frequency oscillations (HFOs, 80-500 Hz) seem better biomarkers for epileptogenic tissue than spikes. We studied how HFOs and spikes in combined pre- and post-resection ECoG predict surgical outcome in different tailoring approaches.

METHODS

We, retrospectively, marked HFOs, divided into fast ripples (FRs, 250-500 Hz) and ripples (80-250 Hz), and spikes in pre- and post-resection ECoG sampled at 2048 Hz in patients with refractory focal epilepsy. We defined four groups of event occurrence: pre+post- (+/), pre+post+ (+/+), pre-post+ (-/+) and pre-post- (-/-). We subcategorized three tailoring approaches: hippocampectomy with tailoring for neocortical involvement, lesionectomy of temporal lesions with tailoring for mesiotemporal involvement and lesionectomy with tailoring for surrounding neocortical involvement. We compared the percentage of resected pre-events, the occurrence of events and the different tailoring approaches to outcome (seizure-free versus recurrence).

RESULTS

We included 54 patients (median age 15.5y, 25 months follow-up, 30 seizure-free). The percentage of resected FRs, ripples or spikes in pre-ECoG did not predict outcome. Only the occurrence of FRs in post-ECoG, given FRs in pre-ECoG (+/-, +/+), predicted outcome ($p=0.03$). The absence of spikes in pre-ECoG might indicate good outcome ($p=0.06$). The highest predictive value for seizure recurrence was the presence of FRs in post-ECoG for all tailoring approaches.

CONCLUSION

FRs which persists before and after resection predict poor post-surgical outcome. These findings hold for different tailoring approaches. FRs can thus be used for tailoring epilepsy surgery with repeated intra-operative ECoG measurements.

INTRODUCTION

Intra-operative electrocorticography (ECoG) can measure epileptiform activity directly from the cortex during epilepsy surgery, aiming to delineate the epileptogenic tissue. This so called 'tailoring' searches for interictal spikes before and after initial resection and can be repeated until no epileptiform abnormalities remain[13,75]. A post-resection ECoG (post-ECoG) can verify completeness of the resection and should thus indicate the prognosis towards surgical success[15]. The value of tailoring has been challenged; the removal of tissue with interictal spikes in pre-resection recordings (pre-ECoG) has been associated with seizure freedom by some[28, 29, 48, 60-62], but not by others[65, 76, 77]. Spikes in pre-ECoG extended outside the resected tissue in about 75% of frontal lobe cases, and in 91 % of surgeries in the central, parietal and occipital lobes[78]. Several studies have suggested that residual spikes in the final post-ECoG predict poor surgical outcome[29, 63, 64], but this is again contradicted by others[15, 30, 31, 65]. A main concern is that surgical manipulation of the cortex may agitate the tissue, evoking spikes in the resection margin, which are not correlated with seizure outcome[16].

In the last decade a new biomarker for epileptogenic tissue has been suggested: high frequency oscillations (HFOs), consisting of ripples (80-250 Hz) and fast ripples (FRs, 250-500 Hz)[18, 20, 47]. Research on HFOs has focused on chronic ECoG and depth EEG, and to a lesser extent on FRs in intra-operative pre-ECoG[19]. Incomplete removal of tissue with HFOs, especially FRs, was strongly linked to poor surgical outcome whereas this relationship was not found for spikes[19, 20]. Recently, we demonstrated that the presence of residual FRs after resection in post-ECoG, predicted recurrent seizures while spikes and ripples did not[79]. FRs occur more focally than ripples and spikes as they probably arise from local pathological connections of neuron clusters, rather than from the larger scale networks in which spikes propagate. FRs are therefore considered more specific for epileptogenic tissue than spikes[20, 80]. It seems that surgical manipulation of cortex does not provoke 'de novo' FRs at the resection margin[67], so FRs could be a useful clinical biomarker to tailor epileptogenic tissue.

The tailoring strategy is largely determined by the MRI findings. In case of a clear lesion, the primary goal is often a lesionectomy including corticectomy of the surrounding epileptogenic tissue based on ECoG findings. In neocortical temporal epilepsies, ECoG is used to determine hippocampal involvement, and the necessity of a hippocampectomy[15, 81].

We wondered what would be the best ECoG approach to assure successful outcome. Is it time to replace spikes with FRs and would this hold for all pathologies and tailoring strategies? Can we rely on HFOs at resection borders? We attempted to provide a complete picture on the prognosis of intra-operative tailoring including both spikes and HFOs. We compared HFOs, especially FRs, and spikes in pre- and post-ECoG recordings, related them to outcome, and verified our findings for different tailoring strategies.

METHODS

COHORT

We retrospectively included patients with focal refractory epilepsy who underwent surgery with intra-operative ECoG tailoring at the University Medical Center in Utrecht, The Netherlands, between 2008 and 2012[79]. Patients were included if: i.) the ECoG was recorded at a 2048 Hz sampling frequency, ii.) pre- and post-resection ECoG recordings were performed and iii.) at least one year follow-up data on post-surgical seizure outcome was available. Exclusion criteria were: i.) preceding chronic ECoG (grid) monitoring, ii.) a functional disconnection procedure, iii.) ECoG intended for functional mapping only, or iv.) recurrent tumor growth or radiotherapy after surgery. In all patients traditional tailoring was performed based on interictal spikes and ictiform spike patterns, using conventional EEG settings (70 Hz low-pass filter, 10s/page) in a common average reference montage. HFOs were never analyzed during surgery, precluding their influence on per-operative decision-making regarding the extent of resection. The results of the effects of spikes and HFOs in post-ECoG were separately reported[79].

The institutional ethical committee acknowledged the study and waived the need for written informed consent because of the retrospective character of the data analysis, provided that data were coded and handled anonymously.

INTRA-OPERATIVE ECOG DATA

ECoG was recorded using 4x5 or 4x8 electrode grids and additionally 1x6 or 1x8 electrode strips (Ad-Tech, Racine, WI, USA), placed on the brain. Platinum electrodes with 4.2 mm^2 contact surface and 1 cm inter-electrode distances, embedded in silicone, were used. We used a 64-channel EEG system (MicroMed, Veneto, Italy) and recorded at 2048 Hz sampling rate (anti-aliasing filter: 538 Hz). Grids and strips were placed in several configurations before and after resection. Propofol anesthesia was stopped during each ECoG registration until a continuous ECoG background pattern was seen, without waking the patient. We selected recordings preceding the first resection as 'pre-ECoG', whereas the recordings after the final resection were designated 'post-ECoG'. One-minute epochs at the end of each ECoG recording were selected to minimize propofol effects[43]. We used photos taken during surgery to schematize the electrode positions pre- and post-resection. We classified electrode pairs in a bipolar montage as being positioned in the resected area, in the resection margin (≤ 1 cm from the resection margin) or in the extra-marginal tissue (> 1 cm from the resection margin).

ANALYSIS OF FR, RIPPLES AND SPIKES

We used an automated HFO detector, with a high pass finite impulse response (FIR) filter > 80 Hz for ripples and > 250 Hz for FRs, adapted for our intra-operative ECoG to detect HFOs in a bipolar electrode montage[44, 67]. All detected HFOs were visually post-processed in Stellate Harmonie Reviewer (v7.0, Montreal, QC, Canada) by two reviewers (MvtK/MZ/NvK) to correct for artefacts[24], false positive and missed HFOs. A split screen was used to simultaneously visualize ripples (FIR filter > 80 Hz; gain 5 μV/mm) and FRs (FIR filter > 250 Hz; gain 1 μV/mm) at a time interval of 0.4 s/page[18].

We marked spikes in the same bipolar montage, using conventional filter settings 0.5-70 Hz (IIR-filter) at a gain of 75-200 μV/mm and 10 sec/page (by MvtK, in Stellate Harmonie 7.0 Reviewer). Marked spikes were checked by a neurophysiologist (FL). Spikes were defined as paroxysmal sharp transients, with a maximum duration of 80 ms[67] and minimal amplitude of twice the baseline. Sharp waves that co-occurred with spikes on neighboring bipolar electrodes were also marked. Ictiform spike patterns were not analyzed. HFOs were marked independently and blinded for spikes and vice versa. All events were marked blinded for outcome.

PATHOLOGY AND POST-SURGICAL OUTCOME

Pathology results, post-surgical outcome and the use of AEDs after surgery were collected from the clinical reports. Pathology was subdivided into four groups, i) mesial temporal sclerosis (MTS), ii.) glioneural tumors including gangliogliomas and dysembryoplastic neuroepithelial tumors (DNET), iii) malformations of cortical development, including focal cortical dysplasia (FCD) and tuberous sclerosis, and iv.) other pathology, e.g. gliosis and cavernomas.

Post-surgical outcome was determined for the most recent follow-up with a minimum of one year, and dichotomized into complete seizure freedom ever since surgery (Engel IA) and recurrent seizures (Engel 1B–4)[5]. We classified the anti-epileptic drug (AED) use of seizure-free patients into three groups i) continuing pre-surgical AED(s), ii) started withdrawal of AED(s), or iii) stopped AED use.

DATA ANALYSIS

We analyzed numbers of FRs, ripples and spikes ('events') in pre- and post-ECoG. We calculated for each patient: the total number of events on all channels (# events), the number of channels with events (# channels with events), the mean event rate per minute over all channels with events (mean # events/minute/channels with events), the percentage of events in the resected tissue (for pre-ECoG events only), in the resection margin (1 cm), and extra-marginal tissue (for pre- and post-ECoG data).

Pre-ECoG and outcome prognosis

First, we analyzed the pre-ECoG, data comparing the presence (+) or absence (-) of ECoG events before resection with outcome (Fisher exact). We tested for confounding patient characteristics for post-surgical outcome and for differences in those characteristics between the pre+ and pre- groups for FRs, ripples and spikes and outcome (X^2-test). We tested for significant differences between the percentage of resected events found in the pre-ECoG and outcome (Mann-Whitney U) and tested this for different pathology groups. The total number of patients with events, number of events and the mean event rate were compared for different pathology groups (Kruskal-Wallis).

Post-ECoG events in relation to pre-ECoG events

Second, we added the post-ECoG findings to the pre-ECoG findings resulting in four groups for each event: pre+post- (+/-), pre+post+ (+/+), pre-post+ (-/+) and pre-post- (-/-). We compared post-resection events to outcome, given the presence or absence of pre-events (Fisher exact). Note that exact sampling locations of pre-ECoG and post-ECoG could differ.

Additionally, we compared the mean event rate, the percentage of events in the resected tissue, in the resection margin (1 cm), and extra-marginal tissue of the pre+ groups (+/-,+/+), as well as for the pre- groups (-/+, -/-) to outcome, in order to find a variable that might explain the predictive value of the combined pre- and post results in the four groups (Kruskal Wallis).

Complete resection of pre-ECoG events in relation to post-ECoG events

For the group that showed events (FRs, spikes) in pre-ECoG (pre+), we described whether these events were completely resected. We related this to occurrence of post-ECoG events and assessed how these combinations ($pre_{complete}$/ post-, $pre_{complete}$/ post+, $pre_{incomplete}$/post-, $pre_{incomplete}$/post+) predicted surgical outcome (Fisher exact).

Tailoring approach: spikes versus FR

We investigated the clinical impact of the ECoG findings for tailoring. Tailoring with ECoG can be used for several clinical situations: 1.) a primary mesio-temporal source where tailoring is performed to estimate the extent of neocortical involvement; 2.) a (temporal) neocortical lesion, where tailoring is performed to determine whether the mesio-temporal structures should be included in the resection 3.) a neocortical lesion, where tailoring is performed to estimate the extent of the neocortical resection; 4.) combinations of the above, or multiple lesions. We wanted to investigate the potential clinical value of HFOs versus spikes in the first three situations. We related the presence of pre- and post-resection events in the area of interest to outcome. For the first situation we selected the neocortical events over the remaining cortex, so on the grid electrodes that were not covering the resection area before and after resection and compared them to outcome. For the second situation we compared the pre- and post-resection events recorded with reed electrodes over the hippocampus, so we selected only those patients in whom the hippocampus remained. For the third situation we compared the pre- and post-resection neocortical events surrounding the resection, so in the resection margin, to outcome. We assessed the highest positive predictive value (PPV) per event type- i.e. the percentage of patients who showed pre- or post-resection events in the selected area who had bad outcome - and negative predictive value (NPV) – i.e. the percentage of patients who showed no events and good outcome.

Statistical analysis was performed in IBM SPSS Statistics 23 (IBM Corp, Armonk, NY, USA). We considered p-values ≤ 0.05 significant.

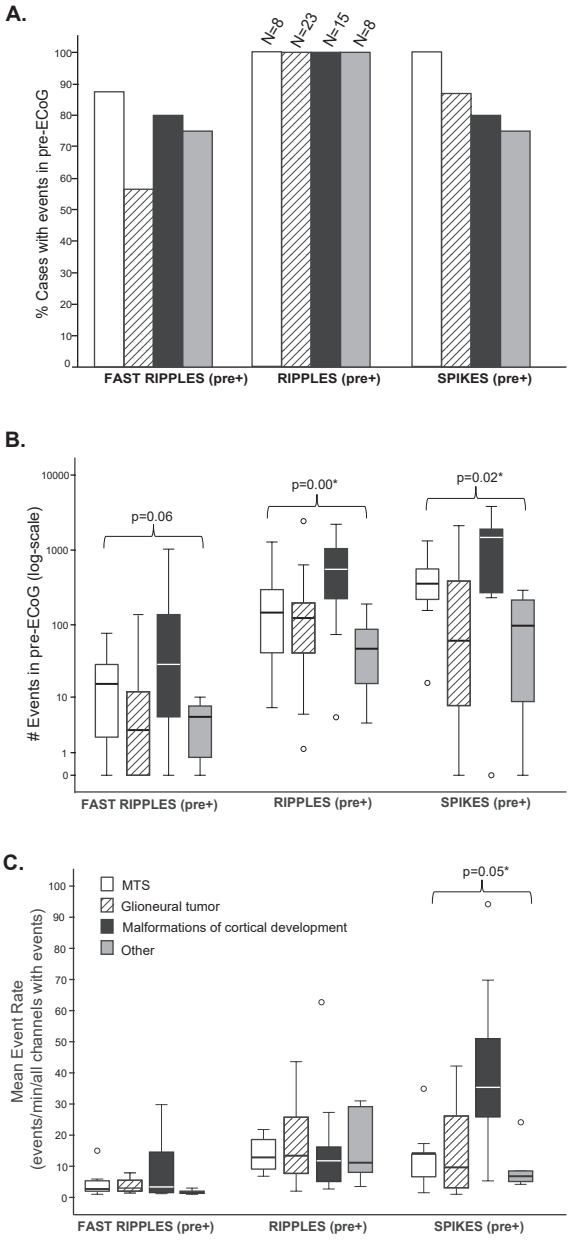

FIGURE 4.1

FRs, ripples and spikes in pre-ECoG for the four pathology groups. A.) Percentage patients with events in pre-ECoG per pathology group.

Comparison of events in pre-ECoG revealed that there is a significant difference between the group pathology groups that in general can be attributed to congenital abnormalities, that differ from the other three pathology groups; B.) Congenital abnormalities showed an overall higher number of events in pre-ECoG (p_{FR}=0.06, p_R=0.00, p_S=0.02, Kruskal Wallis) C.) a higher mean spike rate (p_S=0.05, Kruskal Wallis).

TABLE 4.1
Patient characteristics in relation to the presence or absence of FRs and spikes in pre-ECoG

			FR findings pre-ECoG			Spike findings pre-ECoG		
	Total	**patient characteristics vs. outcome p-value (test)**	**FRs pre+**	**FRs pre-**	**p-value**	**Spikes pre+**	**Spikes pre-**	**p-value**
No. Patients (Good/Poor)	54 (30/24)		38 (20/18)	16 (10/6)	0.56 [a]	46 (23/23)	8 (7/1)	0.06 [a]
Gender (No. M/F)	29/25	0.54 [a]	25/13	12/4	0.01[a,c]	28/18	1/7	0.01[a,c]
Age at surgery in years (median(IQR))	15.5 (12.0-30.5)	0.06 [b]	16 (12.0-31.8)	13.5 (12.0-27.0)	0.44[b]	15.5 (12.0-29.5)	16.5 (7.0-43.8)	0.84[b]
Age at seizure onset in years (median (IQR))	9.5 (0.0-21.5)	0.05 [b,c]	8.5 (1.0-23.0)	10.5 (6.3-16.5)	0.89[b]	8.5 (1.0-22.0)	13 (1.3-19.3)	0.83[b]
Duration of epilepsy in years (median (IQR))	6.5 (2.3-11.8)	0.68 [b]	8 (3.8-12.0)	3 (2.0-12.0)	0.27[b]	7 (2.0-11.3)	6 (2.3-18.3)	0.73[b]
Follow-up period in months (median(IQR))	25 (17.0-35.8)	0.40 [b]	25 (16.5-34.3)	25.5 (15.5-43.0)	0.43[b]	20 (17.0-36.0)	25.5 (15.0-46.8)	0.74[b]
TLE vs. extra-TLE	33/21	0.45 [a]	23/15	6/10	0.89[a]	29/17	4/4	0.49[a]
Resected region (%, No.):		0.14[a]			0.65[a]			0.82[a]
- Temporal	61.10%		23	10		29	4	
- Frontal	22.20%		7	5		9	3	
- Central	13.00%		6	1		6	1	
- Parietal	1.90%		1	0		1	0	
- Occipital	1.90%		1	0		1	0	

FRs= Fast Ripples, No. =number of patients, M=male, F=female, IQR= interquartile range, TLE=temporal lobe epilepsy, extra-TLE= epilepsy outside the temporal lobe, Good= seizure free (Engel 1A), Poor= recurrent seizures (Engel 1B-IV).
Note that we chose to report only the results for FR and spikes as almost all patients showed abundant ripples in their pre- ECoG. No correction for multiple comparisons was performed. [a]Pearson X^2-test/Fisher exact test, [b]Mann-Whitney U-test, [c]significant ($p \leq 0.05$)

RESULTS

COHORT

We included 54 people with focal refractory epilepsy (median age 15.5y, 33 temporal lobe epilepsy, 25 female) who underwent tailored surgery with intra-operative pre- and post-resection ECoG who met the inclusion criteria[79]. Their median (interquartile range, IQR) follow-up period was 25.0 (17.0-35.8) months (table 4.1). Three patients were MRI negative, but considered good candidates for tailored surgery based on other clinical information. Twenty-four patients (44%) had recurrent seizures after surgery. Five of the 30 seizure-free patients continued using their pre-surgical AEDs at latest follow-up, while 8 had started AED withdrawal, and 17 had already stopped using AEDs. A younger age at seizure onset was associated with seizure freedom, accompanied by a trend towards a younger age at surgery. We found no interaction of these patient characteristics for the pre-resection FR and spike findings and outcome, apart from an unexplained difference for gender (table 4.1). Note that all patients had ripples in the pre-ECoG

DATA

We included 2744 bipolar channels for pre-ECoG and 1325 for post-ECoG recordings analysis, resulting in a median (IQR) of 41.0 (36.0-63.5) channels per patient in pre-ECoG and 19.5 (15.0-28.0) in post-ECoG. We excluded channels with artifacts from analysis; a median (IQR) of 4.0 (1.0-7.0) channels per patient for pre-ECoG and 4.0 (2.0-8.0) channels per patient for post-ECoG. The total number of analyzed bipolar channels in the resection margin was 540 for pre-ECoG, with a median of 8.0 (5.0-11.8) channels per patient, and 279 for post-ECoG, with a median of 4.0 (3.0-7.0) channels per patient. The selected one-minute epochs were 11:13 minutes after propofol was stopped for pre-ECoG and 10:13 minutes for post-ECoG.

PRE-ECOG

We found FRs in 38 patients, ripples in all 54, and spikes 46 patients in pre-ECoG. The number of events in pre-ECoG, event rate and percentage of events in the resection, in the margin and in extra-marginal tissue can be found in table 2. FRs, ripples and spikes were found in equal number of patients with different pathology types (figure 4.1). Congenital abnormalities differed from the other three pathology groups showing an overall higher number of events (p_{FR}=0.06, p_R<0.001, p_S=0.02, Kruskal Wallis) in combination with a high rate of spikes per channel (p=0.05).

PRE-ECOG AND OUTCOME PROGNOSIS

The absence of spikes in pre-ECoG seemed related to seizure freedom as seven out of eight patients became seizure free (p_S=0.06, Fisher exact). This group of seven showed diverse pathology and localization. None had mesiotemporal sclerosis. We found no significant differences in the presence or absence of FRs or ripples in pre-ECoG in relation to outcome. The percentage of resected FRs, ripples and spikes in the pre-ECoG did not differ between the seizure free and recurrent seizure group (p_{FR}=0.71, p_R=0.49,p_S=0.67, Mann Whitney U) (figure 4.2). We found no differences within the four pathology groups for outcome (figure 4.2C).

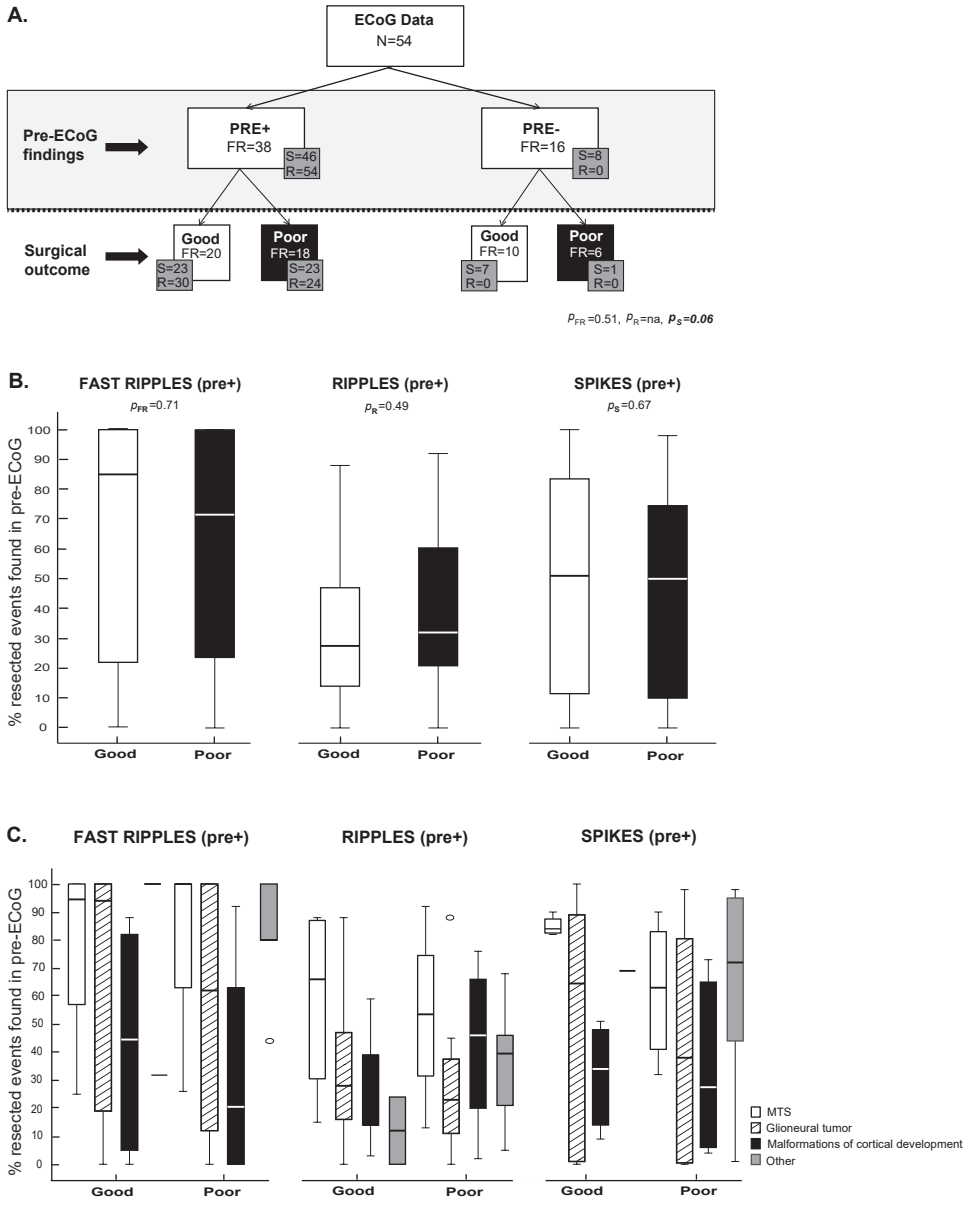

FIGURE 4.2

Pre-ECoG findings. A.) Distribution of FRs, ripples (R) and spike (S) in pre-ECoG, in relation to post-surgical outcome (Good=Seizure free, Poor=Recurrent seizures). There is a trend towards an association between the absence of spikes in pre-ECoG and post surgical seizure freedom (p_S=0.06, Fisher exact). B.) Boxplot of the percentage FRs, ripples and spikes in the pre-ECoG included in the resection, compared to outcome. No significant differences for FRs, ripples nor spikes in the percentage of resected events in pre-ECoG were found (p_{FR}=0.74, p_R=0.42,p_S=0.80, Mann Whitney U). C.) The percentage resected events in pre-ECoG for the four pathology groups compared to outcome. Note that the percentage of resected events is in general lower in malformations of cortical development, followed by glioneural tumors. However, no significant differences between nor within the four pathology groups were found.

TABLE 4.2
Pre- and post-ECoG findings

	Pre-ECoG			Post-ECoG		
	FRs	**Ripples**	**Spikes**	**FRs**	**Ripples**	**Spikes**
No.	38	54	46	12	51	38
# Events (median (IQR))	13.0 (5.0-51.5)	467.5 (183.3-802.5)	306 (92.5-1356.0)	7.0 (2.3-48.0)	85.0 (34.0-216.0)	67.0 (14.5-227.8)
# Channels with events (median,(IQR))	6.0 (2.0-14.2)	32.5 (22.0-50.3)	24.0 (11.8-38.3)	2.5 (2.0-6.3)	12.0 (8.0-20.0)	10.0 (4.8-19.0)
% Channels with events (median,(IQR))	12.1% (5.0-19.2)	83.1% (58.2-92.3)	54.6% (35.1-78.7)	11.3% (7.5-16.5)	65.0% (44.4-83.3)	47.1% (21.3-78.3)
Event rate (events/min/channels with events) (mean (SD))	4.95 (±6.0)	15.6 (±11.9)	20.1 (±19.4)	8.4 (±14.6)	9.9 (±11.1)	13.7 (±18.3)
% Events resected (mean,(SD))	61.1% (±40.4)	35.6% (±26.6)	49.8% (±36.4)	-	-	-
% Events in resection margin (mean, (SD))	15.5% (±21.4)	19.3% (±17.1)	18.1% (±17.1)	24.4% (±31.8)	12.8% (±14.1)	15.3% (±23.5)
% Events in extra-marginal tissue (mean, (SD))	23.4% (±33.7)	45.0% (±26.6)	32.1% (±25.0)	75.6% (±31.8)	87.1% (±14.7)	82.0% (±7.0)

ECoG= electrocorticogram, FRs= Fast Ripples, No. =number of patients, SD=standard deviation, IQR=inter quartile range.

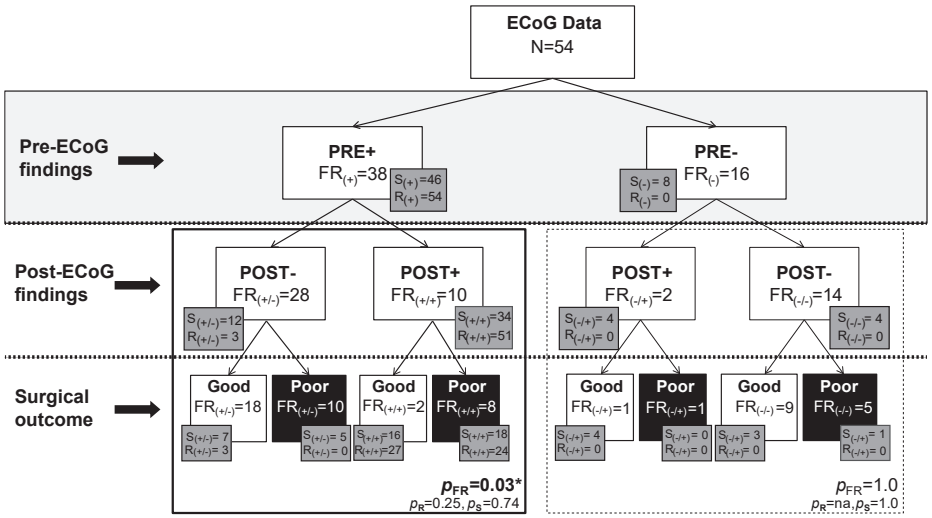

FIGURE 4.3
Combined pre- and post-ECoG findings in relation to outcome (Good=Seizure free (white), Poor=Recurrent seizures (black).The presence of FRs in post-ECoG, given the presence of pre-ECoG FRs (+/- and +/+) was significantly associated with outcome (p_{FR}=0.03, Fisher exact) (left solid lined box). The presence or absence of residual FRs had no predictive value in the absence of pre-resection FRs (-/+ and -/-) (right dotted lined box). There was no association with outcome for post-ECoG findings given the presence or absence of pre-ECoG findings for ripples (R), and spikes (S) (in gray blocks). For statistical analysis a Fisher exact test was used.

POST-ECOG EVENTS IN RELATION TO PRE-ECOG EVENTS

Patients with events in pre-ECoG

Twenty-eight patients with FRs in pre-ECoG, did not have FRs in post-ECoG (pre+post-). For ripples these were only three patients, and for spikes 12. The number of patients with events in both pre- and post-ECoG (pre+post+) was 10 for FRs, 51 for ripples, and 34 for spikes (figure 4.3).

Patients without events in pre-ECoG

We found 'new' events in post-ECoG, in absence of pre-ECoG events (pre-post+), in two patients for FRs, none for ripples, and four for spikes. New FRs were found in one patient (recurrent seizures, tumor in frontal operculum) in remote extra-marginal frontal lobe tissue within a larger area of spikes. In the other patient one single new FR was found close to the resection margin (seizure-free, cortical malformation). Fourteen patients had no FRs at all (pre-post-) which included 39.5% (9 out of 23) of patients with a tumor; none of these patients showed ripples, and four showed no spikes in post-ECoG (figure 4.3).

COMBINED PRE- AND POST-ECOG WITH OUTCOME

The presence and absence of FRs in post-ECoG, given the presence of pre-ECoG FRs (pre+post- and pre+post+) was significantly associated with outcome (p_{FR}=0.03, Fisher exact) (figure 3). Presence or absence of post-resection FRs had no predictive value in the absence of pre-resection FRs (pre-post+ and pre-post-) (p_{FR}>0.99, Fisher exact). For ripples and spikes we found no indication that post-ECoG findings, given the presence of pre-ECoG events (+/- and +/+), were predictive for outcome (p_R=0.25 and p_S=0.74, Fisher exact), neither were post-ECoG findings in case of absence of pre-ECoG events (-/+ and -/-) (p_R=na and p_S=1.0, Fisher exact). We found no difference for the percentage resected events, events in- and outside the margin and mean event rate for FRs, ripples nor spikes between the pre+ and post+ groups with regard to outcome

COMPLETE RESECTION OF PRE-ECOG EVENTS IN RELATION TO POST-ECOG EVENTS

We assessed whether post-ECoG findings in the group of patients showing events in pre-ECoG (pre+) were influenced by the fact that these pre+ events were completely resected or not. Only the presence of FRs in the post-ECoG, given incomplete removal of FRs in the pre-ECoG, was indicative for outcome ($p_{incomplete}$=0.04, Fisher exact). We found no statistical evidence that incomplete or complete resection of ripples and spikes in pre-ECoG was related to their post-ECoG findings.

TAILORING APPROACH: SPIKES VERSUS FR

Three MRI negative patients and three patients who underwent a combined tailoring approach (situation 4) were excluded from this analysis. Note that we chose to report only the results for FRs and spikes as almost all patients showed abundant ripples in their pre- and post-ECoG. We used the PPV and NPV to enable comparison of performance of FRs versus spikes per tailoring strategy. Fifteen patients (8 had poor outcome) underwent primary anterior temporal lobectomy plus (amygdala-) hippocampectomy and had grid recordings over the neocortex (situation 1). The highest PPV for recurrent seizures in situation 1 was found for the presence of neocortical FR in post-ECoG (67%) and the highest NPV for the absence of spikes in pre-ECoG (67%). Twelve patients underwent resection of a temporal neocortical lesion (situation 2), only 3 out of 6 patients had recordings over the mesio-temporal area which was left in situ (2 poor outcome). Spikes and FRs performed equally well for the PPV of pre- and post- ECoG (100%), but spikes had the highest NPV for pre- and post-ECoG (100% vs. 100% (pre) and 50% (post) for FRs). Twenty-one patients (7 poor outcome) underwent neocortical lesionectomy only (situation 3). The highest PPV was for FRs in post- (100%) and NPV for spikes in post-ECoG (90%) in the resection margin (table 4.3).

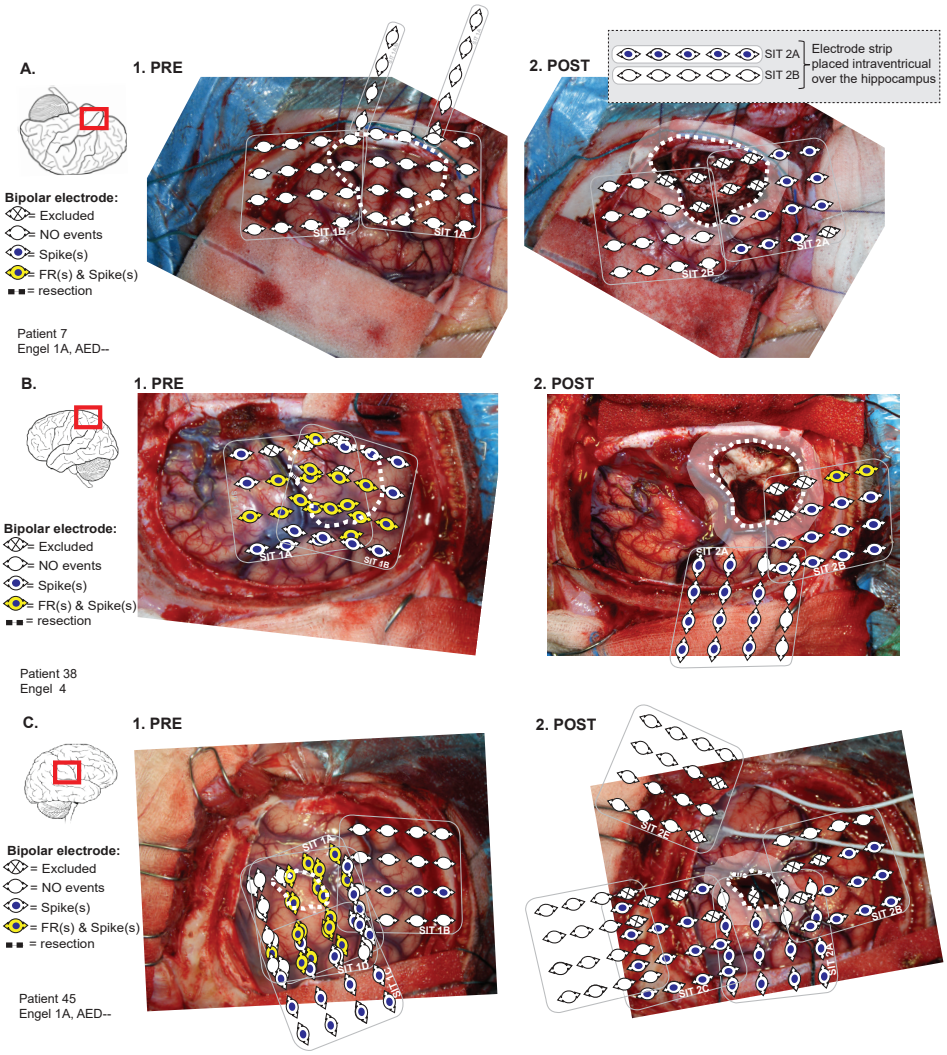

FIGURE 4.4

Patient examples of combined pre- and post-ECoG findings. Note that pre- and post-ECoG recording did not always (re-)sample the same region. A.) Patient with a tumor left baso-temporal. Pre-ECoG recordings were without events. Post-resection the ECoG showed residual spikes. Most likely these spikes are caused by surgical manipulation, as they disappeared over time on the intra-ventricular strip in the second post-resection recording (sit 2B) and the patient became totally seizure free after surgery. B.) Patient with tuberous sclerosis who showed FR and spikes in pre-ECoG surrounding the dominant epileptic tuber in the left parietal lobe. These FRs in pre-ECoG were incompletely removed, and new FRs and spikes were found in post-ECoG that covered an area with a second tuber. The patient had recurrent seizures after surgery. C.) Patient with a right sided central lesion that showed a large area with spikes and a smaller area with FRs overlapping with the lesion. In the post-ECoG, after lesionectomy, residual FRs were absent but residual spikes remained in what was considered eloquent cortex. Although FRs and spikes in pre-ECoG were incompletely removed, FRs were abolished after resection. The patient became totally seizure free.

TABLE 4.3
PPV and NPV for poor outcome for spikes and FRs per tailoring approach

		Pre-ECoG		Post-ECoG	
		PPV (tp/tp+fp)	**NPV (tn/tn+fn)**	**PPV (tp/tp+fp)**	**NPV (tn/tn+fn)**
Tailoring situation 1 (N=15, 8 RS) (events in neocortex outside mesio-temporal resection)	**Spikes**	58.3%	66.7	50.0%	42.9
		(7/12)	66.7%	(4/8)	42.9%
	FRs	50.0%	45.5	66.7%	50.0
		(2/4)	45.5%	(2/3)	50.0%
Tailoring situation 2 (N=3, 2 RS) (events in mesio-temporal tissue)	**Spikes**	100%	100	100%	100
		(2/2)	100%	(2/2)	100%
	FRs	100%	100	100%	50.0
		(2/2)	100%	(1/1)	50%
Tailoring situation 3 (N=21, 7 RS) (events in neocortical marginal tissue)	**Spikes**	42.9%	85.7%	54.5%	90.0%
		(8/14)	(6/7)	(5/11)	(9/10)
	FRs	37.5%	69.2%	100%	77.8%
		(3/8)	(9/13)	(3/3)	(14/18)

Abbreviations: Tailoring situation 1: A primary mesio-temporal source where tailoring is performed to estimate the extent of neocortical involvement, situation 2: A (temporal) neocortical lesion, where tailoring is performed to determine whether the mesio-temporal structures should be included in the resection, situation 3: A neocortical lesion, where tailoring is performed to estimate the extent of the neocortical resection. Excluded from this analysis are combinations of the above or multiple lesions (situation 4). RS=recurrent seizures, PPV= positive predictive value for poor outcome, tp= true positive, defined as recurrent seizures and events present, fp=false positive, defined as seizure free and events present, NPV=Negative predictive value for poor outcome, tn=true negative, defined as seizure free and events absent, fn=false negative, defined as recurrent seizures and events absent. FRs= Fast Ripples. Note that the PPV and NPV depend on the prevalence of surgical outcome, and so they are not comparable across the tailoring strategies.

DISCUSSION

In tailoring based on pre- and post-ECoG the best post-surgical outcome predictor is the presence or absence of FRs after resection, given the presence of FRs before resection. Pre-resection events alone did not show predictive value. HFOs were found in all types of pathologies. Overall the post-resection FRs predicted recurrent seizures best, especially in the tailoring situation of neocortical lesions. Absence of spikes tended to predict seizure freedom. Our results, and that of our previous study[79], suggest that FRs could be used to tailor epilepsy surgery and favors the use of repeated intra-operative ECoG, including recordings after final resections. We hypothesize that complete resection of FRs will yield good outcome. This holds for different tailoring strategies.

Our pre-ECoG results contradict previous studies in subdural and depth EEG in which removal of the majority of FRs in pre-resection recordings was linked to good surgical outcome[19, 20]. Wu et al (2010). showed first intra-operative FRs to be linked to outcome[19]. A combination of population characteristics and definition of outcome can explain differences with literature. We included

strictly focal resections, whereas Wu et al. included hemispherectomies and lobar disconnections[19]. HFOs in depth EEG were found to predict better outcome if the majority was resected[20]. We found no such relationship for ripples and only a slight trend in this direction for FRs. ECoG samples different areas including functionally eloquent areas, yielding more ripples than depth EEG and our outcome measure was more strict. Epilepsy surgery is increasingly performed in young children, as successful surgery improve social, psychological and cognitive development[90]. We found seizure freedom to be associated with a younger epilepsy onset age and accompanying younger age at surgery. Literature reports a wide variation of pre-operative predictors for outcome[206]. We did not adjust our statistical analysis for potential confounders. Future large data studies could model these effects.

It has been suggested that tailoring with or without ECoG in temporal lobe surgery led to similar postsurgical outcome[15]. A recent study showed, however, that tailored surgery with repeated ECoGs and stopping after abolishment of spikes led to better surgical outcome[82]. This is supported by our analysis of tailoring of temporal neocortical lesions with potential hippocampal involvement. Our finding that incomplete resection of spikes in pre-ECoG is not related to post-ECoG findings and seizure recurrence is in concordance with Tran et al. (1997)[77].

We found FRs in pre-ECoG in 70% of patients. FRs are focal phenomena which makes them prone to be missed due to undersampling, especially when they occur in the sulcus depth. These FRs might go unnoticed during pre-ECoG, and might (re-)appear on the recording surface after cortisectomy, which might explain finding 'de novo' FRs in post-ECoG.

The underlying pathophysiology of focal epilepsy can be considered a network problem[82]. FRs are thought to represent local intrinsic fast synchronizing behaviour of damaged neurons resulting in out-of-phase firing of neuronal clusters. Candidate mechanisms for fast interneuronal connections are ephaptic interactions, gap junction coupling, or fast synaptic transmission[32, 80]. Combined microelectrode and subdural ECoG recordings showed that HFOs mark the core of the seizure onset, as they correspond to intense synchronized multi-unit burst firing in the underlying cortex and do not relate to the ictal spreading wave front (penumbra), while spikes behave similar in both areas[83]. The network theory might explain why we do not have to remove distant pre-ECoG FRs which abolish after resection (figure 4)[85, 86]. Interictal spikes may be generated in areas secondarily involved in the epileptogenic network and might even protect against seizures[18, 85]. We found post-excision activated spikes, which -as previously suggested - appeared to be a benign process unrelated to outcome[86]. The deviation in pathophysiological origin between interictal spikes and HFOs is also underlined by their different responses to AEDs[69].

METHODOLOGICAL LIMITATIONS

We compared events found in ECoG to the most recent post-surgical outcome. Traditionally, post-surgical outcome is evaluated twelve months after surgery, and re-evaluated every year. The reliability of the post-surgical outcome increases over time[207]. Discontinuation of anti-epileptic drugs is usually considered after one year, but always with the patients consent, so is difficult to standardize. The high heterogeneity within people with focal refractory epilepsy leads to fragmentation when one wants to take into account underlying aetiology, localization, anti-

epileptic drugs usage or other patient characteristics. Detailed studies on subtypes might lead to distinct HFO properties, e.g. event rate or morphology[36, 87, 88], which can be used to further improve the tailoring strategy at individual patient level. Future larger studies should also focus on identifying the best Boolean combination of different types of activity to specify the best tailoring strategy. Second, we chose to focus on the presence or absence of events to perform statistical group level analysis. The number of recorded channels and resection size differ per patient. Normalization for numbers of channels would not fit clinical practice. We used mean values per patient which is a commonly used approach[18, 20, 79]. Third, we assumed precise localization of our resection and resection margins, but matching of the intra-operative photos may have resulted in a small but relevant localization bias. Re-sampling of events in the resection margin post-resection may be hampered by a shift of the brain tissue. Tailoring is an iterative process, only regions where spikes were seen in previous recordings were re-sampled, while FRs occur in smaller areas and might be missed due to sampling errors. Pre-and post-resection recordings differ due to the time between the recordings and the anatomical changes after resection. A prospective study based on fast ripples would decrease this discrepancy. Fourth, we used an unsupervised HFO detector, characterized by over-detection. A stringent definition for ripple size is lacking[208]. We kept large and small ripples seen at 5 μV gain. Marking only large ripples might increase specificity. We deliberately chose an all-or-none criterion for the amount of events as we hypothesized that presence of any FRs represents epileptogenic tissue [79]. Post-hoc ROC analysis showed that the combined sensitivity and specificity for this all-or-none cut-off criterion was indeed highest for post-resection FRs (38% and 90%) and pre-resection spikes (96% and 23%). A more liberal detection threshold for FRs might have increased the number of patients showing FRs, but might lower specificity. From the clinical perspective of using FRs to extent the resection, we chose specificity over sensitivity. Another limitation is that, spikes influenced the surgical decision making. We found spikes post-resection in the majority of patients, unrelated to outcome suggesting that the clinical decision also depended on other factors. Finally, we considered all HFOs pathological, but there may be physiological HFOs, especially ripples which are difficult to distinguish. We found ripples and FRs in eloquent areas, such as the sensorimotor, Broca or Wernicke areas and in the occipital regions analogous to literature[37, 67, 79, 89]. We thus have to be careful when assessing FRs in eloquent areas. Fortunately, when weighing those locations against the presumed epileptic focus, we saw that these FRs often occur in more remote areas than the 'resection area of interest'.

CLINICAL IMPLICATIONS AND FUTURE PROSPECTS

We may conclude four main lessons for clinical tailoring: 1.) FRs surpass spikes in their specificity in outcome prognosis; 2.) To assure the highest chance of surgical success it is important to repeat ECoGs throughout surgery, as control recordings after (extended) resection is the only way to ensure complete resection of epileptic biomarkers and thus seizure freedom; 3.) The area with pre-resection FRs might be larger than the area that needs to be removed to yield a 'clean' ECoG after the resection and to achieve seizure freedom. This suggests that we should not just chase FRs, but remove the clinically suspected lesion first; 4.) Spikes are less suited for the tailoring approach than FRs. However, clinicians should not be led by FRs alone as the absence of spikes

seemed to predict good outcome. The review of spikes and FRs on top of spikes can help to distinguish epileptiform FRs from artifacts and physiological FRs.

Clinical implementation of HFOs is hampered by fear of the inability to discriminate between physiological and pathological HFOs. Our findings support the idea that ripples are closer related to physiological processes while FRs are related to pathology. We believe that similar restrictions used for spikes for tailoring are valid for HFOs, as the intra-operative ECoG is used to adjust a pre-existing surgical plan , which is often limited by eloquent cortex or anatomy[90]. The first randomized controlled clinical trial (The HFO trial) to evaluate our hypothesis that use of HFOs intra-operatively can improve outcome is on its way[90]. This will lead to guidelines on how to handle technical challenges of recording HFOs in the operating room to avoid abundant artifact sources (e.g. the diathermy and the surgical microscope)[43]. A second limitation for clinical implementation is the offline visual analysis of HFOs resulting in delays of minutes before the surgeon can proceed. Several automatic HFO detectors have been developed, but most still require extensive computing time[91-93]. The ideal detection algorithm for intra-operative use can quantify HFOs real-time[94] in individual channels and in temporal and spatial relation to HFOs in other channels[95]. Finally, unraveling the patho-physiological relation at cell-level between the 'new' HFOs and the 'old' epileptic spikes is an important missing link for the international acceptation of the clinical use of HFOs during epilepsy surgery.

PART II

INTERVENTION WITH HFOS

CHAPTER 5

HIGH FREQUENCY OSCILLATIONS IN THE INTRA-OPERATIVE ECOG TO GUIDE EPILEPSY SURGERY ("THE HFO TRIAL"): STUDY PROTOCOL FOR A RANDOMIZED CONTROLLED TRIAL

Trials 2015;16:422.

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ABSTRACT

BACKGROUND

Intra-operative electrocorticography (ioECoG), based on inter-ictal spikes and spike patterns, is performed to optimize delineation of the epileptogenic tissue during epilepsy surgery. High frequency oscillations (HFOs, 80-500 Hz) have been identified as more precise biomarkers for epileptogenic tissue. Aim of the trial is to determine prospectively if ioECoG-tailored surgery using HFOs, instead of inter-ictal spikes, is feasible and will lead to an equal or better seizure outcome.

DESIGN

We present a single blinded multi-center randomized controlled trial "the HFO trial", including patients with refractory focal epilepsy of all ages who undergo surgery with intra-operative electrocorticography. Surgery is tailored by HFOs (arm 1) or inter-ictal spikes (arm 2) in the intra-operative electrocorticography. Primary outcome is post-operative outcome after one year, dichotomized in seizure freedom (Engel 1A&B) versus seizure recurrence (Engel 1C- 4). Secondary outcome measures are the volume of resected tissue, neurologic deficits, surgical duration and complications, cognition and quality of life. The trial has a non-inferiority design to test feasibility and at least equal performance in terms of surgical outcome. We aim to include 78 patients within three years including one year follow-up. Results are expected in 2018.

DISCUSSION

This trial provides a transition from observational research towards clinical interventions using HFOs. We address methodological difficulties in designing this trial. We expect that the use of HFOs as a biomarker for tailoring will increase the success rate of epilepsy surgery while reducing resection volume. This may reduce neurological deficits and yield a better quality of life. Future technical developments, such as validated automatic online HFO identification, could, together with the attained clinical knowledge, lead to a new objective tailoring approach in epilepsy surgery.

TRIAL REGISTRATION

This trial is registered at the US National Institutes of Health (ClinicalTrials.gov) #NCT02207673 (31 July 2014) and the Central Committee on Research Involving Human Subjects, The Netherlands #NL44257.041.13 (18 March 2014).

BACKGROUND

The current success rate of epilepsy surgery in patients with refractory focal epilepsy lies between 36 and 84 % after one year[9]. Intra-operative electrocorticography (ioECoG) can be performed during surgery to optimize delineation of the epileptogenic area by taking into account inter-ictal spikes or spike patterns. This so called 'tailoring' affects surgical decision making[13]. Resection of areas with inter-ictal spikes has been associated with seizure freedom[29, 60, 96], whereas remaining spikes after resection have been suggested to indicate poor surgical outcome[13, 49, 96], while other studies contradict this[15, 31, 65]. The value of tailoring by inter-ictal spikes can be questioned as they represent the irritative zone rather than the seizure onset zone[49], spikes can spread into non-epileptic surrounding areas[49], and can also arise from surgical manipulation[16]. This leaves tailoring based on spikes in the ioECoG under international debate. Incomplete resection of so called 'ictiform spike patterns', consisting of recruiting patterns, repetitive bursting patterns or continuous rhythmic spiking, has been reported to predict poor outcome[62].

High frequency oscillations (HFOs, 80-500 Hz) are proposed as a new and more precise biomarker for epileptogenic tissue than spikes[18-20, 32, 35, 46, 72, 97, 98]. HFOs are an indicator of the seizure onset zone[18]. Removal of tissue with HFOs, especially fast ripples (FR: 250-500 Hz), is linked to good surgical outcome[19, 20, 67, 79]. The area showing HFOs usually overlaps with, but is smaller than the irritative zone showing spikes[18]. HFOs mirror epileptic disease activity as they are prominently found in FCD, with increased numbers in the more epileptogenic FCD type 2 compared to type 1[46], and they increase when anti-epileptic drugs are tapered[99]. HFOs can be recorded during surgery, after anesthetics are reduced[43].

Two decades after their first discovery there is a rising demand to know whether HFOs can be used in clinical practice and decision making[32, 100, 101]. Tailoring based on HFOs in the ioECoG could improve the chance of seizure freedom. Other beneficial aspects could be a smaller resected area, with less chance of neurological deficits, which – together with an equal or increased seizure freedom rate – may lead to an overall better quality of life. Last but not least, the identification of epileptic HFOs can be automated[25, 71, 92, 93, 98] and therefore could yield a future objective approach that is easily implemented.

"The HFO trial" is a randomized controlled trial (RCT) designed to investigate the feasibility and safety of using HFOs during intra-operative tailoring of epilepsy surgery. Primary objective of this RCT is to validate if tailoring based on HFOs versus inter-ictal spikes in the ioECoG during surgery will lead to the same post-surgical seizure outcome one year after surgery (non-inferiority design). Secondary objectives are comparison of the volume of resected tissue, the duration of the surgery, the occurrence of complications and neurologic deficits, and changes in cognitive functioning and quality of life. RCTs in the field of epilepsy surgery are not trivial[102, 103]. We report the details of our study design, and share our considerations for the trial set-up.

FIGURE 5.1
Logotype "The HFO trial".

DESIGN

TRIAL DESIGN

"The HFO trial" is a single blinded randomized controlled Dutch multi-center clinical trial. This trial is based on a non-inferiority design with an allocation ratio of 1:1. We chose a non-inferiority design, as we considered the primary objective of the study, to demonstrate that the intra-operative prospective use of HFOs to tailor surgery is feasible and will not lead to worse outcome than the widely applied method based on spikes. Secondary objectives of this study are to investigate whether the HFO-based, compared to spike-based, tailoring will lead to differences in the volume of resected tissue, neurologic deficits, surgical duration and complications, cognition and quality of life.

PARTICIPANTS

Participants are candidates for epilepsy surgery who are referred to the Dutch Collaborative Epilepsy Surgery Program (DCESP) and are selected to undergo epilepsy surgery with ioECoG based tailoring. Members of the DCESP come from the two Dutch epilepsy centers and three Dutch university medical centers performing epilepsy surgery. The two participating surgical centers in this trial are: 1.) the UMC Utrecht, specialized in pediatric epilepsy surgery with 75% of the patients being <18 years of age at surgery, and 2.) the VUmc Amsterdam, that operates on adult patients only (≥18years). Patients and parents or care givers will be asked to participate if they comply with the following eligibility criteria:

- Refractory focal epilepsy, defined as at least two seizures in the past 24 months, in spite the use of two or more different anti-epileptic drugs (AEDs).
- Epilepsy surgery planned with ioECoG to tailor the resection.
  Note that in the participating centers, this automatically excludes standard right sided temporal resections, disconnections and hemispherectomies in which no ioECoG is performed.
- Command of the Dutch language by the patient or parents\legal representatives and capability of completing the Dutch questionnaires.
- Able to give informed consent.

Exclusion criteria are:

- Previous chronic ECoG (grid) monitoring preceding epilepsy surgery.
  This is a biased population, because results of the extensive pre-surgical work-up and the results of the monitoring period determine the outline of the resection. This includes precise knowledge of the seizure onset zone, the inter-ictal spikes and HFO areas.
- Patients with an occipital focus undergoing ioECoG.
  Physiological FRs have been described to occur in the occipital lobe[37]. We deemed it unsafe to perform HFO guided resections in these patients, as it is still difficult to discriminate between pathological and physiological HFOs.

INTERVENTIONS

Participating patients who will undergo ioECoG tailored surgery will be randomized into an HFO guided (arm 1) or standard epileptiform spikes guided (arm 2) resection.

The ioECoG is routinely recorded at a sampling frequency of 2048 Hz using clinical EEG software. A dedicated HFO team, including two experienced HFO observers, will perform the analysis during surgery when the patient has been allocated to the HFO arm. The HFO reviewers need to achieve consensus about the HFOs and distinguish them from artifacts. This team will, together with the clinical neurophysiologist in charge, advice the neurosurgeon on the extent of the tissue to be resected. HFO analysis is performed visually and off-line using Stellate Harmonie Reviewer (v7.0, Montreal, QC, Canada), as this is currently the only clinical EEG software that provides HFO visualization and marking. A split screen modus will be used to simultaneously visualize ripples (settings: FIR filter >80 Hz, gain 5 μV/mm) and fast ripples (settings: FIR filter >250 Hz, gain 1 μV/mm) at an elongated time interval of 0.4 s/page, according to the settings described in other studies[18, 19, 67, 79]. Based on pilot data, we expect that the visual analysis of HFOs will require 50% more time compared to the visual analysis of epileptogenic spikes, which usually takes three to five minutes per recording.

This is an intention to treat study. HFOs in eloquent regions will not be resected, similar to standard tailoring practice based on spikes. In absence of HFOs, ictiform spike patterns (for definition see introduction)[62] will always be resected, irrespective of treatment allocation. If no HFOs or ictiform spikes are found a planned resection is performed according to size and location of epileptogenic structural lesion(s), similar to standard clinical practice when no spikes could be identified.

OUTCOMES

Primary outcome parameter is the post-surgical seizure outcome after one year based on the Engel classification[5]. Seizure outcome will be dichotomized in seizure freedom (Engel 1A&1B) versus seizure recurrence (Engel 1C-4). We decided to include possible auras (Engel 1B) in the seizure-free group, because it can be difficult to distinguish true auras from aura-like non-epileptic sensations. Post-surgical outcomes at six to eight weeks, six and twelve months will be determined by means of a follow-up questionnaire completed in by the patient or legal representatives. So called 'running down' seizures, seizures occurring only during the first two weeks after surgery are not considered as seizure recurrence.

Secondary outcome parameters are:

- **Volume of resected tissue (in cm^3):** The volume of resected tissue is determined by voxel-based volumetrics of the routine pre- and post-surgical 3D whole head T1 MRI scan.
- **Neurological deficits:** Neurological deficits are assessed by neurological examination and translated into the National Institutes of Health Stroke Scale (NIHSS) before surgery and before hospital discharge after surgery. The cumulated NIHSS score ranges between 0-42; a difference of 1 point on the NIHSS scale between the two tests is considered clinically relevant. In that case the NIHSS will be repeated at follow-up moments. Additionally, neurological deficits will be classified as 'pre-existing' (either improved or aggravated) or as 'new' (either anticipated or unexpected).

- **Duration of surgery:** Post-hoc analysis of duration of surgery (start-stop time neurosurgeon, in minutes) and ioECoG recording time (in minutes).
- **Surgical complications:** Accounts are kept of the number of (post-) operative complications, such as bleeding, infection, unexpected or aggravated neurological deficits. These events will also be reported as (serious) adverse events ((S)AE).
- **Cognition:** Comparison of results from pre- and twelve month post-operative neuropsychological evaluation. This routine neuropsychological evaluation includes testing of IQ, working memory and processing speed. All tests performed are chosen according to the age of the patient, but report on the same domains. Per domain individual patients' results will be dichotomized into negative, no, or positive change compared to pre-surgical baseline.
- **Quality of life (QoL):** QoL will be determined pre-, and post-operatively after six to eight weeks, and six and twelve months using a visual analogue scale (VAS) on overall self-perceived quality of life, or by the parents in case of children <12 years of age.

Data collection, management and storage is done in open source clinical trial software OpenClinica (OpenClinica, LLC, Waltham, USA; www.openclinica.com), managed by the IT division of the Neurology & Neurosurgery department of the UMC Utrecht. Figure 5.2 shows the timeline of the study procedures.

SAMPLE SIZE

The sample size calculation is based on a success rate of surgery (defined as Engel 1A or B at 1 year) in the control group (tailoring based on spikes) of 65%, and the expected success in the experimental group (HFOs) of 80%. The resulting margin is 15%. Using a one sided 95% confidence interval and a non-inferiority limit of 10%, to acquire an 80% power we need 39 patients per group. This results in a total of 78 patients. We anticipate that 80% of the eligible patients will participate in the study.

We expect the potential loss-to-follow-up after the initial intervention to be low. Routine clinical visits to the neurosurgeon and neurologist are scheduled after surgery at 6 to 8 weeks, 6 months and 12 months. Our FU questionnaires coincide with these visits. Inclusions will proceed until the target sample size is achieved to address loss to follow-up. Loss to follow-up can be expected from; 1.) withdrawal by the subject from the study during follow-up before the first preliminary outcome determination at 6-8 weeks, and 2.) withdrawal from the study during the surgery by the surgeon or dedicated team due to urgent medical or technical issues.

Subjects are replaced if they withdraw from the study during follow-up before the first preliminary outcome determination at 6-8 weeks. Subjects that are withdrawn from the study during the surgery by the surgeon or dedicated team due to urgent medical or technical issues are also replaced.

INTERIM ANALYSIS

Safety and efficacy analysis will be performed by the statistician of the independent data monitoring committee (DMC) after the first 20 and 40 included patients. This analysis will be based on the available, preliminary, post-surgical outcomes and also includes the number of

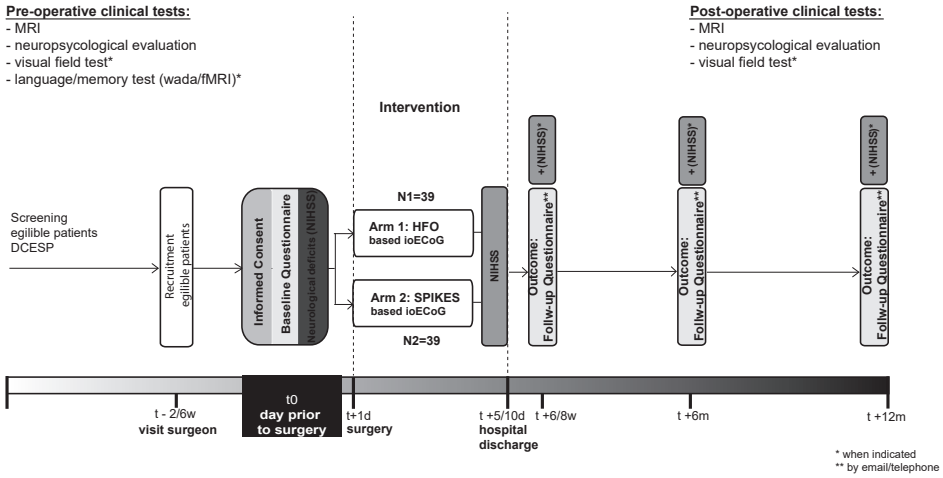

FIGURE 5.2
Timeline of the study procedures. On the day prior to surgery, the patient signs the informed consent form and subsequently the baseline questionnaire and neurological examination (by means of NIHSS) are completed and the patient is enrolled in the study by randomization. Note that the follow-up questionnaire collects information about (preliminary) outcome, AED use, quality of life and occurrence of (serious) adverse events. Additional information is collected from routine clinical tests that are performed during the pre- and post-surgical period.

surgical complications and (serious) adverse events (SEA/AE). The DMC can advise on premature termination of the study in case of harmful effects, or superiority, in one of the treatment arms or in case of non-feasibility issues.

RANDOMIZATION

Eligible epilepsy surgery patients and/or parents or caregivers will be informed about this study by the researcher in person, minimally two weeks in advance of the surgery. Informed consent is asked on the day prior to the day of surgery. After written consent, the patient will be randomized into the treatment allocation (ALEA version-release 2.2). Stratification for participating site and epilepsy type (extra- vs. temporal lobe epilepsy) is performed using block randomization of two, four and six patients. The stratification for participation site includes an indirect stratification for age, as in the VUmc only adults and in the UMCU predominantly children are operated.

BLINDING

Blinding of the treating physicians and neurosurgeons for treatment allocation is not feasible because of the character of the intervention. Therefore, this is a single blinded trial as patients will be blinded for the treatment allocation to avoid bias of the follow-up results. To guarantee the blinding of the patient, the outpatient physicians, neurologist and psychologist involved in the follow-up procedures are blinded for the ioECoG report during the entire period of follow-up. After study completion or termination patients who wish so will be de-blinded.

STATISTICAL METHODS

The primary 'intention to treat' analyses at study completion are based on the difference between the treatment arms with respect to surgical outcome after twelve months. The primary outcome is a categorical outcome, seizure freedom vs. seizure recurrence. Risk ratios (RR) and risk difference with 95% confidence interval will be calculated (X^2 –test, one-sided).

Secondary outcomes are the amount of tissue resected, neurological deficits, duration of surgery, complications, cognition and quality of life. For the secondary outcome parameters consisting of continuous variables, a T-test with 95% confidence interval is calculated and tested (one-sided). For the secondary outcome parameters consisting of categorical variables, the RR and risk difference with a 95% confidence interval are calculated and tested using a Chi-square test (one-sided).

Logistic regression analysis will be performed 1.) to adjust for the stratification factors, by including site and epilepsy type as variables in analysis, and 2.) to investigate if there are relations between seizure outcome and subject variables, such as age, gender, and pathology, or with experimental variables, such as the operating surgeon and the amount of anesthesia received. Note that we are not going to use a mixed model to adjust for site, as we consider two sites a too small sample to be representative for other sites. Regression analysis is also performed between seizure outcome and the secondary outcome parameters.

In the logistic regression analysis we will, after screening of the number of missing data, perform multiple imputation. For the primary and secondary endpoint no imputation will be performed. Statistical analysis will be performed in SPSS version 21 or higher and/or R version 3.1 or higher. The number of eligible patients withholding consent will be registered, and demographic information will be collected anonymously for post-hoc explorative analysis to understand potential bias in study outcomes.

SAFETY ASPECTS

Adverse events (AEs) are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the experimental treatment. A serious adverse event (SAE) is defined as any untoward medical occurrence or effect that results in death, is life threatening, requires (prolonged) hospitalization, results in persistent or significant disability or incapacity, or is a new event of the trial that is likely to affect the safety of the subjects. We will not report the majority of direct postsurgical complaints, such as nausea, headache, abdominal pains or pain related to the surgical scar during the hospitalization period, as AEs, as those result directly from brain surgery and will resolve before discharge. Similarly, direct post-operative functional deficits, due to surgical induced brain edema, will often resolve prior to discharge; these will not be reported as (S)AE. SAEs will be closely monitored by the researchers within the timeframe of hospital admission (normal range ≤10 days after surgery). The neurological deficits will be assessed with the NIHSS questionnaire (see also neurological deficits). During the one year follow-up period all reported AEs and SAEs, independent of the site, will be registered by the researchers and reported to the DMC. SAEs are reported to the medical ethical committee.

ETHICAL CONSIDERATIONS

Informed consent will be obtained from all participants and/or their legal representative(s), in writing, before inclusion in the trial. "The HFO trial" protocol has been approved by the medical ethics committee of the UMC Utrecht (MEC-13-389). "The HFO trial" is registered at the US National Institutes of Health (ClinicalTrials.gov) #NCT02207673 and the Central Committee on Research Involving Human Subjects, The Netherlands #NL44257.041.13.

DISCUSSION

We announce, by reporting our study design, the start of the "The HFO trial", a multi-center randomized controlled trial in epilepsy surgery. This is the first clinical trial investigating tailoring based on biomarkers in the ioECoG, i.e. HFOs versus inter-ictal spikes, with respect to post-surgical seizure outcome.

TRIALS IN EPILEPSY SURGERY

RCTs are considered the gold standard for evaluating therapeutic interventions, but surgical RCTs are challenging and only few have successfully been completed in the field of epilepsy surgery. So far thirteen RCTs were performed in the period 1992-2012, and five of them, all in temporal lobe resections, investigated a new surgical strategy or compared AED prescription versus (early) surgery[102, 104]. Currently, five ongoing RCTs in the field of epilepsy surgery are registered on the international trial register *"clinicaltrials.gov"*. Two of these RCTs investigate a new surgical strategy, including the trial reported here.

CONSIDERATIONS

An important consideration in RCTs in epilepsy surgery is feasibility, which is influenced by the (in)ability of recruiting enough eligible patients with sufficient speed of recruitment. The difficulty to standardize diagnostic testing, medical treatment and surgical interventions across multiple centers is of great influence[102-104]. In the Netherlands, all epilepsy surgery is performed in three medical centers and is embedded in the Dutch Collaborative Epilepsy Surgery Program (DCESP)[105]. Cases are discussed within this national team of (pediatric) neurologists, clinical neurophysiologist, (pediatric) neuropsychologists, nurse practitioners, physician assistants, radiologists and surgeons. This unique collaboration secures more or less standardized protocols and consistent indications for eligibility and treatment. The small distance (<50 km) between the two centers in Amsterdam and Utrecht gives us the opportunity to have one dedicated HFO team to execute the trial in both centers, including the HFO analysis during surgery.

We chose post-surgical outcome as primary outcome parameter, as the ultimate goal of epilepsy surgery is to achieve seizure freedom. This is the most relevant clinical parameter as the number of seizures and the side effects of AED use are the main determinants of Qol in epilepsy patients[9]. We chose to differentiate between seizure freedom ± auras (good outcome) and seizure recurrence (poor outcome) although in the literature, good outcome is often defined as Engel 1 & 2[18, 20]. We deliberately applied this stricter definition, because we aim to find and remove all epileptogenic

tissue based on HFOs and thus aim for seizure freedom as completely as possible. We included auras in the good outcome group, because distinction of the presence or absence of aura sensations in the first year after surgery, while still using AEDs, may be subjective. We will add post-hoc analyses using other dichotomies of the outcome score (for example Engel 1A vs. all others). These analyses might help to design future prospective studies. Recent studies have suggested that early AED withdrawal does not affect long-term seizure outcome or cure. It might unmask incomplete surgical success sooner[3]. Definite proof of complete removal of potentially epileptogenic tissue requires enduring seizure freedom over many years without the use of AEDs. We will collect information about AED use during follow-up for post-hoc analysis.

Previous epilepsy surgical RCTs included patients with intractable epilepsy with a minimal age of twelve years[102]. Including children in clinical trials is inextricably connected to ethical concerns[100]. A shift in epilepsy surgery towards the pediatric population has, however, been set in motion over the last years and the proportion of young children who undergo epilepsy surgery is increasing[105]. Children are considered for early epilepsy surgery nowadays, because successful resection of epileptic foci may lead to seizure- and medication freedom, and may improve social, psychological and cognitive development[10, 106]. We chose to include children, irrespective of age, because the research question about seizure outcome and the expected smaller resection that might reduce neurological and cognitive deficits are most relevant in this population. Limiting inclusion to solely adults would not only provide a lower number of eligible patients for participation, but would exclude the patient population who might benefit most.

We chose for a non-inferiority design to support feasibility of the trial, although findings in literature suggest that HFOs are the superior biomarker for epileptogenic tissue rather than spikes[18-20, 25, 35, 67, 71, 72, 97, 98]. The limiting factor of a superiority design is the large sample size needed, which would require participation of more than two centers. Currently, the few HFO experts and the fact that the surgeries and HFO analyses are time consuming limit the number of participating centers and patients. We consider the most important aspect of the study to assess the feasibility of using HFOs for tailoring. We think it is also important, at the same time, that automated ways of analysis are developed and implemented[92, 93], since these are indispensable for clinical application in other centers. Together with this trial this could set the stage for a larger, international RCT aimed at proving actual superiority of HFOs.

Our power calculation is based on estimated success rates deduced from retrospective studies up till 2013. These studies consisted of different patient populations. The current success rate of epilepsy surgery based on the old treatment, tailoring based on spikes, lies around 65%; in resections for temporal lobe epilepsy (TLE) 60-90% of patients achieve seizure freedom while in extra-temporal epilepsy it is around 40-65%[107, 108]. We expected an increase in success percentage of 15% leading to 80% for HFOs based on retrospective studies in which part of the patients, with surgery based on spikes, had incomplete removal of HFO tissue and poor outcome[19], while incomplete removal of spikes does not predict seizure recurrence[18, 20]. We are aware that we chose a relatively large effect size. An overestimation of the success rate for the outcome in the HFO arm could lead to non-significant finding due to a too small population. Before we started with this RCT, we retrospectively studied our own ioECoG data in order to validate previous findings[79]. We found good outcome (Engel 1A and B after one year) in 70%

of patients and tailoring based on FRs could have improved outcome in 15% of these patients[79]. This study also gave rise to the question whether or not we need to discriminate between epileptic and physiological HFOs, as we found some FRs, although far away from the area of resection, in functional eloquent areas, such as the sensorimotor and Broca's areas, after resection in two patients who were seizure and medication free. This means that we have to be careful when assessing HFOs in eloquent areas. In our retrospective data analysis we found that residual FRs were strongly related to epileptic tissue and poor outcome, while for ripples we found no association[79].

Tailoring based on spikes in the ioECoG is not an evidence-based method, as no RCT has compared tailoring to resection without ioECoG, but we relate to it as the "gold standard" [49]. The same restrictions for tailoring are valid for HFOs as for spikes. The ioECoG might either confirm the surgical plan or change the surgical plan, but reduction or extension of the planned resection might be limited by eloquent cortex or anatomy. Events, spikes or HFOs, occurring remote from the resection site are usually not considered indicative for removal. Upfront it may be hard to tell if tailoring will really influence the surgical plan, even though in our population we aim to perform ioECoG only with a valid indication. We will, prospectively, collect data on the influence of events on the actual clinical decision and perform a post-hoc analysis to clarify in which patients tailoring actually changed the surgical plan.

The visual analysis of HFOs requires experienced observers, and additional analysis time due to the offline analysis and expanded time settings for ECoG display. We estimate this additional analysis time is five to ten minutes per ioECoG recording, compared to spike analysis. A disadvantage of using spikes is that they have a lower inter-observer agreement compared to HFOs[45]. An associated risk of the use of HFOs is undersampling and underdetection of HFOs compared to spikes, as HFOs are a more local phenomena than spikes[18], and the negative effect of anesthetics on the number of HFOs[43]. These concerns are preempted similar as for the procedures during tailoring based on spikes; multiple recordings are made before and after resection, where the sampling strategy is based on the results in previous recordings to guaranty optimal sampling. All recordings are made while tapering the propofol until a continuous EEG pattern can be seen.

EXPECTED BENEFIT

We believe that an RCT is the proper way to prospectively test the beneficial properties of HFOs as a new biomarker for delineation of epileptogenic tissue and eventually improve the success rate of epilepsy surgery. Retrospective research suggests that HFOs are more specific and precise biomarkers for the epileptogentic zone than spikes. Complete resection of the epileptic focus may lead to seizure- and medication freedom, and may improve social, psychological and cognitive development, especially in children. Potential benefits from a smaller or more precise resection would be reduced neurological deficits and, combined with equal or better seizure outcome, should improved quality of life. The identification of HFOs can be standardized and automated and thereby potentiate an objective tailoring approach for international implementation.

TRIAL STATUS

The first patient was enrolled in "The HFO trial" on November 6th 2014 in the UMC Utrecht. At the moment of acceptance of this manuscript, August 2015, in total 10 patients have been included. Enrolment at the second site, VUmc Amsterdam, will start in Autumn 2015. Results are expected in 2018.

SUPPLEMENTS

STEERING COMMITTEE

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Case Report File data monitoring is performed, twice a year per site, by an independent Clinical Research Associate from Julius Clincial, Zeist, The Netherlands.

PART III

INNOVATION DRIVEN BY HFOS

CHAPTER 6

TIME-FREQUENCY ANALYSIS OF SINGLE PULSE ELECTRICAL STIMULATION TO ASSIST DELINEATION OF EPILEPTOGENIC CORTEX

Brain 2011;134:2855-2866.

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ABSTRACT

INTRODUCTION

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 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.

METHODS

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 to seizure onset zone and post-surgical outcome.

RESULTS

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 550% of fast ripples removed by resection had a poor outcome.

CONCLUSION

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.

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, about 20-30% of the patients with epilepsy are not satisfactorily controlled with anti-epileptic drugs. Resective surgery is a potentially curative option for 12.5% of these patients with refractory focal epilepsy[8, 21]. Unfortunately in these patients, the area responsible for seizure generation, represented by a hypothetical epileptogenic zone, is difficult to define precisely. Although several pre-operative techniques, for example inter-ictal and ictal EEG, PET, MRI, SPECT and MEG, are available, these are still time-consuming and only partly accurate[8, 109]. New reliable pre-surgical localizers of epileptogenic tissue are therefore still very 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; SOZ) and localize the cortical regions responsible for their generation while preserving the eloquent brain areas. The success of these chronic subdural electrocorticography (ECoG) registrations depends on the occurrence of the patient's typical seizures[1, 8]. Waiting for spontaneous occurrence of seizures makes this a 'long term' invasive procedure. Other biomarkers for the epileptogenic zone have been suggested, such as inter-ictal spikes and the more recently discovered spontaneous high frequency oscillations (HFOs). Inter-ictal spikes are related to the epileptogenic zone, but their specificity is not very high[8]. Recently it has been found that spontaneous HFOs (80-500Hz) specify the epileptogenic zone better than spikes. Generally these HFOs can be divided into ripples (80-250 Hz) and fast ripples (250-500 Hz)[17, 18, 110]. Total removal of HFO generating tissue, in particular fast ripples, correlates with a good surgical outcome . Still, the occurrence of HFOs is unpredictable and their visual detection extremely time consuming[18].

To reduce invasive monitoring time, with its risks of complications and patients discomfort, one wishes to be independent of random spontaneous events such as spikes, HFOs 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[21]. The location of these evoked epileptic responses is related to the SOZ and removal of this area is associated with better surgical outcome for temporal and frontal lobe epilepsy[21-23, 111]. A recent finding, in an animal model, is that single pulse stimulation can also evoke epileptic HFOs[112]. SPES evoked HFOs 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, the waveform definitions, and the 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[21]. Review of the expected evoked HFO responses in the time domain by the method described by Jacobs et al. (2008) would complicate visual evaluation of SPES responses even further[18].

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 (TF) analysis is based on wavelet transformation, and also automatically takes statistics into account. In a retrospective study, electrodes showing TF-SPES evoked responses in different frequency ranges are compared to the SOZ and surgical outcome in a number of patients undergoing long-term invasive monitoring. We wanted to see if our TF-SPES analysis would provide a new tool for the delineation of the epileptogenic cortex in these patients.

TABLE 6.1
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 tumor resection 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	Tumor	80	7	I
9	13/F	Frontocentral	L&R	Tuberous sclerosis (multiple tubers)	96	5	IV
10	13/M	Frontal	L	Focal cortical dysplasia	78	6	IV
11	8/M	Frontocentral	L	Tumor	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, M = Male, L = Left, M = Male, R = Right

METHODS

PATIENTS

Chronic ECoG data were recorded from 13 patients (7 male, 6 female, 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 Center of Utrecht, The Netherlands, between 2008 and 2010. The patients were monitored for 5 to 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 visual analyzed

SPES results, in line with recommendations of Valentín et al. (2002)[21], were included in the clinical decision making. There were three patients with temporal, three frontal, one parietal, three frontocentral, one centroparietal, one central and one occipital lobe epilepsy. Most patients were on multiple anti-epileptic drugs which were tapered during the registration. Patient information is given in table 6.1. The procedure was approved by the hospital's Medical Ethics Committee.

ELECTROCORTICOGRAPHY

Chronic ECoG was performed with subdural electrodes (Ad-Tech, Racine, Wisconsin, USA) 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 mm^2 contact surface, embedded in silicone, with an inter electrode distance of 1 cm. In one patient, also two depth electrode strips were implanted, each with 8 cylindrical contacts with 7.9 mm^2 contact surface and 5 mm inter electrode distance (Ad-Tech, Racine, Wisconsin, USA). For 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 SOZ and the resected area of the eventual surgery were marked by the involved clinical neurophysiologists (CF, FL). They were aware of the conventional visual analyzed SPES results as part of our clinical practice, but unaware of the TF-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 artifacts, that was matched to an MRI surface rendering acquired pre-operatively[113]. The SOZ was defined as the set of electrode contacts that showed the earliest ictal activity in the ECoG. Early ictal activity was defined as the first ECoG pattern consisting of rhythmic spikes, rhythmic sharp waves, spike & slow waves complexes, increasing gamma activity, regular or sharpened delta or theta activities, or low-amplitude activity in the beta range[21, 114, 115] preceding or coinciding with a habitual clinical seizure.

For patients with a follow-up longer than 12 months or immediate continuation of the seizures, the outcome of surgery was determined (Engel classification)[5]. 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 (Micromed IRES 600, Micromed, Veneto, Italy). 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 Valentín et al. (2002, 2005) 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[21, 23].

PRE-PROCESSING

The pre-processing of the data files was performed with dedicated software implemented in Matlab (Mathworks, Natick, MA). The data was not initially recorded keeping the proposed analysis method in mind. Therefore a pre-processing step detecting exact stimulus timing was required. Other steps included stimulus artifact 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 two seconds for each pair of stimulated electrodes. For subsequent computations a sub-interval of [-1 s: -0.2 s] was defined as computational baseline to avoid interference of the residual stimulus artifact 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)[116] 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, respectively 3 and 0.5. 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 LEVELED 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)[116].

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 assumed surrogate data distribution with a chosen significance level (p=0.05). Non-significant ERSP values were discarded by setting them to zero[116].

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] color scale with saturation of values above the limit of ±15 dB (accommodating the stimulus artifact). Note that this color scaling shows changes in power relative to the baseline for each frequency. In figure 6.1 a schematic representation, by means of a flow chart, of the different steps in this time frequency analysis method, from now on called TF-SPES, is given.

EVENT-RELATED SPECTRAL PERTURBATION VISUAL ANALYSIS

The TF-SPES evoked responses present in the ERSP images (64 electrodes x 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 Valentín et al. (2002, 2005)[21-23] 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) which we will call the Spike band (S), 80-250 Hz, referred to as the Ripples band (R) and 250-520 Hz, referred to as the Fast Ripples band (FR). In a joint meeting, the observers were shown several examples of ERSP images and corresponding delayed responses and HFOs as visually marked in the raw time domain SPES data for one patient by an experienced observer (MZ), following the methodology of Jacobs et al. (2008)[18]. 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 artifacts, like the spectral scattering >80 Hz due to muscle artifacts[99, 117].

Then visual classification of TF-SPES evoked responses in all ERSP images was performed by two observers (CF, FL, GH or MK) independently. Inter observer agreement was calculated using Cohen's kappa (κ) using SPSS 18 (PASW Statistics 18, Rel. 18.0.0, 2009, Chicago; SPSS Inc.). Kappas were calculated separately for the number of responses classified as S, R and FR. A kappa score > 0.4 was interpreted set as sufficient agreement[118]. For the final counts of S, R, and FR per patient only responses for which there was agreement between the two independent observers were accepted. The total amount of responses was calculated for S, R and FR and for each recorded electrode. Datasets with kappa <0.4 for all three frequency regions were excluded from further analysis.

STATISTICAL ANALYSIS

Statistical analysis was performed for S, R, and FR separately and for each individual patient. The correspondence between electrodes showing a TF-SPES evoked response was related to the clinically defined SOZ electrodes. The populations' median sensitivity, specificity, positive (PPV) and negative prediction value (NPV) of TF-SPES evoked responses for the SOZ 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 $\frac{\text{number of positive electrodes within SOZ}}{\text{total number of positive electrodes}}$ (Eq 6.1)

NPV_{el} is defined as $\frac{\text{number of negative electrodes within SOZ}}{\text{total number of negative electrodes}}$ (Eq 6.2)

The PPV was also calculated for the TF-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 $\frac{\text{number of evoked responses on positive electrodes within SOZ}}{\text{total number of evoked responses on all positive electrodes}}$ (Eq 6.3)

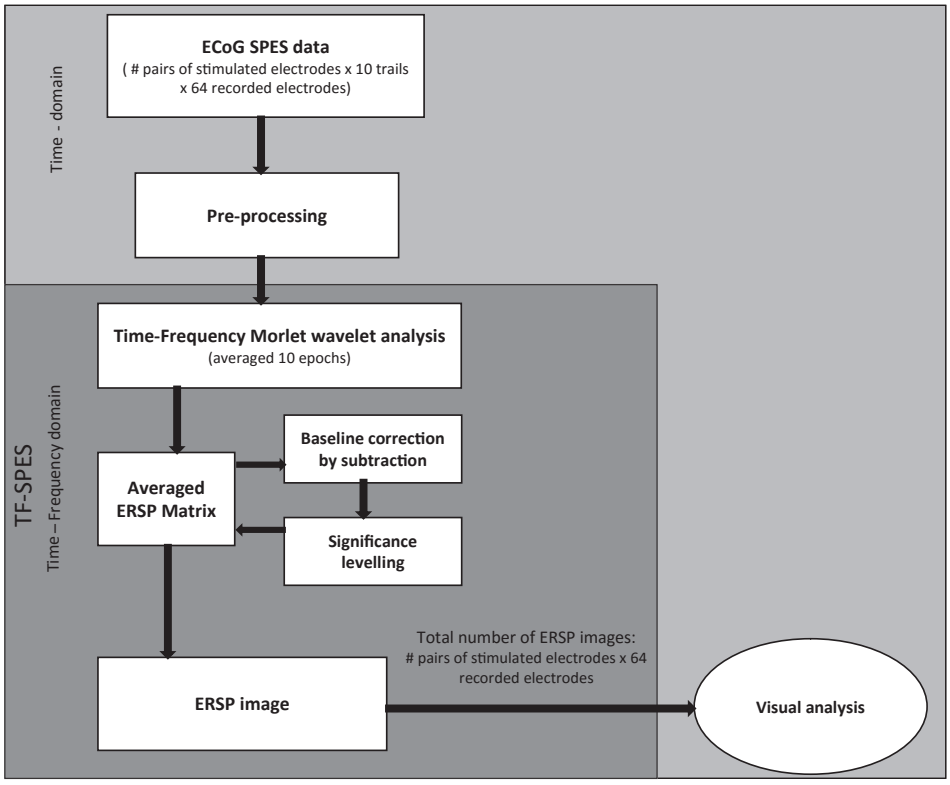


FIGURE 6.1
Flow chart different steps in the time-frequency analysis. ECoG=electrocorticography.

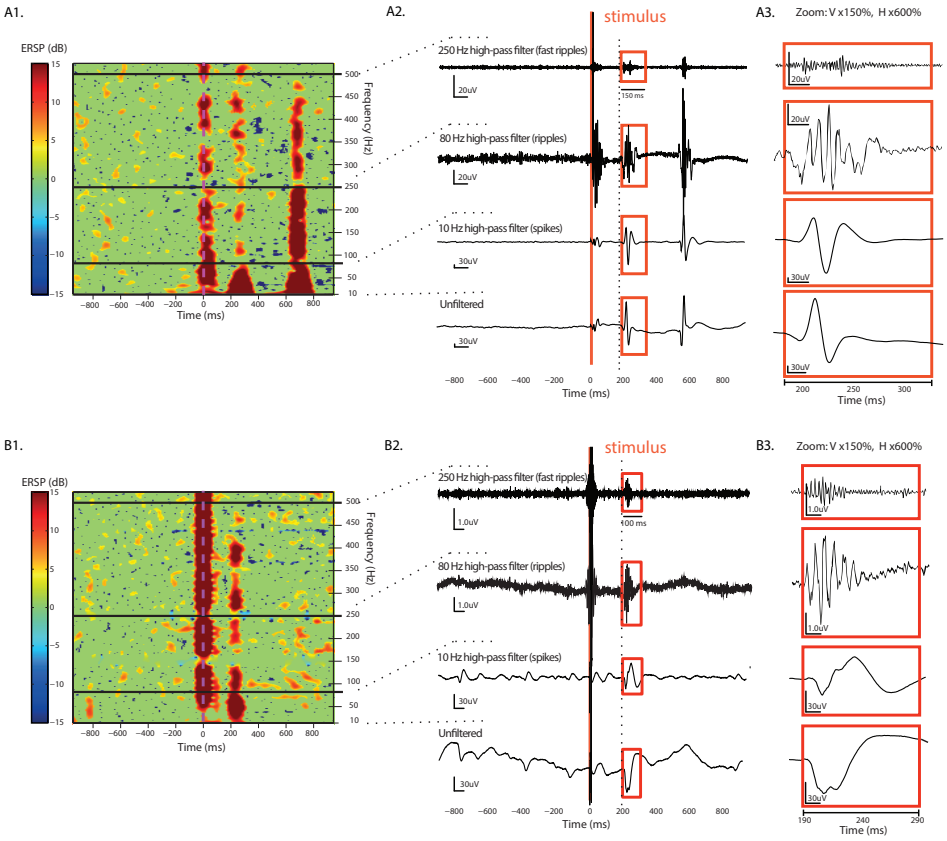


FIGURE 6.2

Two examples (A and B) of the correspondence of the same single epoch between (left) a significant ERSP image as result of time-freqency SPES analysis and (center) time domain ECoG data with the high pass filtered (Stellate Harmonie 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 μV) with superimposed R (72 μV) and FR (15 μV) and a second larger spontaneous spike with accompanying large R and FR. Note that the second spontaneous spike shows relatively more ripple component compared to the evoked spike, a phenomena which is described as potential side effect of filtering (Urresterazu et al. 2007) in which false HFOs show a significant ripple. The example in B.) (Patient 4) shows at ~200ms an evoked low amplitude spike (unfiltered 80 μV) with superimposed ripple (4.3 μV) and fast ripple (1.3 μV). Both evoked spikes result in differentiated small blobs with significant FR blobs in the ERSP, in absence of large (elongated) blobs in the R band.

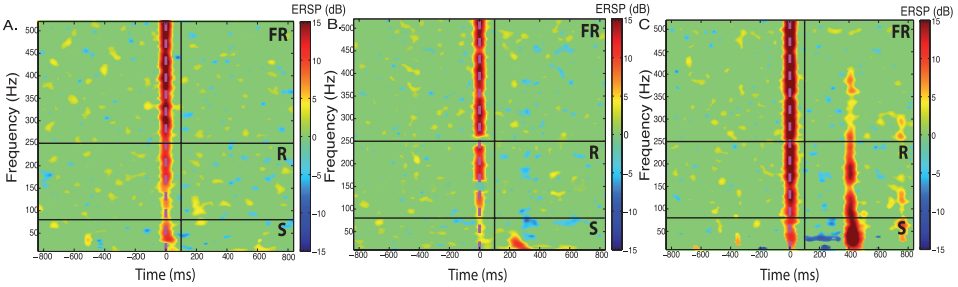


FIGURE 6.3
Significant averaged ERSP images marked as A. no response, B. a time-frequency SPES evoked response in the spike region, C. time-frequency SPES evoked responses in spike (S), ripple (R), and fast ripple (FR) regions. Note that these ERSP images are all of different patients.

RESULTS

In figure 6.2 two examples are given of the correspondence for the same single epoch of an evoked response as analyzed by TF-SPES and the conventional visually marked S, R, and FR and an expanded view of these oscillations. These were used in the joint meeting preceding the visual classification of TF-SPES evoked responses of all data by the independent observers.
In 13 patients, a median of 46 (range of 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 figure 6.3 three examples are given of significant averaged ERSP images classified as no response, a TF-SPES evoked spike response only, and a combined evoked S, R and FR response. Note that these examples are chosen from results of different patients.
Visual classification of TF-SPES evoked responses was performed for all ERSP images of the 13 included patients. The inter observer agreement (IOA) for one patient (Patient 3) resulted in a $\kappa<0.4$ for S, R and FR and this data was rejected from further analysis. So inter-observer consensus scores for S, R and FR remained for 12 patients and were further analyzed. Within this group of 12 analyzed patients the IOA for 5 patients demonstrated a $\kappa< 0.4$ for solely one frequency range, but were still analyzed. For one patient this concerned S, and for four patients this concerned FR. In total, 6553 responses were classified for 12 patients. For all 12 patients each of three defined responses S, R and FR were found. The median number per patient of S was 320 (range 75-790), of R 125 (range 26-465) and of FR 29 (range 2-112).

RELATION SPIKES, RIPPLES AND FAST RIPPLES WITH THE SOZ

For the 12 analyzed patients the median number of SOZ electrodes of the ictal ECoG was 5 (range 0-30). No clear SOZ could be identified in one patient. In the remaining 11 patient the median sensitivity of TF-SPES for SOZ decreased and its median specificity increased with higher frequency range. There is a high sensitivity (100%) and low specificity (17%) for S versus a lower sensitivity (67%) but higher specificity (79%) for FR. A median PPV_{el} value was found of 17% for S, 19% for R

and 27% for FR. The PPV_{resp} improves this PPV_{el} by 7% for R and by 10% for FR, resulting in a PPV_{resp} of 37% for FR. There was no difference for S. This effect is illustrated in figure 4 and 5, where TF-SPES responsive electrodes are classified as highly responsive (defined as electrodes with ≥50% of the maximal number of responses present on a single electrode) and moderately responsive (<50%) for S, R and FR. The median NPV_{el} was 100% for S, 100% for R and 96% for FR.

RELATION SOZ, SPIKES, RIPPLES AND FAST RIPPLES WITH OUTCOME

The post-surgical outcome after one year is available in 9 patients; the other patients had been operated too recently. Four patients (Patients 1, 7, 8 and 11) had good surgical outcome in Engel class I, but still treated with AEDs. In 5 patients (Patients 4, 9, 10, 12 and 13) surgery resulted in an outcome of Engel class II-IV. In these 9 patients the resection area had been covered by a median number of 9 electrodes (range 5-36). The SOZ was totally resected in 4 patients (Patients 1, 7, 10 and 12), of which two patients (Patients 1 and 7) had a good surgical outcome of Engel class I. These two patients both suffered from TLE. Total removal of the area showing TF-SPES evoked FR, which best correlated with SOZ, was retrospectively achieved in one patient (Patient 7) who showed good post-surgical outcome. The other TLE patient (Patient 1) had 94% of the number of evoked FR removed. Of the remaining seven patients, in whom less than 50% of evoked FR were removed, 5 patients had a poor post-surgical outcome. Removal of area showing evoked S and R, which are less specific for the SOZ, showed the same trends as FR but with lower percentages.

Examples of TF-SPES evoked responses, SOZ and RA in four patients (Patients 1, 4, 10 and 13) are depicted in figure 6.4 and figure 6.5. The results of the other nine patients are available as supplementary material.

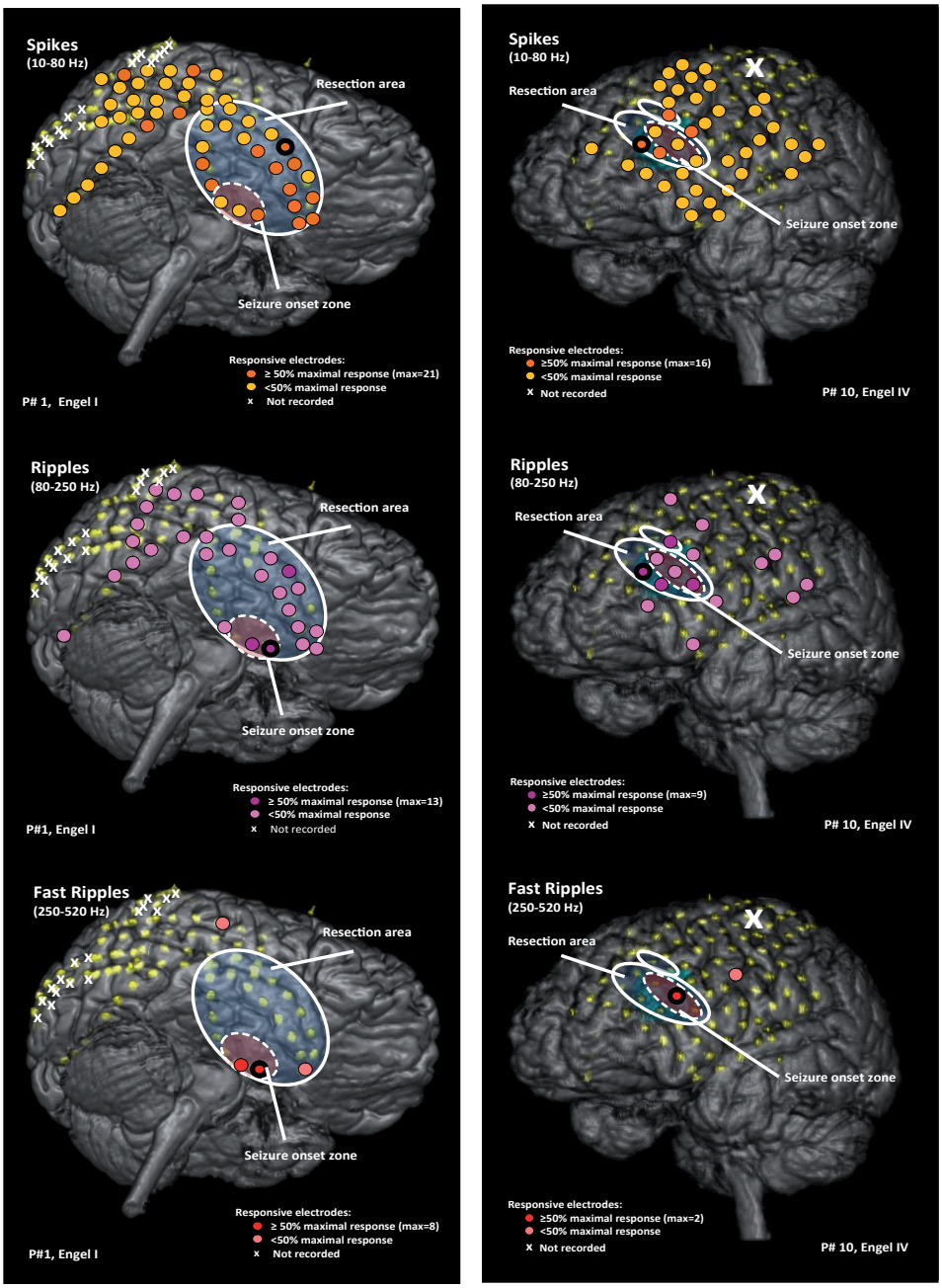


FIGURE 6.4
An example of total seizure onset zone removal by resection for Patient 1 (temporal lobe epilepsy) with good post surgical outcome Engel class I (left), and Patient 10 with poor post surgical outcome Engel class IV (right).

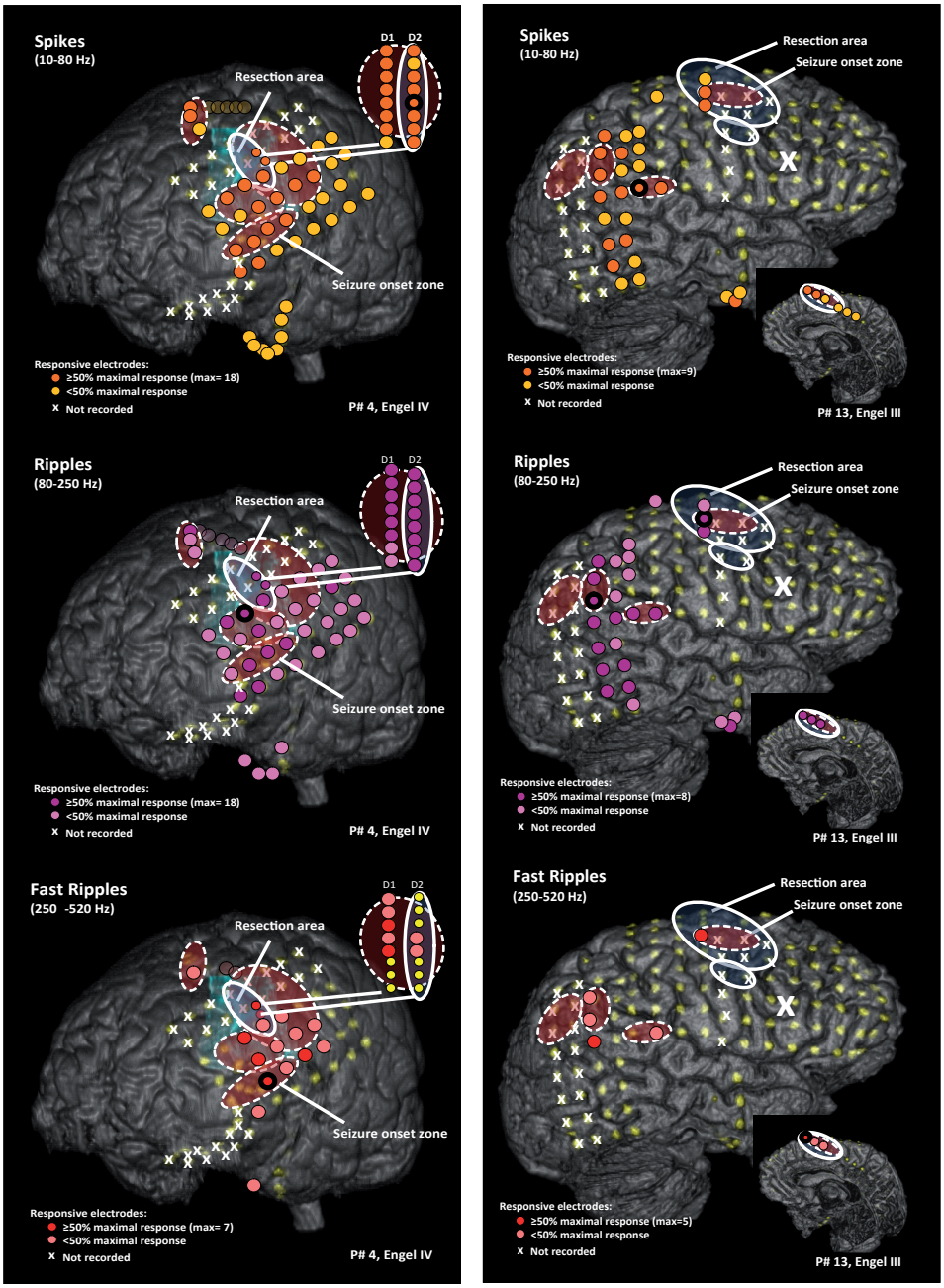


FIGURE 6.5
An example of incomplete seizure onset zone removal, incomplete fast riple removal by resection and poor post surgical outcome. Patient 4 (left), Engel class IV, D1 and D2 represent depth electrodes, and Patient 13 (right), Engel class III.

DISCUSSION

We demonstrate a novel approach for analysis of SPES based on wavelet transform time-frequency analysis. In this TF-SPES approach the significance of evoked responses is incorporated in the results and data reduction is achieved by averaging of 10 trails into a single image. TF-SPES shows that not only spikes, but also responses in the HFOs range can be evoked by SPES and can be readily recognized whereas visual identification of spontaneous HFOs in chronic ECoG 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 FR may be the most useful marker of the epileptogenic zone of the three. With a median specificity of 79% for the SOZ and their incomplete removal being related to poor outcome, TF-SPES analysis assists in delineation of the epileptogenic cortex, with evoked FR being the most promising new marker. If this promise is confirmed in larger studies, this will make interpretation of chronic ECoG independent from spontaneously occurring events, and thus shorten these invasive recordings.

METHODOLOGICAL ASPECTS

Time Frequency analysis

In the recent years Morlet wavelet based time-frequency decomposition has been proposed and used for analysis of spectral properties of spontaneous inter-ictal events like spikes and HFOs in the ECoG[44, 45, 84, 119]. In this literature the discussion of how many false HFOs 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[57]. The Morlet Wavelet used in our time-frequency analysis resembles the finite impulse response filter used in visual HFO detection following the methodology of Jacobs et al. 2008 and Bénar et al (2010)[18, 57]. In the ERSP images, we found evoked HFOs (R, FR) superimposed on spikes, but also spikes without concomitant R or FR[18, 84], as is also the case in the time domain in spontaneous HFOs. But also, as is the case in spontaneous HFOs, whether TF-SPES evoked power in the R and FR band reflects specific properties of spikes or separate HFOs activity is still under debate. Figure 2 shows two examples: in 2A a relatively steep (spontaneous) spike that can be characterized by its high power extending in the R- and FR band and an earlier evoked HFO 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 (2B), with an even smaller amplitude and steepness of the initial evoked spike and relatively large FR component. Urrestarazu et al. (2007) described that false HFO detection as a filter effect of sharp spikes reveals itself showing large R components superimposed on spikes[33]. For practical purposes, regardless of the interpretation, the presence of significant power in the R and FR band, shows an increased specificity for the SOZ 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 for averaging 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 inter-ictal activity in the data. Compared with this statistical correction method in Valentín's original paper (2002)[21] our method seems less objective, but is more time-efficient. Yet, the advantage of TF-SPES is that power spectra for multiple epochs are accumulated, evoked responses with approximately the same latency show a larger power increase compared to spontaneous inter-ictal events[116]. 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 ERSP 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 TF-SPES evoked responses. To avoid subjectivity we took a conservative approach and let the relevance of scored S, R and FR be based on agreement between two independent observers. The kappa values of the IOA resembled those for inter-ictal discharges in MEG and ECoG[45, 118, 120, 121]. Some low kappa values were encountered and reflect poor signal-to-noise. Especially FR suffered from low kappa 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 TF-SPES fully automated.

Stimulation protocol

When SPES was first proposed by Valentín et al. (2002, 2005) as a clinically useful tool, their results were described for temporal lobe and frontal lobe epilepsy patients separately. They showed that the localization of the evoked events was most specific in temporal lobe epilepsy[21, 23]. 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 FR is a most promising new finding, regional differences in prediction of SOZ and outcome may be different in TF-SPES from tradition SPES looking at spikes only. Our number of patients is insufficient to draw conclusions with regard to regional differences.

A further difference in the analysis of SPES responses compared to Valentín et al. (2002, 2005)[21-23] is that they established both association of pathology with the responsive electrodes and

association with the site of stimulation that evoked a response[21-23, 111]. At first sight in our data we found no indication of a clear relation between the site of stimulation and the SOZ. However, a thorough analysis requires a different approach based on the identification of epileptic networks which is currently still unpractical for surgery[122].

Rolston et al. (2010) in a more fundamental study showed the existence of evoked HFOs <100 ms after stimulation in an animal rat model[112]. In this study evoked HFOs 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[21]. In our clinical study we wanted to process the SPES protocol and resulting data in line with the rationale of Valentín et al. (2002, 2005) who described only late responses >100 ms as pathological[21-23]. We showed that the TF-SPES analysis increases specificity for the SOZ when incorporating signals in the HFO 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 HFO responses, also in conjunction with a thorough analysis of the early normal responses that have been described by Valentín et al (2002)[21].

CLINICAL FINDINGS

Although our population represents a small and heterogeneous group, our results resemble those by Valentín et al. (2002-2005) and by Flanagan et al. (2009) on the one hand, and recent HFO research on the other. They showed that delayed spike-like responses on SPES and spontaneous HFOs are related to the SOZ and surgical outcome[18, 21-23, 33, 111]. Our results suggest that TF-SPES evoked FR may have the same clinical value. We could evoke R and FR in all our patients, suggesting that evoked HFOs, like spontaneous HFOs, mark epileptogenicity independent of underlying pathology[20], although we, like others, have no normal controls.

As noted by Lesser et al. (2010), R and FR may occur simultaneously at sites separated by centimeters[18, 84] which might explain why some patients have a poor post-surgical outcome (figure 6.5 and Patients 9, 10 and 12 in supplementary material). Partial removal of the epileptogenic zone might have been the case[8].

TF-SPES results in 2 out of 9 patients with follow-up were remarkable in that S, R, or FR 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 SOZ 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) was 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 one year and medication withdrawal is not the endpoint[2].

TF-SPES showed a decrease in sensitivity for R and FR. FR demonstrated the lowest sensitivity for the SOZ. Electrode coverage in subdural chronic ECoG may be insufficient to pick up all evoked

responses. Also incomplete coverage may result in seizure activity propagated from unexplored areas being indicated as seizure onset. But it should be kept in mind that seizure onset zone may not always predict the epileptogenic zone[123].

A weakness of our study may be that the clinicians taking care of the patients, although unaware of TF-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)[21]. We did not comprehensively compare this type of visual evaluation with our TF-SPES classification of the evoked S frequency range.

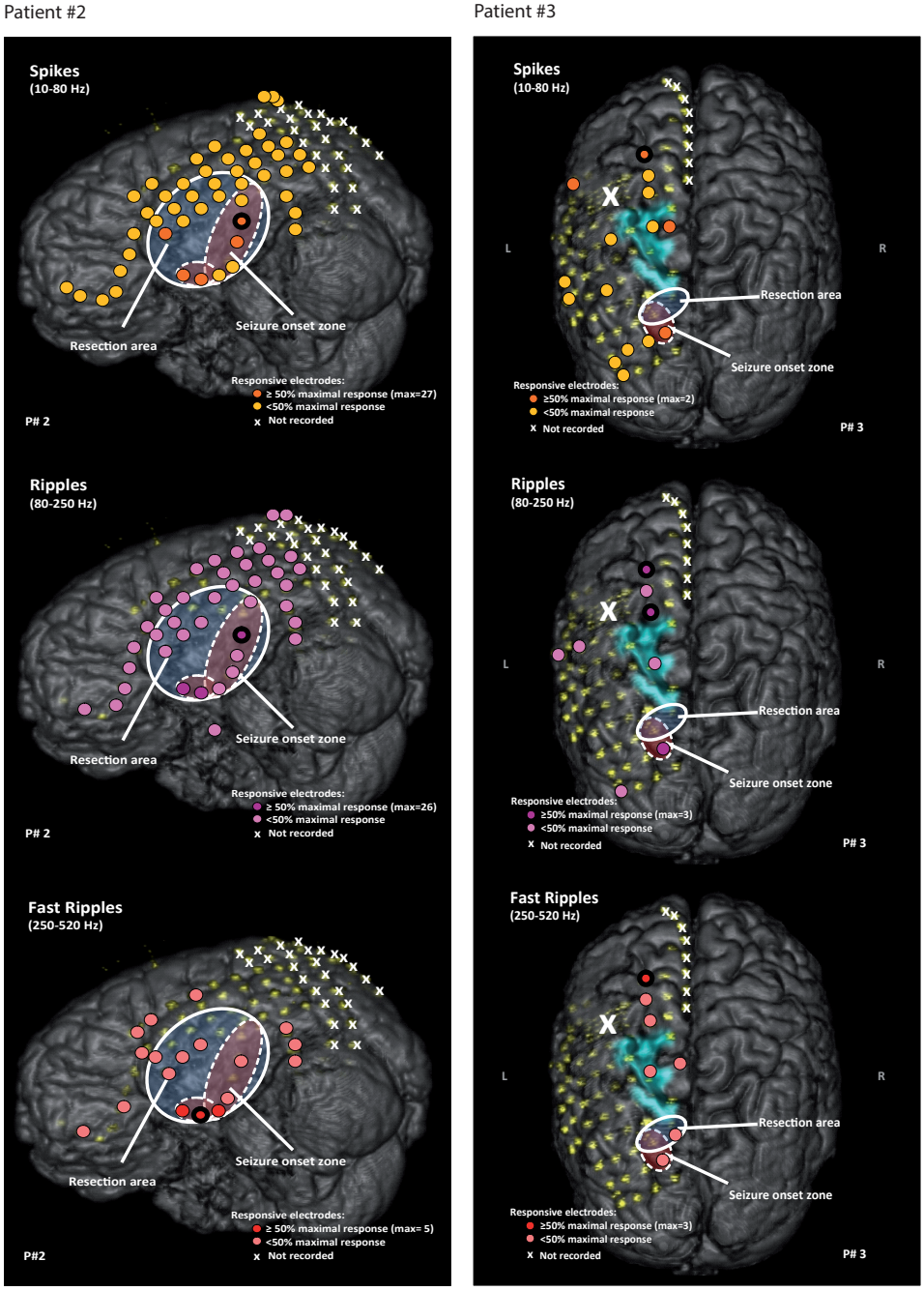
Our first results are encouraging in patients who are among the most difficult in epilepsy surgery. In the Netherlands, the work-up of epilepsy surgery is traditionally non-invasive and subdural electrode implantations are restricted to a small number of the most complex patients[12]. In patients who 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 showed need to be confirmed in a larger and more general group of focal refractory epilepsy patients. To establish the validity of TF-SPES as a method for delineation of the epileptogenic zone would ideally require a prospective study. Then the influence of TF-SPES results on resection and long-term outcome after medication withdrawal can be compared to outcome without TF-SPES results available to surgical decision-making.

IMPLICATIONS AND FUTURE DIRECTIONS

TF-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 (S, R and FR) that can be used to delineate the epileptogenic cortex without depending on their spontaneous occurrence and the occurrence of seizures. The inclusion of HFO responses in TF-SPES seems to increase specificity of the method and mirrors the experiences with spontaneous HFOs compared to inter-ictal spikes. A next step would be to automate interpretation of the ERSP images, with which TF-SPES could become a time-efficient way to use SPES in clinical practice. TF-SPES could be used to reduce the region of interest and grid placement in invasive chronic ECoG monitoring, and even in acute ECoG for tailoring surgery intra-operatively. Larger, preferably prospective studies are needed to establish the full clinical potential.

SUPPLEMENTS

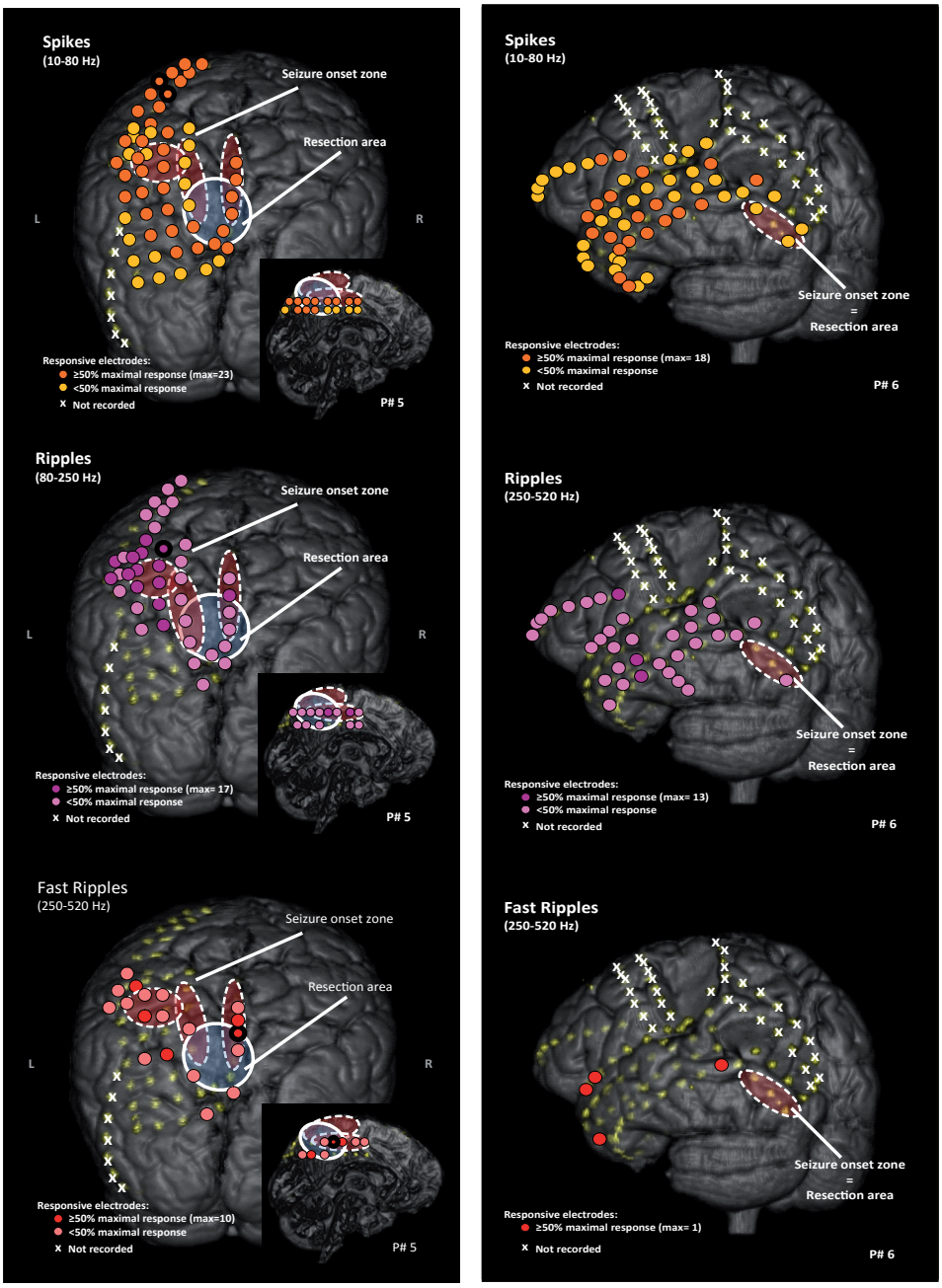
TF-SPES results of the other patients included in this study.
(see next pages)



SUPPLEMENTARY FIGURE 6.1
Patient P#2 (TLE) with complete SOZ removal, outcome unknown (right), and patient P#3, excluded from analysis due to $\kappa<0.4$ for S, R and FR (left).

Patient #5

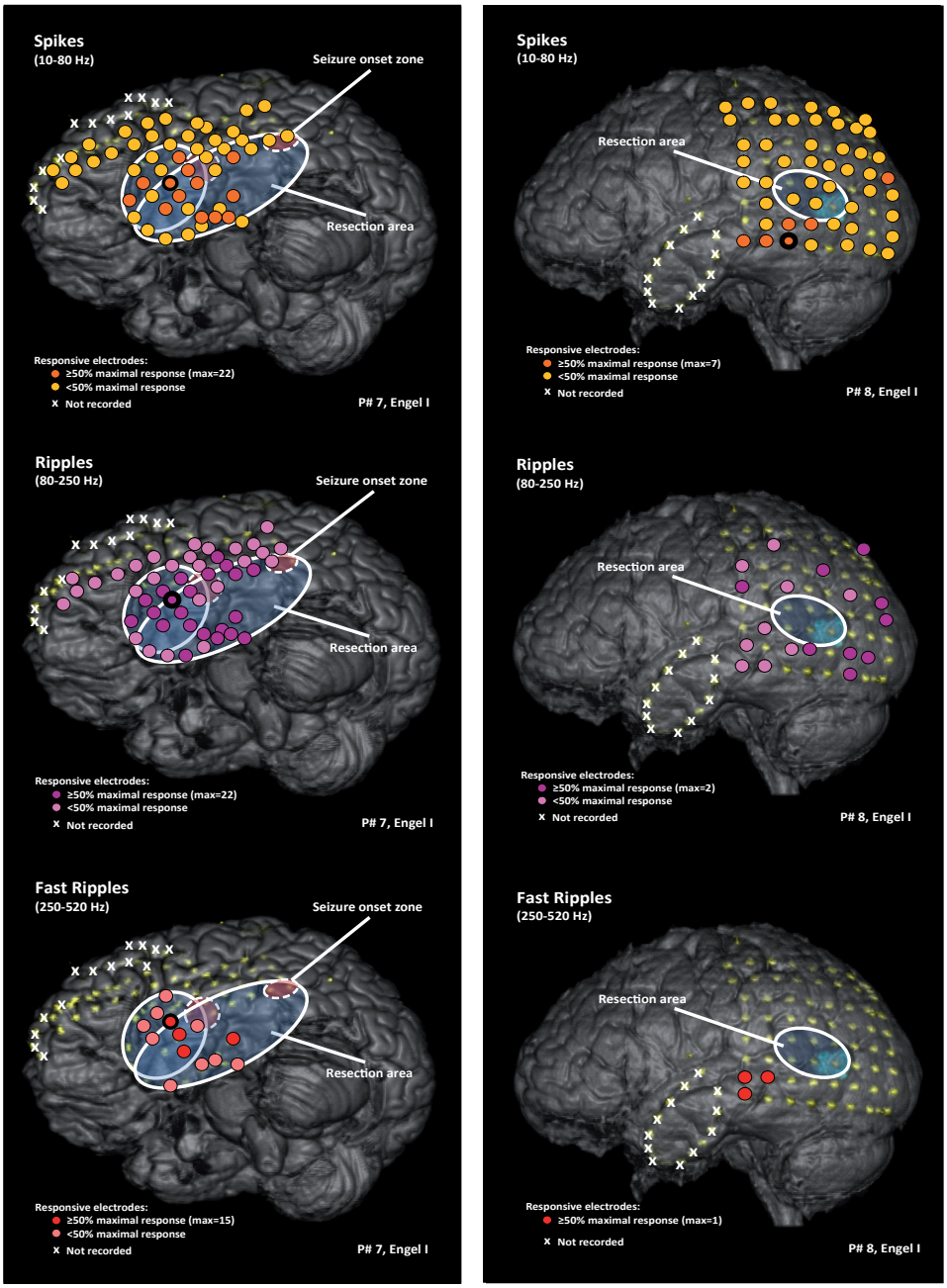
Patient #6



SUPPLEMENTARY FIGURE 6.2
Patient P#5 incomplete SOZ removal and FR removal, outcome unknown (left), and patient P#6 (severe ischemic brain damage) with contradictory TF-SPES results (right).

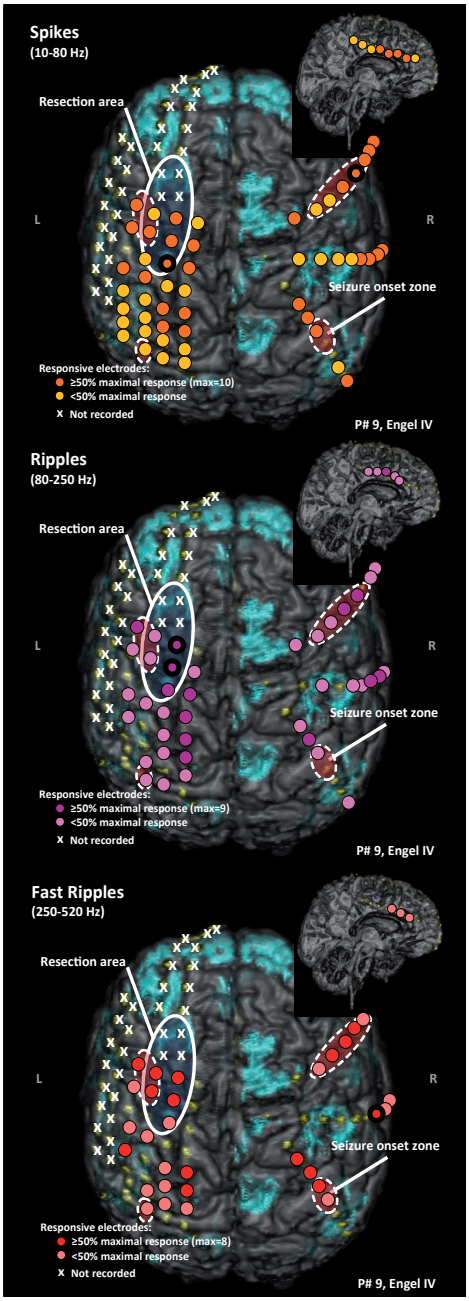
Patient #7

Patient #8

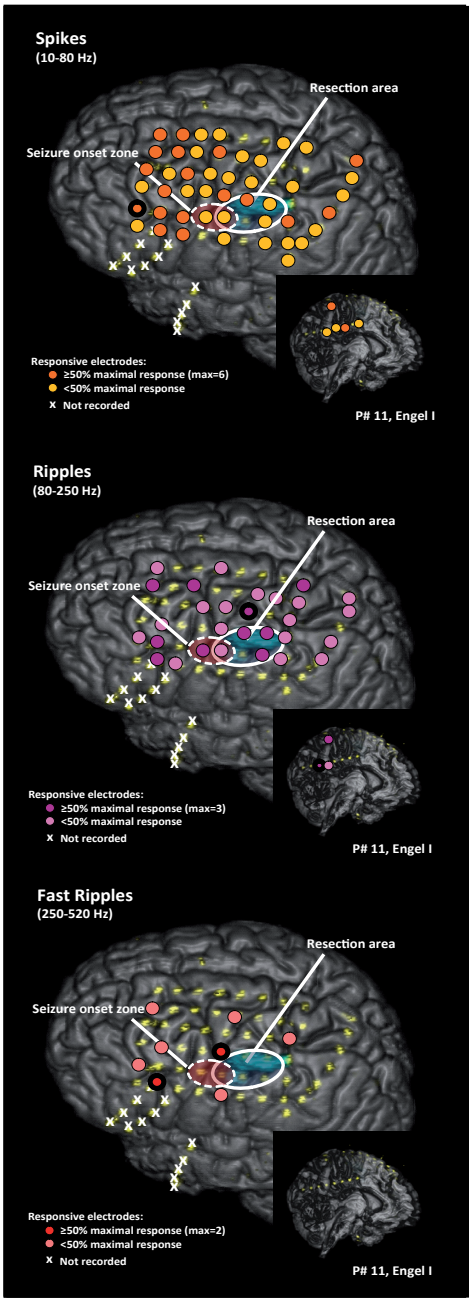


SUPPLEMENTARY FIGURE 6.3
Patient P#7 (TLE) complete SOZ removal and FR removal, and good post-surgical outcome (left), and patient P#8 with undefined SOZ but good outcome as discussed in the paper (right).

Patient #9

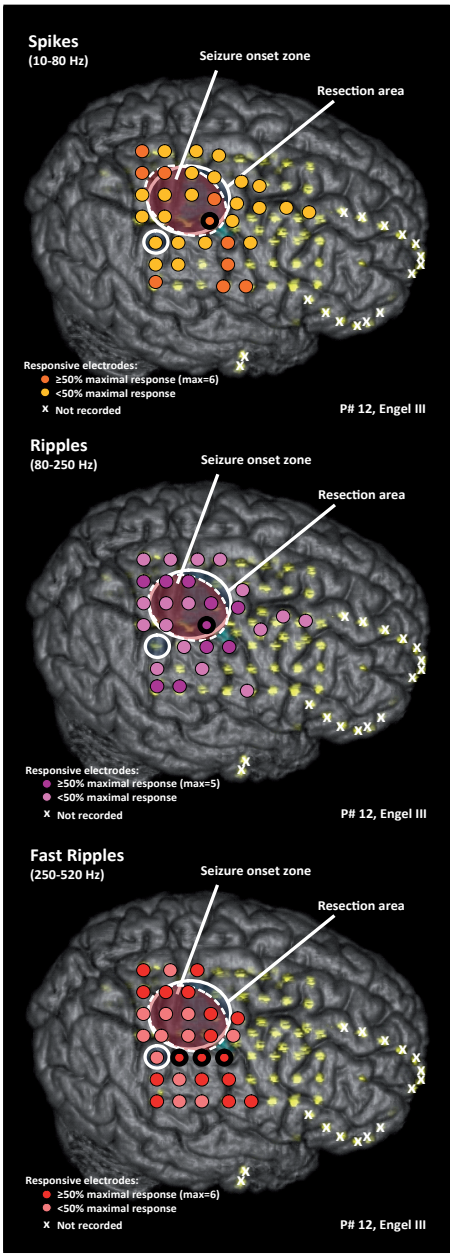


Patient #11



SUPPLEMENTARY FIGURE 6.4
Patient P#9 with multiple lesions (Tuberous Sclerosis) and bilaterally placed electrodes (left), and patient P#11 with incomplete SOZ removal and FR removal, but good outcome, limited number of stimulated electrode pairs as discussed in paper (right).

Patient #12



SUPPLEMENTARY FIGURE 6.5
Patient P#12, almost total SOZ removal but incomplete removal of FR with poor outcome.

CHAPTER 7

SINGLE PULSE ELECTRICAL STIMULATION TO IDENTIFY EPILEPTOGENIC CORTEX: CLINICAL INFORMATION OBTAINED FROM EARLY EVOKED RESPONSES

Clin Neurophysiol 2016;127:1088-1098.

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ABSTRACT

INTRODUCTION

Single pulse electrical stimulation (SPES) probes epileptogenic cortex during electrocorticography. Two SPES responses are described: pathological delayed responses (DR, >100 ms) associated with the seizure onset zone (SOZ) and physiological early responses (ER, <100 ms) that map cortical connectivity. We analyzed properties of ERs, including frequencies >80 Hz, in the SOZ and seizure propagation areas.

METHODS

We used data from 12 refractory epilepsy patients. SPES consisted of 10 pulses of 1 ms, 4-8 mA and 5 s interval on adjacent electrodes pairs. Data were available at 2048 samples/s for six and 512 samples/s (22 bits) for eight patients and analyzed in the time-frequency (TF) and time-domain (TD).

RESULTS

Electrodes with ERs were stronger associated with SOZ than non-SOZ electrodes. ERs with frequency content >80 Hz exist and are specific for SOZ channels. ERs evoked by stimulation of seizure onset electrodes were associated with electrodes involved in seizure propagation.

CONCLUSION

Analysis of ERs can reveal aspects of pathology, manifested by association with seizure propagation and areas with high ER numbers that coincide with the SOZ. Not only DRs, but also ERs could have clinical value for mapping epileptogenic cortex and help to unravel aspects of the epileptic network.

INTRODUCTION

Cortical single pulse electrical stimulation (SPES) and its responses yield information about the epileptic tissue in the brain. SPES was first described by Valentin et al. (2002) in focal refractory epilepsy patients who underwent chronic electrocorticography (ECoG) preceding surgery. The stimulation protocol consists of ten brief pulses of 1 ms and 4-8 mA amplitude with a 5 s interval given over two neighboring electrodes[21]. SPES evokes two types of responses: early responses (ERs) within 100 ms after stimulation and delayed responses (DRs) after 100 ms up to 1 s after stimulation[21-23]. SPES research has mainly focused on DRs. DRs are associated with the seizure onset zone (SOZ) and contain pathological high frequency (80 Hz-500 Hz) information. These high frequency DRs are more specific for the seizure onset zone compared to DRs in the low frequency band (<80 Hz)[124]. ERs are assumed to be a physiological phenomenon originating from stimulation of cortico-cortical association fibres (u-fibres). ERs resemble the N1 potential in cortico-cortical evoked potentials (CCEP; general settings 0.3 ms pulses, 1 Hz, 1-15 mA, 20-70 stimuli averaged). The N1 potential provides information regarding cerebral functional connectivity[125-132]. It has been suggested as a method for the identification of functional areas during surgery[133]. As such, CCEPs, and ERs, may reveal regions of rich network connectivity. On the other hand , it has been shown that seizure propagation proceeds locally through neocortical cells as well as over longer distances through the deeper lying u-fibers that are stimulated by CCEP[134, 135]. ERs might mirror these seizure propagation pathways, thus revealing an important aspect of the pathology of epilepsy.
We investigated ERs, including higher frequency responses above 80 Hz, evoked by stimulation out- and inside the seizure onset zone (SOZ) and analyzed their properties in the SOZ and in areas of seizure propagation, respectively. We used two approaches; Analysis in the time-frequency (TF) domain of high temporal resolution data and analysis in the time-domain (TD) of high dynamic range data.

METHODS

PATIENTS

Data from 12 patients (5 males, mean age 19.7 years, range 8-42 years) with refractory epilepsy who underwent chronic ECoG preceding epilepsy surgery were used. All patients were admitted to the intensive epilepsy monitoring unit of the UMC Utrecht in the Netherlands in the period 2008-2012. SPES was routinely performed as a clinical protocol. SPES results were included in the medical decision making after visual inspection in line with recommendations of Valentin et al. (2002)[21].
Monitoring time ranged from three to eight days. All 12 patients underwent resective surgery of a presumed epileptic focus. Five patients had temporal, three had frontal, two had frontocentral, and two had parietal lobe epilepsy. Most patients were on multiple anti-epileptic drugs that were tapered during the registration. Patient information is summarized in table 7.1.
The institutional ethical committee indicated that no explicit approval was necessary because of the retrospective character of this study, provided that data were coded and handled anonymously.

ELECTROCORTICOGRAPHY DATA

Subdural grids and strips (Ad-Tech, Racine, Wisconsin, USA) were placed under general anesthesia, after craniotomy. The circular platinum electrodes, imbedded in silicon, had a contact surface of 4.2 mm^2 and an inter-electrode spacing of 1 cm. In two patients, additional depth electrodes were implanted with eight cylindrical contacts with 7.9 mm^2 contact surface and 5 mm inter-electrode distance. Electrode placement was based on clinical pre-operative diagnostics, covering both the suspected epileptogenic region(s) and eloquent areas. Electrode positions on the cortex were obtained by co-registration of post-implantation CT with preoperative 3D MRI images[113]. The median number of implanted electrodes was 96 (range 88-120) per patient (table 7.1).

CLINICAL INFORMATION

Per patient a recording of a typical spontaneous clinical seizure was analyzed retrospectively by two neurologists (chosen from CF/FL/MZ). They were asked to mark independently; 1.) the one electrode with the first ictal activity as the seizure onset electrode (SO-electrode), 2.) all electrodes on which seizure propagation was found (SP-electrodes) within the first 30 seconds after initial onset. Ictal activity was defined as the first ECoG pattern consisting of rhythmic spikes, rhythmic sharp waves, recruiting gamma activity, regular or low-amplitude activity in the beta range prior to or coinciding with the clinical manifestation of the seizure[114]. In case of a generalizing seizure, observers marked all electrodes showing ictal activity up to the point of generalization. Disagreement in the marked onset or propagation between two observers was solved in a consensus meeting. Additionally, a clinical SOZ area was defined, based on all recorded seizures from the total monitoring period (by FL/CF). This SOZ typically contained multiple electrodes.

SINGLE PULSE DATA ACQUISITION

Single pulse electrical stimulation (SPES) was performed using a manually controlled cortical stimulator (IRES 600 surgical, Micromed, Treviso, Italy). Monophasic SPES stimuli were given, ten pulses with a duration of 1 ms, separated by 5 s intervals, on pairs of adjacent electrodes. Stimulation was performed at an intensity of 8 mA and only in stimulation pairs where twitches or pain occurred the intensity was gradually reduced to as low as 4 mA. In six patients SPES was available at a high sampling rate of 2048 Hz and a hardware anti-aliasing filter of 538 Hz in a subset of 64 electrodes simultaneously (SD128, Micromed, Treviso, Italy). Subset selection was based on the monitoring result of previous days, and included the clinical SOZ. In eight patients SPES was sampled at 512 Hz (anti-aliasing filter 134 Hz) with a high dynamic range at 22 bits resolution, simultaneously in all implanted electrodes. In two patients both types of recordings were available. Data were recorded with respect to an extra-cranial reference. All recordings showed stimulus artifacts in most electrodes that needed to be dealt with. Electrodes with other artifacts were excluded from analysis.

TIME-FREQUENCY PROCESSING OF SPES

We used the same SPES datasets and a similar analytical approach as in our previous study on time-frequency analysis of evoked DRs[124]. The aim of the current study is time-frequency analysis of evoked ERs instead of evoked DRs. To enable analysis of ERs we made the following

methodological changes: 1.) the time-interval of interest was changed to <100 ms, 2.) time-frequency decomposition was based on Hilbert Huang Transformation instead of Wavelet transform in order to create a higher time resolution, and 3.) additional processing was required in order to obtain images similar to the ERSPs in the previous study[124]. Further details are provided in the following sections.

Preprocessing

Time-frequency (TF) analysis was done only on data sampled at 2048 Hz. Preprocessing of the data files was performed in Matlab® (The MathWorks, Natick, MA) as described in our previous study[124]. Preprocessing steps included: stimulus detection, epoching of the data and re-referencing to average reference. Re-referencing was performed in order to exclude contamination of the data by frequencies above 70 Hz, mostly muscle artifacts, which could be present in the extra-cranial common reference. Epochs with interval of [-1 s : 1 s] covering pre-stimulus baseline were selected. This resulted into ten epochs for each stimulated electrode pair and all recorded response electrodes. An additional steep low-pass finite impulse response filter with a cut-off frequency of 500 Hz (f_{stop}=520 Hz, f_{pass}=500 Hz, attenuation >60 dB/octave) was applied to limit any interference of higher frequencies.

Time-frequency decomposition

The Hilbert-Huang transform (HHT) was used to detect ERs in the proximity of the stimulus artifact. HHT allows time-frequency analysis with a high time resolution that prevents overlap of the artifact with the ER time-window <100 ms we are interested in[136]. The Hilbert-Huang transform provides a decomposition of the signal into a finite number of components. These so called modes are not directly related to a specific frequency band, but when combined they result in a coverage, albeit incomplete, of the time-frequency matrix. Frequencies not present in the modes are absent in this matrix and their power is automatically set to zero.

HHT was implemented in Matlab® (The MathWorks, Natick, MA) using a customized script that is freely available online (http://perso.ens-lyon.fr/patrick.flandrin/emd.html)[137]. Default values for stop criteria and number of iterations were used as described there. HHT time-frequency analysis (range 5-500 Hz) was performed for each epoch of each stimulus pair. The frequency and time resolution were set at 1 Hz and 0.488 ms. Each analysis resulted in a time-frequency matrix of 496 rows by 4096 columns.

Construction of time-frequency images

Color coded event related spectral perturbation (ERSP) images were constructed from the time-frequency matrix [116]. First, the data were smoothed using a 15x15 weighted Gaussian filter. ERSP images were then calculated by averaging each set of ten stimulus epochs, generating one ERSP image for each stimulus pair for each set of response electrodes (total # ERSP images= # pairs of stimulated electrodes x 64 recorded electrodes). Significance ($p<0.05$) of spectral perturbations was determined by bootstrapping based on a pre-stimulus baseline interval [-1 s: -0.2 s]. Intensity values were expressed in power on a logarithmic power scale [-20 dB: 20 dB]. Additional stimulus masking was performed, by constructing a template based on the symmetry of the HHT of the

stimulus artifact. The template was subtracted in each image to mask the artifact and enable better visualization of ERs. A schematic overview of the TF analysis can be found in figure 7.1A.

Visual analysis of ERSP images

ERSP images were visually classified for events in three frequency bands, spike (S) <80 Hz, ripple (R) 80-250 Hz and fast ripple (FR) band (250-500 Hz)[124]. The time interval of interest for ERs was defined as [0 s: 0.1 s] after stimulation, based on the latency definitions by Valentin et al. (2002)[21]. To avoid bias in interpretation, the DR interval [0.15-1 s] was removed from the final image. ERs were defined as clusters of increased power (coded orange-red) that stand out from the baseline for the same frequency band. Classification of the ER responses in all ERSP images for all stimulus electrode pairs was performed independently by two observers (chosen from CF/JH/GH/BM) for each patient. Inter-observer agreement was calculated by Cohen's kappa (κ) using SPSS 21 software (IBM SPSS Statistics, Rel. 21.0 2012, Chicago; SPSS Inc.). The κ-scores were calculated separately for the S, R and FR events. A $\kappa > 0.4$ was considered as reasonable agreement[124, 138]. Final counts of ERs in the spike, ripple and fast ripple band were based on consensus events of two observers. Datasets with $\kappa < 0.4$ for all frequency bands were excluded from analysis.

TIME-DOMAIN PROCESSING OF SPES

Pre-processing

Time-domain (TD) analysis was done only for data recorded with high dynamic range (22 bits). These recordings contain all implanted electrodes, and include both the SOZ and the seizure propagation area. Preprocessing consisted of stimulus detection and epoching as described above, but no re-referencing was performed. For TD-analysis, as opposed to TF-analysis, contamination of frequencies above 70 Hz present in the extra-cranial common reference does not pose a problem.

Artifact correction and event detection

An algorithm was developed to detect ERs in the time-domain consisting of two steps (Matlab®,The MathWorks, Natick, MA). Step 1 is removal of the stimulus artifact. This was based on Wiener filtering, assuming that the shape of the artifact is the same in all response electrodes for a given stimulus pair. A major determinant of the shape of the stimulus artifact in our data is cross-talk of the leads carrying the stimulus current to leads of recording channels in the same connecting cable. Therefore the stimulus artifact has generally the same shape, however, the amplitude may differ per electrode. First, a grand average over 10 pulses and over each response electrode was taken. Note that the large number of electrodes (X_0=N, which ranges 80-120 electrodes) provides that the number of electrodes without ERs exceeds the number of electrodes with ERs, implying that this average is dominated by artifact data. Therefore it was used as the first template to construct a Wiener filter, which, when applied, removed the main part of the stimulation artifact. Next, ER responses were detected automatically in the filtered data by a quick peak detection algorithm (PeakFinder, N. Yoder, Matlab file-central). A more accurate template artifact was then constructed by excluding electrodes with ERs (N-X_i) from a new grand average over the original

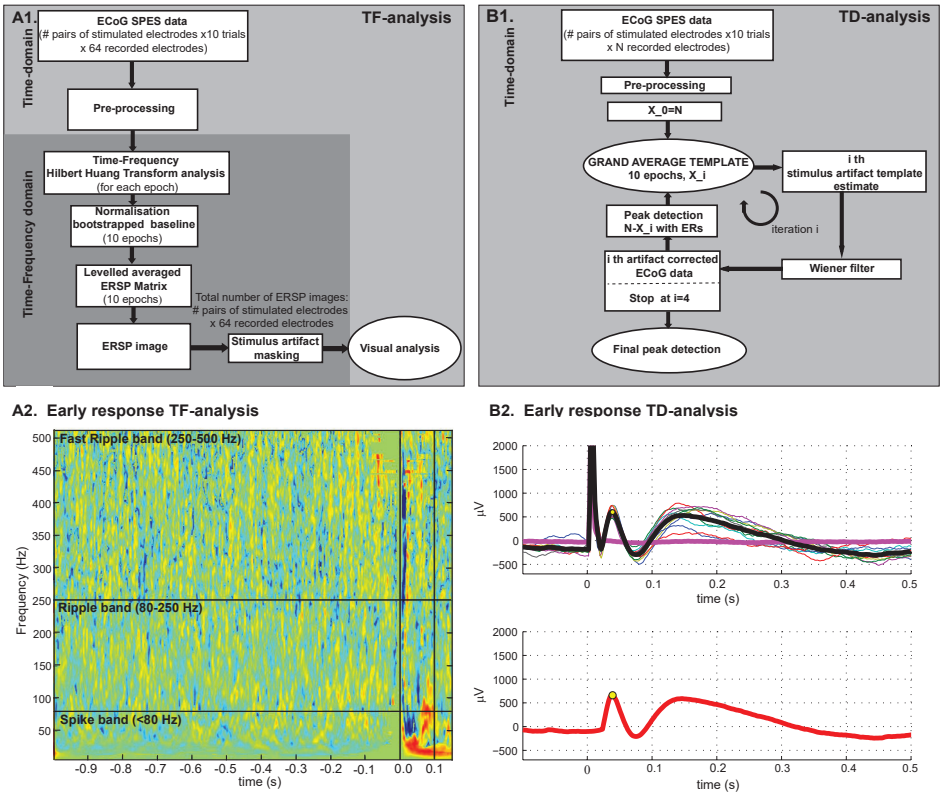

FIGURE 7.1
Schematic representation of two analyses performed. A1.) TF-analysis based on HHT constructed ERSP images. A2.) Resulting HHT based ERSP image of TF-analysis, as presented to the observers. Red colors indicate enhanced power compared to the pre-stimulus baseline on a power scale of [-20 : 20] dB. Intense red=20dB, green (not significant)=0 dB, and intense blue (decreased power) =-20 dB. The vertical axis represents frequency, with the horizontal lines separating the S, R and FR frequency band. The horizontal axis is time, with the stimulus at t=0. A pronounced spike and ripple event is shown around 50 ms after stimulation. B1.) TD-analysis based on iterative Wiener filtering and peak detection of ER responses. B2.) TD-analysis, thin lines are raw data for one electrode of responses evoked by 10 consecutive stimuli. The average is shown in black, the purple line shows the optimal Wiener filter estimate of the stimulus artifact. In the lower panel the artifact corrected average response (red) with the ER detected by the peak detection (yellow dot).

data. The new template was then again used for Wiener filtering. This process is repeated four times (iteration i=1:4) to further refine the template artifact. Step 2 of the algorithm is the final ER peak detection in the interval 0.02-0.1 s after stimulation. This interval was chosen to avoid potential bias for incomplete artifact correction that interferes with the onset of the ER. For final peak detection the same algorithm as mentioned above was used, but now with an adaptive amplitude threshold determined by visual inspection. The choice of amplitude threshold was made by favoring over-detection over under-detection; When visually clear responses were not adequately detected due to an inadequate threshold setting this was lowered by 10 μV steps.

A schematic overview of the TD-analysis can be found in figure 7.1B. ER response electrodes were those that exceeded the threshold for a particular stimulus electrode pair. For the TD- analysis of ERs and seizure propagation, stimulation of a single electrode pair was considered: one electrode was the electrode marked as SO-electrode (see section clinical information) and the other a neighboring electrode that was located on the same gyrus. In cases of ambiguity, the stimulation pair that showed the largest total number of responses was chosen.

STATISTICAL ANALYSIS

Statistical analysis was performed in IBM SPSS Statistics 21 (IBM Corp., Armonk, NY, USA). For all tests we considered p-values <0.05 significant. TF-analyses were done for the spike, ripple and fast ripple band separately. The following analyses were done for TF and TD, when appropriate:

Early response counts

For the TF-analysis we counted the number of ERs per electrode (ER_{count}) for the total of all stimulus pairs. We normalized the number of ERs in each response electrode with respect to the maximal count (ER_{max}) found, expressed as percentage:

$$ER_{norm} = \frac{ER_{count}}{ER_{max}} * 100\% \qquad \text{(Eq 7.1)}$$

We then defined electrodes with a high occurrence of ERs, ER_{norm} >50%, as ER_{50} electrodes. So while the detection of a single ER in a particular band reflects the excitability of the underlying tissue with respect to the stimulus, high values of ER_{norm} will reflect the richness of connections to that electrode.

Association of ER counts with SOZ and ERs with propagation

We tested for differences in the value of ER_{norm} between SOZ and non-SOZ channels using a non-parametric Mann-Whitney U test (two-tailed). Differences between association of ER_{50} electrodes with SOZ and with non-SOZ channels was assessed using Fisher's exact test (two-tailed) (TF-analysis). We tested for differences in association between ERs, following stimulation of SO-electrode, in SP-electrodes and non-SP-electrodes using Fisher's exact test (two-tailed) (TD-analysis).

Sensitivity and specificity of ERs

To further quantify results, sensitivity, specificity, positive and negative predictive value (PPV and NPV) of ER_{50} for the clinical SOZ (TF-analysis) and ER for seizure propagation (TD-analysis) was determined. Sensitivity was calculated as tp/(tp+fn), specificity as tn/(tn+fp), PPV as (tp/tp+fp) and NPV as (tn/tn+fn).

For TF-analysis an electrode was considered as:

- a true positive electrode (tp) if involved in the SOZ and classified as ER_{50} electrode,
- a false positive electrode (fp) if NOT involved in the SOZ but classified as ER_{50} electrode,
- a true negative electrode (tn) if NOT involved in the SOZ and NOT classified as ER_{50} electrode, and
- a false negative electrode (fn) if involved in the SOZ but NOT classified as ER_{50} electrode.

For TD-analysis an electrode was considered as:

- a true positive electrode (tp) if marked as SP-electrode and showing ERs,
- a false positive electrode (fp) if NOT marked as SP-electrode but showing ERs,
- a true negative electrode (tn) if NOT marked as SP-electrode and NOT showing ERs, and
- a false negative electrode (fn) if marked as SP-electrode but NOT showing ERs.

Cross-check TD- and TF-analysis

Finally, we performed a cross-check of the ER results found by the TD- and TF-analysis in the patients for which SPES data of both types, high temporal resolution data and high dynamic range, were available. This includes: a.) association between ERs marked in the spike band in TF-analysis with SP-electrodes when stimulating the SO-electrode, b.) calculation of the ER_{50} electrodes for the total number of stimulus pairs of the TD data, and determining their association with the clinical SOZ electrodes. We computed sensitivity, specificity, PPV and NPV for seizure propagation and seizure onset zone respectively.

RESULTS

OVERALL PATIENT RESULTS

For six patients (pt 1-6) SPES data were sampled at 2048 Hz in 64 channels allowing the TF-analysis and association of ER counts with SOZ for different frequency bands. For eight patients (pt 1, 2, 7-12) data were recorded in up to 120 channels recorded at high dynamic range (22 bits) that allowed the TD-analysis and association of ERs with seizure propagation. In two patients (pt 1, 2) both SPES data types were available.

For the six patients recorded at 2048 Hz the mean number of analyzed channels was 62 (±2). The median number of stimulated electrode pairs was 46 (range 16-55). The median number of electrodes in the SOZ was 5 (range 2-30). For the eight patients recorded at 22 bits a mean of 92 (±13) channels was analyzed. All patients had seizures with a focal gamma onset. For each patient a SO-electrode was marked. For detailed patient characteristics we refer to table 7.1.

TABLE 7.1
Patient characteristics

Pt	TD/TF	M/F	Age (y)	Region	Side	Pathology	Total # electrodes	Monitoring period (days)	# seizures (onset activity, types)	SOZ resected?	Outcome (1y) Engel class
1	TD+TF	M	42	T	L	MTS	96	7	2 (focal gamma onset, 1 type)	complete	I
2*	TD+TF	F	23	T	L	Glioneural heterotopia	96	6	6 (gamma burst onset, 1type)	complete	I
3	TF	M	9	Fr	L	FCD	96 (16 depth)	5	12 (focal beta-gamma onset,1 type)	incomplete	IV
4	TF	F	31	T	R	MCD	88	8	1 (gamma onset)	complete	I
5	TF	F	13	FrC	L&R	TS	96	8	6 (gamma burst onset, 1 type)	incomplete (multiple tubers bilateral)	IV
6	TF	M	8	FrC	L	Tumor	96	5	>100 (gamma onset, 1 type)	complete	I
7	TD	F	17	Fr	R	FCD	120	3	6 (gamma onset, 1 type)	complete	I
8	TD	M	8	P	R	FCD	94 (6 depth)	5	1 (gamma onset,1 type)	incomplete (eloquent regions)	I
9	TD	F	18	Fr	L	Reactive changes	112	7	19 (gamma onset, 1 type)	complete	I
10	TD	F	15	P	L	FCD	96	5	3 (gamma onset, 1 type)	incomplete	IV
11	TD	M	26	T	L	MTS	120	6	6 (gamma onset, 1 type)	complete	I
12**	TD	F	27	T	L	MCD	96	6	4 (gamma onset, 2 types)	incomplete	IV

TD/TF= analysis performed; TD/TF= analysis performed; TD=time domain analysis, TF=time-frequency analysis, F=Female, M = Male, Fr = Frontal, T=Temporal, C=Central, FrC=Fronto-Central, P=Parietal L = Left, R=Right, TS=Tuberous Sclerosis, MCD=Malformation of Cortical Development, FCD=Focal Cortical Dysplasia, MTS=Mesial Temporal Sclerosis, # seizures= number of spontaneous clinical seizures, Outcome (1y)= post-operative outcome after 1 year using the Engel classification. *Patient 2 had a number of different electrodes on a single strip from which the clinically identical seizures originated. We choose one of these electrodes as SO-electrode, this could explain the poorer results in this patient compared the other patients. **In patient 12 two separate onset zones were found. Only one seizure onset zone could be resected, therefore we selected a seizure of that type.

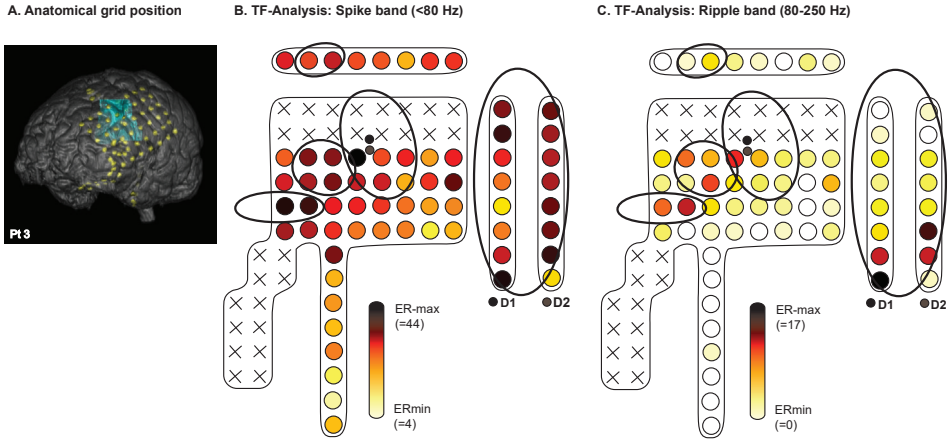

FIGURE 7.2
Patient example (pt 3) of ER_{norm} distribution. A.) MRI and CT merged images depicting the anatomical grid positions. B.) ER_{norm} distribution in the spike band (<80 Hz) and relation with the SOZ (encircled areas). C.) ER_{norm} distribution in the ripple band (80-250Hz) and relation with the SOZ. Note: D1 and D2 are depth electrodes.

TF- AND TD-ANALYSIS

In figure 7.1A a TF-domain ERSP image based on HHT is shown for one response electrode as presented to the observers. In figure 7.1B, the TD-analysis of ERs for one electrode of ten responses to consecutive stimuli is shown. ER responses were found in all patients, irrespective of the used analysis method.

ER findings and association with SOZ and propagation

TF-analysis for the six patients resulted in a kappa ≥0.4 in five patients (pt 1-4, and 6) for the spike band, in six patients (pt 1-6) for the ripple band but in only one patient (pt 2) in the FR band(four ERs in the FR-band on three electrodes), therefore the FR-band was excluded from further analysis. In figure 2 an example of the ER_{norm} distribution in relation to the clinical SOZ is represented. Note that the SOZ is characterized by high values of ER_{norm} (figure 7.2).

In three out of five patients with k>0.4 (pt 1, 3 and 4) there was a significant difference between ER_{norm} counts in- and outside the SOZ ($p<0.05$, Mann-Whitney U) for the S-band and in three out of five patients (pt 1, 3 and 5) for the R-band ($p<0.05$, Mann-Whitney U). See figure 7.3.

Similar results were found at individual patient level for electrodes marked as ER_{50} electrodes. ER_{50} electrodes were significantly associated with SOZ electrodes in the spike band in two patients (pt 1and 3; $p<0.05$, Fisher exact) and in three patients in the ripple band (pt 1, 3 and 5; $p<0.05$, Fisher exact). At group level the association of ER_{50} and the SOZ was significant for both the spike and ripple band ($p<0.05$, Fisher exact) (table 7.2).

In figure 7.4 an example is given for an individual patient (pt 1) of ERs detected using the TD-method when stimulated in the SO-electrodes. Note that ERs are mostly present in SP-electrodes. ERs detected when stimulating in SO-electrodes were significantly associated with SP-electrodes in four patients (pt 8, 9, 10 and 12; $p<0.05$, Fisher exact), and at group level ($p<0.05$, Fisher exact) (table 7.3).

TABLE 7.2
Results of ER analysis in time-frequency (TF) domain and their relation with seizure onset

			SOZ (ER_{50})									
			Sens (%)		Spec (%)		PPV (%)		NPV (%)		p-value*	
#Pt	TF/TD	# elec	S-band	R-band	S-band	R-band	S-band	R-band	S-band	R-band	S-band	R-band
1	TF	61	100	75	56	95	14	50	100	98	***0.046***	***0.002***
2	TF	62	83	50	18	63	10	13	91	92	1.000	0.661
3	TF	64	87	23	56	100	63	100	83	60	***0.001***	***0.003***
4	TF	58	100	33	47	94	9	25	100	96	0.245	0.195
5	TF	64	x	55	X	83	x	40	x	90	x	***0.015***
6	TF	64	50	0	42	87	3	0	96	96	1.000	1.000
mean(±SD)/ median (range)		62 (±2)	87 (50-100)	42 (0-75)	44 (17-57)	91 (64-100)	9 (3-63)	33 (0-100)	96 (83-100)	95 (60-98)	***<0.001***	***<0.001***

Pt=patient, TF=Time-Frequency analysis, TD=Time–Domain analysis, # elec= the number of electrodes included in the analysis, Sens=sensitivity, Spec= specificity, PPV=positive predictive value, NPV=negative predictive value, S-band= spike band (<80Hz), R-band=ripple band (80-250 Hz), SD=standard deviation. *Tested for association ER_{50} with SOZ, using Fisher exact test (two tailed), with $p<0.05$ considered significant. Note: Results for the FR-band in TF-analysis are not reported because of the single finding in only one patient (pt 2).

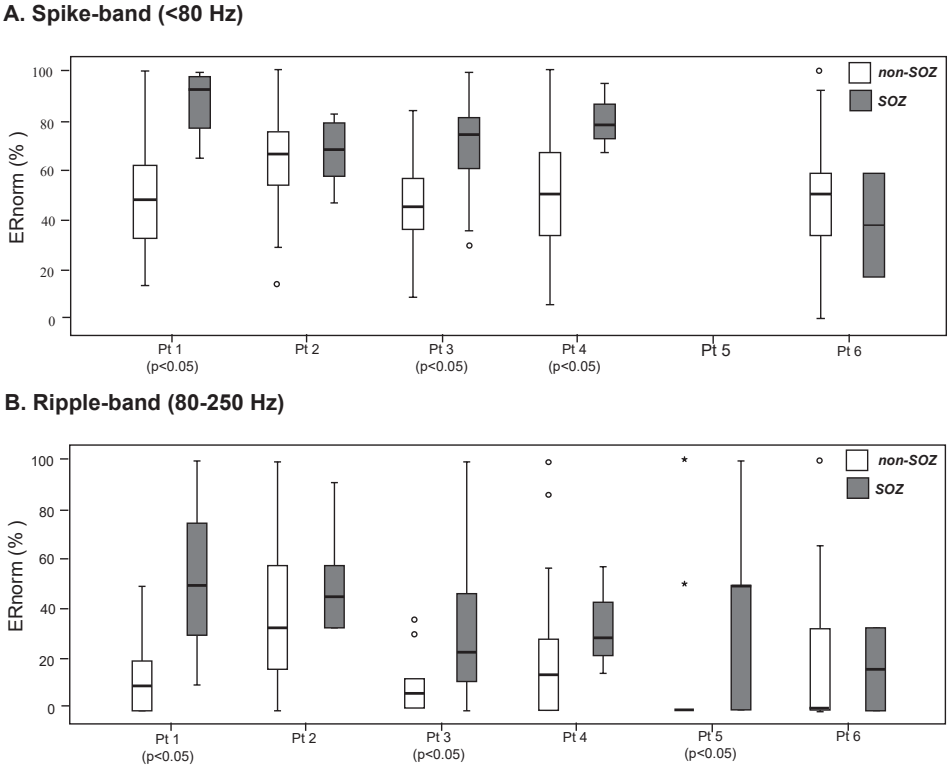

FIGURE 7.3
Boxplots for difference in ER_{norm} between channels in and outside SOZ for A.) the spike band (<80 Hz); three patients (pt 1, 3 and 4) had a significant higher ER_{norm} in the clinical SOZ., B.) the ripple band (80-250 Hz); In the ripple band a significant higher ER_{norm} in the clinical SOZ was found for three patients (pt 1, 3 and 5).

Sensitivity and specificity of ER counts for SOZ and ERs for propagation

In the TF-analysis group of six patients, we found a median sensitivity and specificity of ER_{50} in the spike band for the SOZ of 87% and 44% respectively. For the ripple band this sensitivity and specificity was 42% versus 91% (table 7.2). Median sensitivity and specificity of ERs for the SP-electrodes was 32% and 94% respectively in the TD-analysis group of eight patients (table 7.3).

Cross-check TD- and TF-analysis

In two patients (pt 1 and 2) we compared the results of both TD- and TF-analysis. Note that comparisons were for different datasets of the same patients. We found that:

- Association of evoked ERs with seizure propagation is significant for ER detected by TD-analysis but not for ERs detected in the spike band by TF-analysis in patient 1 (p_{TD}=0.011 vs. p_{TF}=0.435, Fisher exact). The opposite was found for the relation between evoked ERs counts and the SOZ; a significant association between ER_{50} and the SOZ was found by TF-analysis but not in TD-analysis (p_{TF}=0.046 vs. p_{TD}=1.000, Fisher exact). Results for patient 2 were not significant

(propagation: p_{TD}=0.103 vs. p_{TF}=0.263, Fisher exact; SOZ: p_{TF}=1.00 vs. p_{TD}=0.509, Fisher exact). TD- and TF-analysis showed similar, low, sensitivity of ER responses for propagation (range: 24-42%).

- ERs detected by TD analysis had a higher specificity for SP-electrodes than ERs detected by TF-analysis (figure 7.5).
- Sensitivity values of ER_{50} for the clinical SOZ were lower in both patients for TD- (47% and 43%) compared to TF-analysis (100% and 83%) . Specificity values were comparable in patient 1 (54% vs. 56%), but higher for the TD-analysis in patient 2 (66% vs. 18%) (Supplementary table 7.1).

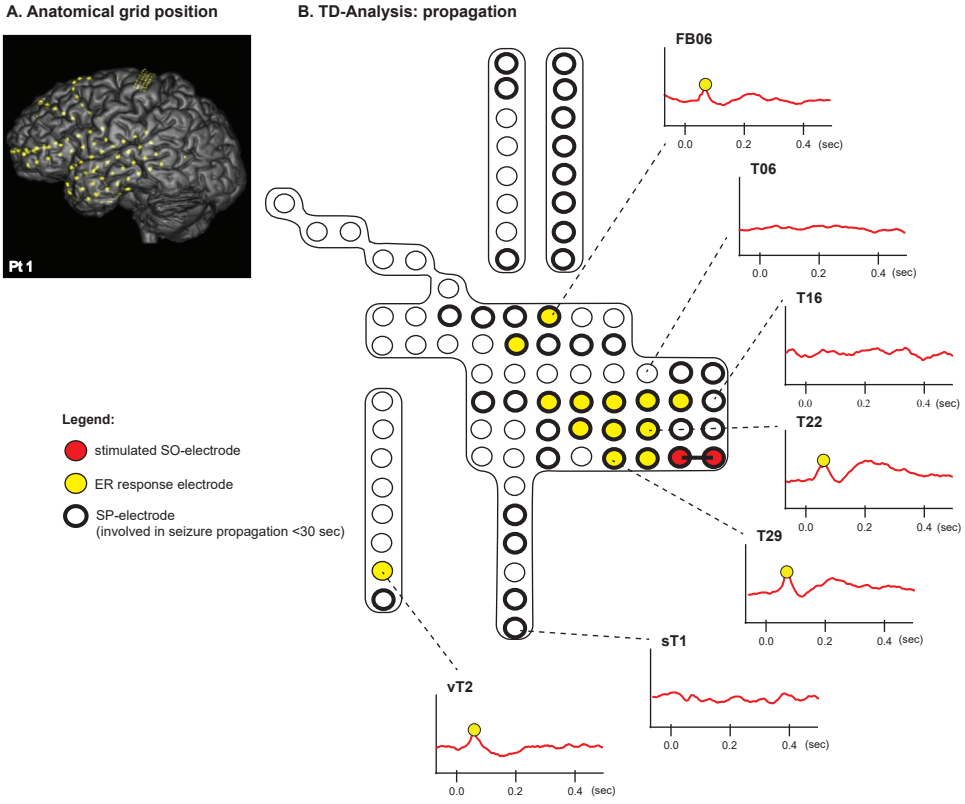

FIGURE 7.4
Patient example (patient 1) of ER response electrodes for stimulation in the SO-electrode (TD-analysis) and the correlation with propagation (SP-electrodes). Included are examples of the ER waveforms in selected electrodes. Sensitivity here is 42%, specificity is 86%, and PPV and NPV are 40% and 87% respectively.

Table 7.3
Results ER analysis in time-domain (TD) and relation with seizure propagation

		Propagation								
#Pt	**TD/ TF**	**# Elec**	**Threshold (μV)**	**Mean latency (ms)**	**Mean amplitude (uV)**	**Sens (%)**	**Spec (%)**	**PPV (%)**	**NPV (%)**	**p-value***
1	TD	102	120	46	301	42	86	40	87	***0.011***
2	TD	91	120	64	378	40	77	52	67	0.103
7	TD	88	90	35	262	24	93	44	84	0.066
8	TD	86	140	47	355	22	98	89	64	***0.003***
9	TD	77	120	44	443	60	84	47	90	***0.001***
10	TD	94	120	59	299	36	100	100	72	***0.000***
11	TD	118	140	44	286	19	94	55	76	0.067
12	TD	82	130	39	206	28	97	92	55	***0.002***
mean (±SD)/ median (range)		92 (±13)	123 (±16)	47 (±10)	316 (±73)	32 (19-60)	94 (77-100)	54 (40-100)	74 (55-90)	***<0.001***

Pt=patient, TD=Time–Domain analysis, TF=Time-Frequency analysis, # Elec= number of electrodes included in analysis, Threshold=amplitude threshold used (see methods section 2.6.2, step 2), Mean amplitude= mean amplitude of ER response (baseline to peak), Sens=sensitivity, Spec= specificity, PPV=positive predictive value, NPV=negative predictive value, S-band= spike band (<80Hz), R-band=ripple band (80-250 Hz), SD=standard deviation, * Tested for association ER response electrodes with SP-electrodes, using Fisher Exact test (two-tailed), with $p < 0.05$ considered significant.

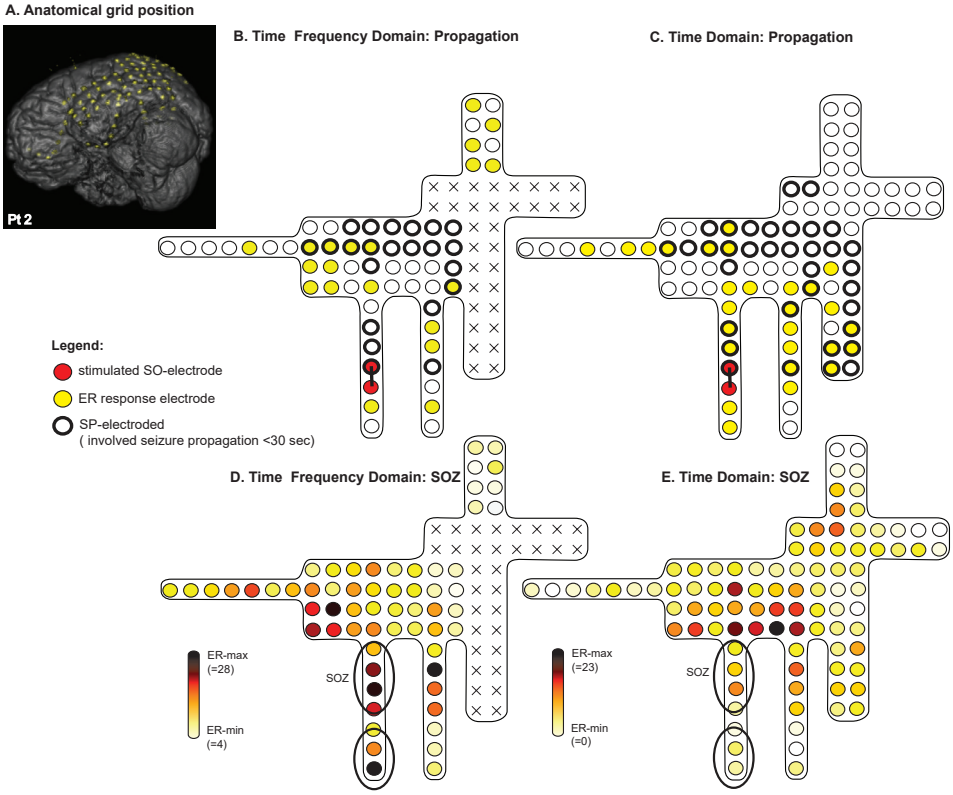

FIGURE 7.5
Results of the cross-check of TF- and TD-analysis in one of the two patients (patient 2). A.) MRI and CT merged images depicting the anatomical grid positions. B & C.) TD- and TF-analysis show similar, low, sensitivity of ER responses for propagation. ERs detected by TD-analysis had a higher specificity for SP-electrodes than ERcounts detected by TF-analysis. D & E.) Sensitivity of ER_{norm} for the clinical SOZ (encircled areas) are comparable for TD- and TF-analysis, but specificity is higher for the TD- analysis.

DISCUSSION

The SOZ is more likely to be located in areas showing high counts of ERs evoked by SPES. ERs evoked by stimulation in seizure onset electrodes are more likely to occur in electrodes that show seizure propagation. ERs in the ripple band (80-250 Hz) exist and electrodes with high ER counts in the ripple band have a high specificity for SOZ channels. So, the analysis of ERs evoked by SPES can reveal aspects of pathology, even if the underlying stimulus-response relation is purely physiological. ERs, besides DRs, could assist in unraveling aspects of the epileptic network.
Based on our results, we cannot suggest a preferred general method of analysis for SPES early responses, as each method has its pro's and con's. For the detection of high frequency content time-frequency analysis is necessary, but results for the cross-check patients show that the TF-method lacks specificity in the detection of lower frequency responses resulting from a single

stimulation site like the SOZ. TD-analysis of SPES yields robust responses with high specificity, but lacks the sensitivity needed to extract useful clinical information about the SOZ from the overall statistics of early responses.

METHODOLOGICAL ASPECTS

We showed that SPES evokes ERs [0.0 s - 0.1 s] with frequency features above the traditional 80 Hz. We used a HHT time-frequency analysis instead of a wavelet analysis as used in our previous study[124]. A strength of time-frequency analysis is that it allows averaging in the frequency domain while retaining time information. The time resolution of wavelet analysis proved to be sufficient for DRs, since their relevant time interval lies between 0.15 s and 1.0 s after stimulation, and interference of the stimulus artifact is not an issue. The advantage of HHT is that it enables the detection of early ripple responses at a high time resolution, close to the stimulus artifact. HHT was successfully used in an earlier study by Kalitzin et al. (2012) to determine 'rippleness' of a signal[139].

We are the first to find that SPES, with its low number of stimuli and low repetition rate, evokes ERs that can be associated with areas of seizure propagation, when stimulating the SOZ. The artifact removal algorithm applied in the time domain allowed us to detect ERs in a semi-automatic way, in spite of the low signal to noise ratio of an average of only ten stimuli.

We were able to show an association between overall counts of ERs (ER_{norm}) and the SOZ at group level, and at individual level in four out of six patients. This finding seems to contradict the general finding of Valentin and coworkers (2002, 2005) that early responses cannot localize epileptogenic cortex. In contrast to the studies of Valentin at al., in our study we count how often an electrode shows an ER response to stimulation throughout the SPES protocol and we relate this measure for the richness of connections to the underlying area to the SOZ[21-23].

Does this mean that we can propose ER analysis as a clinical tool? When it comes to predicting seizure propagation it should be noted that clinically identical seizures in semiology can originate from different foci. Our study is limited by the fact that we studied only one seizure per patient and looked at the resulting ERs when stimulating the corresponding SO-electrode. Including more data or more seizures per patient would have increased the statistical robustness and thus clinical usefulness of our results.

When it comes to predicting the SOZ the sensitivity and specificity, based on ER50, are insufficient for reliable prediction of the SOZ in individual patients. When we thresholded the ER_{norm} values to obtain ER_{50} electrodes, the sensitivity for the SOZ was relatively high (87%) for the spike band, but low for the ripple band (42%). Conversely, specificity is low (44%) for the spike band and high (91%) for the ripple band. Nevertheless information derived from ER counts can be added to that of pathological delayed responses, that have a high specificity and sensitivity[21-23, 124] and can be established during the same SPES session.

Unlike ripples, fast ripples are described as primary pathological events[18, 20, 98, 140, 141]. We found only ERs in the fast ripple band in one patient. This low number of ERs in the fast ripple band cannot refute the assumption that ERs are purely physiological[21, 129, 142], although the lack of fast ripples could be explained by the fact that HHT is less suitable for the noisy high frequency content above 250 Hz[136]. Hardware requirements and the retrospective nature of this study resulted in

small patient groups. Data suitable for both TF- and TD-analysis were not available. Acquiring high frequency data at 2048 Hz meant a sacrifice of recorded ECoG channels to a maximum of 64. The choice of channels to retain was made during the clinical registration and was based on information about the SOZ then available. Capturing seizure propagation over the grid up to 30 sec after onset was not considered when the selection of the 64 electrodes for 2048 Hz recordings was made. Interpretation of the comparison between the TF- and TD-based method is therefore hampered if propagation took place in sacrificed channels for the cross-check patients (see figure 7.5). Additionally, incomplete ECoG coverage of the cortex limits interpretation of results of TF- and TD-based methods in general.

All patients in the TD-analysis showed propagation of both the seizure and the ER responses, not only local but also remote from the SOZ, several sulci and gyri away. We observed that seizures often showed substantial secondary propagation around the site they were initially propagated to. For ERs this was less often the case (see figure 7.4 and figure 7.5D; in the supplementary material a video of an example where secondary ERs do occur is provided). As a result, sensitivity values of ERs for SP-electrodes are relatively low. Probably seizure activity produces more massive secondary activation of connecting fibers than SPES stimulation does. It could be worthwhile exploring local responses to additional SPES stimulation in the electrode where earliest ERs appear when stimulated in the SOZ.

Another limitation is that SPES recordings were not performed in a drug-free state for most patients, although AEDs were tapered during the ECoG monitoring session. Since AEDs may contain the spread of ERs across the cortical mantle, medication may have lowered the sensitivity of both the time-domain and the time-frequency domain method. Both TD and TF approaches rely pre-dominantly on computational signal processing. These methods are, however, not fully automated as they still involve identification by a trained observer as a last step.

RELATION WITH FINDINGS IN LITERATURE

SPES relates to the CCEP stimulation protocol. In Enatsu et al. (2012) CCEPs were used to investigate the relation between evoked cortical responses and seizure propagation. Their CCEP protocol uses more stimuli (50-70) at a higher repetition rate (1 Hz) with pulses of shorter duration (0.3 ms), but an amplitude comparable to SPES (1-15 mA)[127]. Effectively, the injected charge would be equal to using 0.3 - 4.5 mA in SPES. Although this is lower (~50%), the signal to noise ratio for CCEPs is probably higher than for SPES, given the substantially higher amount of averages. Enatsu et al. (2012) found no robust relation between seizure propagation and evoked responses. As in most CCEP studies, focus lied on amplitude and latency rather than occurrence and count of evoked responses. They found no significant difference between amplitude in electrodes with CCEPs and seizure propagation, compared to electrodes where only CCEPs without seizure propagation were observed[127]. Their conclusion that ictal propagation is not necessarily associated with functional connectivity is in contrast to our finding of a high specificity. An explanation could be that the lower SNR of SPES responses results in fewer detections, so fewer false positives with respect to seizure propagation compared to CCEP. On the other hand, the more powerful SPES stimulus current could ensure that a SPES response is more likely a true than a false positive with respect to seizure propagation, compared to CCEP.

On the matter of identification of the SOZ the CCEP literature shows a clear relation between enhanced CCEP response amplitudes when stimulation is inside the SOZ (called iCCEPs) compared to those when stimulating outside the SOZ (called nCCEPs)[127, 131, 132]. There have been no reports on the association of the location of CCEP responses with the SOZ, independent of stimulation side. CCEP studies are characterized by a directional, functional connectivity analysis, whereas our SPES-study is characterized by a cumulative response analysis, rather the sum of all iCCEPs and nCCEPs. The CCEP directionality approach could be of interest in future SPES studies on delayed responses. Valentin et al. (2002, 2005), e.g., showed that a focus in the temporal lobe is represented by tissue showing evoked delayed responses to a stimulus elsewhere, while in the frontal lobe stimulating the focus results in evoked delayed responses elsewhere[21-23].
Recently, Boido et al. (2014) looked specifically at directionality, using a different CCEP protocol (30 pulses at 1 Hz , 2 ms, 4 mA; delivered in depth electrodes (SEEG) with 1.5 mm separation). Note that here current density values exceed those of the CCEP studies mentioned earlier, and also of SPES[143]. They defined electrodes as primarily receivers, activators or bidirectional contacts. Their receivers resemble roughly the ER_{50} electrodes in our study. They did not find, however, an association between primary receiver electrodes and SOZ. Their activators did not show an association with SOZ, nor seizure propagation. However, bidirectional connectivity was a prevalent feature for contacts included in the epileptogenic focus[143]. The large differences in stimulus parameters and detection thresholds hamper interpretations of the mismatch between their and our results. Therefore, studies that combine CCEP and SPES protocols and analysis methods will be important to better understand the underlying physiological mechanisms of the responses each protocol evokes. Evaluating both CCEP and SPES early and delayed responses, including directionality, in the same epilepsy patient could increase the clinical yield of intracranial electrical stimulation.

CONCLUSIONS

To conclude, we found that analysis of ERs evoked by SPES reveals information about the pathology, manifested by the localization of the SOZ in areas of high ER counts and by the ability of ERs to predict seizure propagation. ERs information could be added to that of DRs to improve pre-operative mapping of epileptogenic cortex and the epileptic network. Larger studies, including prospective studies, are needed to establish the full clinical potential of SPES.

SUPPLEMENTS

SUPPLEMENTARY VIDEO 7.1
Video showing single pulse early responses in pt 12. ECoG Grid coverage is left temporal frontal, with additional strips covering parts of the frontal, central and parietal regions. Time range is from 100 ms before to 500 ms after stimulation. Stimulus is at t=0 when black flash appears in the video. Stimulus electrodes are near Wernicke's area, indicated by the neighboring black (+) and white (-) squares. Response amplitude is coded from white (-300 μV) to black (+300 μV). Note early local propagation near the stimulating electrodes, followed by propagation to Broca's area, in the middle of the display. There, responses show addition local spread propagation.

SUPPLEMENTARY TABLE 7.1
Results cross-check time-domain (TD) and time-frequency (TF)-analysis

		Propagation									SOZ (ER_{50})				
#Pt	TF/TD	# Elec	Thresh (µV)	Mean latency (ms)	Mean ampl (uV)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	p-value*	Sens (%)	Spec (%)	PPV (%)	NPV (%)	p-value**
1	TD	102	120	46	301	42	86	40	87	***0.011***	47	54	54	47	1.000
	TF	61	_	_	_	38	50	46	41	0.435	100	56	14	100	***0.046***
2	TD	91	120	64	378	40	77	52	67	0.103	43	66	43	66	0.509
	TF	62	_	_	_	24	52	25	41	0.263	83	18	10	91	1.000

Pt=patient, TD=Time–Domain analysis, TF=Time-Frequency analysis, # Elec= number of electrodes included in the analysis, Thresh=amplitude threshold used (see methods section 2.6.2, step 2), Mean latency= mean time of occurrence after stimulus, Mean ampl= mean amplitude of ER response (baseline to peak), Sens=sensitivity, Spec= specificity, PPV=positive predictive value, NPV=negative predictive value, S-band= spike band (<80Hz), R-band=ripple band (80-250 Hz) *Tested for association ER response electrodes with SP-electrodes, using Fisher Exact test (two-tailed), with $p < 0.05$ considered significant. ** Tested for association ER_{50} with SOZ, using Fisher exact test (two tailed), with $p<0.05$ considered significant. Note: Results are only reported for the S-band because of the limited sampling rate of the TD data.

CHAPTER 8

EVOKED VERSUS SPONTANEOUS HIGH FREQUENCY OSCILLATIONS IN THE ELECTROCORTICOGRAM IN FOCAL EPILEPSY

Submitted.

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ABSTRACT

INTRODUCTION

Spontaneous high frequency oscillations (HFOs; ripples 80-250 Hz, fast ripples (FRs) 250-500 Hz) are biomarkers for epileptogenic tissue in the electrocorticogram (ECoG) in focal epilepsy. HFOs can be evoked with single pulse electrical stimulation (SPES). We hypothesized that stimulation distinguishes pathological from physiological ripples and compared the occurrence of evoked and spontaneous HFOs within the seizure onset zone (SOZ) and eloquent functional areas.

METHODS

Ten patients underwent SPES during 2048 Hz ECoG. Evoked HFOs in time-frequency plots and spontaneous HFOs were visually analyzed. We compared the percentage, sensitivity and specificity between evoked and spontaneous HFOs in the SOZ and functional areas.

RESULTS

Two patients without spontaneous FRs showed evoked FRs in the SOZ. Percentages of evoked and spontaneous HFOs in the SOZ were similar (ripples 32-33%, p=0.77; FRs 43-48%, p=0.63), but evoked HFOs had generally a lower specificity and higher sensitivity for the SOZ-channels. More electrodes with evoked than spontaneous ripples were found in functionally eloquent (54-30%, p=0.028) and 'silent' areas (57-27%, p=0.011) outside the SOZ.

CONCLUSION

SPES can elicit SOZ-specific FRs in patients without spontaneous FRs, but activates ripples in all areas. SPES is an alternative for waiting for spontaneous HFOs, but does not warrant exclusively pathological ripples.

INTRODUCTION

Epilepsy is one of the most common neurological disorders, with a prevalence of 4–10 per 1000 persons per year and an incidence of 50–70 per 100.000 persons per year[4]. One third of people with epilepsy suffer from refractory epilepsy, meaning that their seizures are not sufficiently controlled with the use of, often multiple, anti-epileptic drugs (AEDs). In people with focal epilepsy, surgery is a curative alternative[8]. Delineation of the margins of the epileptic focus is of great importance for the success of the surgery, as well as mapping out neighboring eloquent regions to refrain from harming neurological function. Long-term intra-cranial electrocorticography (ECoG) monitoring is performed to ensure optimal mapping of both regions before surgery[144, 145].

The location of the patient's seizure onset EEG rhythm (seizure onset zone, SOZ) is considered the gold standard for the epileptic focus in the long-term ECoG, but waiting for seizures can prolong the pre-surgical monitoring period, causing stress and increased complication risks[8, 12]. Inter-ictal epileptiform activity can be used as a surrogate marker to define the region of cortex that should minimally be removed to render the patient seizure-free, the so-called epileptogenic zone, which includes the SOZ[1]. Inter-ictal high frequency oscillations (HFOs, >80-500 Hz), visible when the ECoG is recorded at >2000 Hz sampling rate, have been shown to be better inter-ictal predictors of the SOZ than epileptiform spikes[18]. HFOs can be subdivided into ripples (80-250 Hz) and fast ripples (FRs, 250-500 Hz). Spontaneous FRs are presumed to be truly pathological and highly specific for the epileptogenic zone as removal of tissue generating them is associated with seizure freedom[20, 79]. FRs are thought to be generated by small patches of synchronized clusters of hyper-connected neurons, and are considered a relatively focal phenomenon, which can be easily missed[80, 85]. Ripples occur more frequently than FRs and are seen in the majority of patients. Unfortunately spontaneous ripples are found over larger areas, so their clinical value is debated[18, 79, 87]. They occur both pathologically and physiologically, as they are found in healthy animal models and are associated with mesio-temporal memory consolidation[146] and occipital visual processing in humans[37]. Post-resection ripples were found in the non-resected sensorimotor cortex during intra-operative recordings in patients who achieved seizure freedom[67]. This lack of sensitivity for FRs and lack of specificity for ripples hampers the clinical implementation of HFOs in epilepsy surgery practice.

We previously showed that HFOs can be evoked by single pulse electrical stimulation (SPES)[124]. The SPES protocol consists of runs of 10 brief (1 ms) pulses stimulating over neighboring pairs of all electrodes. Delayed evoked responses occurring about >100 -1000 ms after stimulation resemble inter-ictal epileptiform discharges[24] and are considered pathological[21-23]. These delayed responses also contain evoked HFOs, that are specific for the SOZ[124]. It is unknown how evoked HFOs relate to spontaneous occurring HFOs. SPES could help to distinguish pathological from physiological ripples. We hypothesized that delayed responses to SPES are particularly provoked in the epileptogenic network, with the advantage that its stimulus-locked ripples would be truly pathological. This way SPES could provide a valid tool to obtain on demand information about the SOZ, instead of waiting for spontaneous events - be it seizures or inter-ictal events - , to shorten the monitoring period or for use during intra-operative ECoG tailored epilepsy surgery. We wanted

to compare inter-ictal spontaneous HFOs to evoked HFOs, especially ripples, with respect to their ability to correctly identify the SOZ and their occurrence in functionally eloquent areas.

METHODS

PATIENTS

We selected patients with focal refractory epilepsy who underwent long-term subdural ECoG monitoring to determine the SOZ preceding epilepsy surgery of whom SPES results, recorded at 2048 Hz sampling rate, were analyzed in a previous study[124]. We excluded patient in whom no SOZ was found or no spontaneous ECoG data was available at 2048 Hz. Patients were admitted for 5-7 days to the Intensive Epilepsy Monitoring Unit of the University Medical Center of Utrecht, The Netherlands, between 2008 and 2010. During these days, seizures were recorded, the SPES protocol was performed, and functional areas were identified with 50 Hz direct cortical stimulation. The conventional visually analyzed SPES results[21] were available during clinical decision making[21], but HFOs, neither spontaneous nor evoked, were not. Patients were on (multiple) antiepileptic drugs that were tapered throughout the monitoring period.
Data were retrospectively collected and handled coded and anonymously according to the guidelines of the institutional ethical committee.

ELECTROCORTICOGRAPHY

Chronic electrocorticography was performed with subdural electrodes that were placed directly on the cortex. Subdural grids and strips consisted of platinum circular electrodes embedded in silicone, had a 4.2 mm^2 contact surface, an inter-electrode distance of 1 cm. In one patient, also depth electrodes were implanted consisting of eight cylindrical contacts with 7.9 mm^2 contact surface and a 5 mm inter-electrode distance (Ad-Tech, Racine, WI, USA). Multiple subdural grids and strips were implanted making sure to cover the brain areas suspected for epileptic activity or eloquent areas in the affected hemisphere.
ECoG recordings required a high sample rate of 2048 Hz (hardware anti-aliasing filter of 538 Hz) in order to capture HFOs, which at the time meant that a maximum of 64 channels could be recorded by our EEG-system (MicroMed, Veneto, Italy). A sub-selection of electrodes was made that targeted the clinical seizure onset zone as found during earlier recording days.

SINGLE PULSE DATA ACQUISITION

SPES was performed using a manually controlled cortical stimulator (IRES 600, Micromed). Ten monophasic single pulse stimuli of 1 ms width and at a frequency of 0.2 Hz were administered to pairs of adjacent electrodes. This was done while systematically proceeding lengthwise over the electrodes of the selected grids. Typically an intensity of 8 mA was used, but in case of twitches or pain the intensity was lowered to as low as 4 mA. A completed SPES protocol data set consists of all stimulated electrode pairs x 10 stimuli x maximal 64 recorded electrodes.

EVOKED HFO ANALYSIS

We used the evoked HFO results as reported in a previous study[124]. In summary, the analysis of evoked HFOs consists of four steps; 1.) Automated pre-processing of the data in average reference montage resulting in 10 epochs of [-1s:+1s] around the stimulus for each pair of stimulated electrodes. 2.) A Morlet wavelet based time-frequency analysis was performed in the frequency range 10-520 Hz (settings: sliding window of 685 samples with 50% overlap, frequency resolution 1 Hz, two oscillation parameters 3 and 0.5). The ten epochs were summed in the power spectrum (dB). 3.) Event related spectral perturbations (ERSP) images were calculated, using bootstrapping (n=200, $p<0.05$) with respect to pre-stimulus baseline. Non-significant ERSP values were set to zero dB. A color scale [-15dB: 15dB] showed changes in power relative to the baseline for each frequency. The above described steps were performed in Matlab (The MathWorks, Natick, MA), including the EEGlab toolkit[116]. 4.) For each patient evoked ripples and FRs, represented by red blobs in the ERSP images (64 electrodes x stimulated electrode pairs) within the interval of 0.2-1 s after stimulation were visually classified by two observers (chosen from MvtK/CF/FL/GH). Inter-observer agreement (Cohen's kappa, k) was calculated. Only evoked HFOs marked by both observers were used for analysis. ERSP images of the two electrodes of each stimulated electrode pair were classified as artifacts as their signal is saturated by the electrical current of the stimulation. ERSPs images of all other electrodes recorded for each stimulated electrode pair were classified. This analysis resulted in a total amount of ripples and FRs responses per electrode that were evoked by the complete SPES stimulation protocol. We did not correct for the number of stimulated electrode pairs, as these responses elicited by the10 pulses are of stochastic nature and because the number of stimulated electrode pairs is the same for each response channel[124].

SPONTANEOUS HFO ANALYSIS

In addition to the SPES results in previous study, we analyzed inter-ictal spontaneous HFOs in the ECoG of the same patients. One minute epochs of awake resting state at least one hour before or after SPES or seizures were selected for spontaneous HFO analysis. HFOs were visually marked in the same average reference montage in Stellate Harmonie Reviewer (v7.0, Montreal, QC, Canada) by two reviewers (MvtK/NvK). A split screen was used to simultaneously visualize ripples and FRs. Settings used for ripples were a high-pass finite impulse response (FIR) filter of 80 Hz and a gain of 5 μV/mm and for FRs these were a 250 Hz high-pass FIR filter and a 1 μV/mm gain, both were shown at 0.4 s/page. Ripples and FRs had to have at least four cycles clearly standing out from the baseline[18]. Marking of the spontaneous HFOs was done blinded for the evoked HFO results, the SOZ and the post-operative outcome. Inter-observer agreement was calculated. Only HFOs marked in consensus by both reviewers were used for analysis.

CLINICAL DATA

SOZ and resection area

Electrode positions were determined by matching a post-implantation CT with a pre-operative MRI (3 Tesla,3DT1) surface rendering[113] in combination with photographs made during

implantation surgery. The SOZ was clinically defined as those ECoG electrodes that showed the earliest ictal activity, defined as patterns 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[21, 114, 115]. Electrodes covering the SOZ and the resection area were marked by the two involved clinical neurophysiologists (CF/FL). They were blinded for the evoked and spontaneous HFO results.

Functional eloquent areas

Additionally, it was determined whether electrodes covered functional eloquent areas based on the clinically performed cortical 50 Hz stimulation results. During 50 Hz stimulation we routinely tested for the following areas, that are traditionally considered as functionally eloquent: the sensorimotor cortex, the supplementary motor area, the language areas (Broca's and Wernicke's area) and visual cortex. Electrodes where dichotomized in covering eloquent functional areas, when responsive to 50 Hz stimulation, or as covering 'silent' areas, when unresponsive.

Pathology and outcome

Pathology results and post-surgical outcome were collected from the clinical reports. Post-surgical outcome was determined at 1 year follow-up, dichotomized into good outcome (Engel I) and poor outcome (Engel II-IV)[147].

STATISTICAL ANALYSIS

Statistical analysis was performed for ripples and FRs separately. The total number of evoked and spontaneous ripples and FRs were calculated for each recorded electrode. We analyzed HFOs with respect to the following electrode classifications: 1.) in- or outside the SOZ, 2.) in- or outside the resection area, and 3.) covering eloquent functional areas or 'silent' areas.

We analyzed, per patient, the percentage of HFOs in the SOZ with respect to the total amount of spontaneous HFOs or HFOs evoked by SPES. This was done to correct for the individual differences in event counts and to enable comparison between the evoked and spontaneous HFO results.

$$\%\ \text{HFOs in SOZ} = \frac{\#\ \text{HFOs on electrodes in the SOZ}}{\text{total}\ \#\ \text{HFOs on all electrodes}} * 100\% \qquad \text{(Eq 8.1)}$$

We tested for significant differences between the percentages evoked and spontaneous ripples and FRs in the SOZ using a non-parametric test paired samples test (Wilcoxon Signed Rank test). Second, for each patient we calculated the sensitivity, positive specificity, (PPV) and negative predictive value (NPV) of electrodes with evoked or spontaneous HFOs for the SOZ.

$$\text{Sensitivity} = \frac{\#\ \text{electrodes with HFOs inside the SOZ}}{\#\ \text{electrodes inside the SOZ}} * 100\% \qquad \text{(Eq 8.2)}$$

$$\text{Specificty} = \frac{\#\ \text{electrodes with HFOs outside the SOZ}}{\#\ \text{electrodes outside the SOZ}} * 100\% \qquad \text{(Eq 8.3)}$$

$$PPV = \frac{\#\text{ electrodes with HFOs inside the SOZ}}{\#\text{ electrodes with HFOs}} * 100\% \quad \text{(Eq 8.4)}$$

$$NPV = \frac{\#\text{ electrodes without HFOs outside the SOZ}}{\#\text{ electrodes without HFOs}} * 100\% \quad \text{(Eq 8.5)}$$

We tested for differences in sensitivity, specificity, PPV and NPV between evoked and spontaneous ripples and FRs (Wilcoxon Signed Rank test). We compared the percentage removed HFOs during surgery, for each type, between the two outcome groups, similar to Jacobs et al. (2008)[18] (Mann-Whitney U test), as well as between evoked and spontaneous HFOs in the two outcome groups (Kruskal-Wallis test).

We explored if there was an increased percentage evoked and spontaneous HFOs in functional areas outside the SOZ. Second, we compared the proportion of electrodes with HFOs covering functional areas as well as 'silent' areas outside the SOZ.

$$\%\text{ HFOs in functional areas} = \frac{\#\text{ HFOs on electrodes outside the SOZ covering functional areas}}{\text{total \# HFOs on all electrodes outside the SOZ}} * 100\% \quad \text{(Eq 8.6)}$$

Proportion electrodes with HFOs in functional areas (%) =

$$\frac{\#\text{ electrodes with HFOs outside the SOZ covering functional areas}}{\text{total \# electrodes outside the SOZ covering functional areas}} * 100\% \quad \text{(Eq 8.7)}$$

Proportion electrodes with HFOs in 'silent' areas (%) =

$$\frac{\#\text{ electrodes with HFOs outside the SOZ covering 'silent' areas}}{\text{total \# electrodes outside the SOZ covering 'silent' areas}} * 100\% \quad \text{(Eq 8.8)}$$

Statistical analysis was performed in Matlab 2013a (The MathWorks, Natick, MA), and a p-value of ≤ 0.05 was considered significant.

RESULTS

PATIENTS

Ten patients (3 females, mean age 22.4 years, range 8–42 years) were included who had evoked and spontaneous HFO recordings available and in whom the SOZ could be determined. On average 94 electrodes (range 72-128) were implanted per patient. The presumed epileptic focus was temporal in three patients, frontal in two, central in one, parietal in one, fronto-central in two, and occipital in one.

In total we analyzed 587 electrodes, with an average of 59 (range 32-64) per patient. Anatomical distribution revealed a total number of electrodes of 211 in temporal regions, 126 in frontal, 131 in central, 109 in parietal and 10 in occipital regions. In total 80 electrodes were classified as SOZ electrodes, on average 8 (range 2-30) per patient, and 119 electrodes were classified as within

TABLE 8.1
Individual patient characteristics

Pt	Age/ Sex	Localization	Side	Pathology	# Total Implanted Electrodes	# SOZ Electrodes	# Electrodes in functional eloquent areas (# in SOZ)	# Electrodes in resection area	Total # Evoked R/FRs	Total # Spont R/FRs	Recording days	Engel Class at 1y
1	23/M	FrC	R	MCD	128	10	25 (0)	10	111/26	350/20	4	III
2	22/F	CP	R	--	104	8	18 (7)	9	39/43	20/556	6	III
3	31/F	T	R	MCD	88	3	10 (0)	21	129/18	74/0	7	I
4	8/M	FrC	L	Tumor	96	2	36 (2)	5	47/11	16/23	5	I
5	42/M	T	L	MTS	96	6	13 (0)	36	465/58	495/46	7	I
6	23/F	T	L	Glioneural heterotopia	96	4	10 (0)	16	186/39	154/20	8	I
7	9/M	Fr	L	FCD	96 (16 depth)	3	17 (10)	9	430/64	16/56	5	IV
8	29/M	P	L	Lesion tumor resection age of 4	72	2	2 (0)	2	5/2	32/0	4	I
9	11/M	C	R	FCD	72	12	18 (7)	8	309/76	267/108	9	II
10	26/F	O	L	Gliosis and old infarctions	96	3	11 (0)	3	131/4	114/3	8	I

Pt= patient, SOZ=seizure onset zone, Spont=Spontaneous, R=ripples, FRs=fast ripples, F = female, M = male, Fr = frontal, T = temporal, C = central, P = parietal, O = occipital, L = left, R = right, TS = tuberous sclerosis, MCD = malformation of cortical development, FCD= focal cortical dysplasia, MTS=mesio-temporal sclerosis.

the resection area, on average 12 (range 2-36) per patient. In total 160 electrodes covered functional eloquent tissue, on average 16 electrodes (range 2-36) per patient, and 26 of these were also included in the SOZ, which was located frontally or centrally in these cases. One year after surgery six patients were seizure free (Engel class I), one patient had only rare disabling seizures (Engel class II), in two worthwhile improvement was achieved (Engel class III) and in one patient surgery failed (Engel IV) (table 8.1).

EVOKED HFOS

We stimulated on average 42 (range 16–55) electrode pairs per patient. We classified the ERSP images of all electrodes recorded for each stimulated electrode pair, yielding a mean of 2507 (range 864–3584) ERSP images per patient. We found evoked ripples and FRs in all patients, with a total of 1852 evoked ripples, a median of 130 per patient, and a total of 341 evoked FRs, a median of 32.5 per patient. For ripples the mean inter-observer agreement was 0.6 (range 0.2-0.8), and for FRs this was 0.5 (range 0.3-0.6).

SPONTANEOUS HFOS

Spontaneous ripples were found in all patients, while spontaneous FRs occurred in eight patients. We found a total number of ripples of 1538, with a median of 94 per patient. The total number of detected FRs was 832, with a median of 21.5 per patient. Mean inter-observer agreement was 0.4 (range 0.2-0.7) for ripples, and 0.5 (range 0.0-0.7) for FRs.
Inter-observer agreement was higher for evoked than spontaneous ripples (p=0.032), but did not differ for FRs (p=0.97).

EVOKED VERSUS SPONTANEOUS HFOS IN THE SOZ

The two patients that did not show spontaneous FRs, showed evoked FRs within the SOZ. Figure 8.1A shows the percentages of evoked and spontaneous HFOs in the SOZ per patient. We found a similar percentage of evoked and spontaneous ripples in the SOZ (mean 31.9% and 33.0%, p=0.77), and for FRs (mean 42.5% vs. 48.1%, p=0.63) (figure 8.1). Electrodes with evoked ripples had a lower specificity for the SOZ than spontaneous ripples (mean 44.8% vs. 69.7%, p=0.022). We found a higher sensitivity for the SOZ of electrodes with evoked FRs compared to electrodes with spontaneous FRs (mean 51.8% vs. 36.9%, p=0.049) and a lower specificity (mean 83.1% vs. 91.6%, p=0.039) compared to spontaneous FRs (figure 8.1C). No significant differences were found for the PPV and NPV of both ripples and fast ripples (table 8.2).

EVOKED VERSUS SPONTANEOUS HFOS IN RELATION TO OUTCOME

All ten patients underwent surgery. No significant differences were found in percentages of removed HFOs, evoked and spontaneous, between Engel class I and Engel classes II-IV (figure 8.2; table 8.2).

EVOKED VERSUS SPONTANEOUS HFOS IN FUNCTIONAL ELOQUENT AREAS

Ten patients had functional eloquent areas covered by electrodes outside the SOZ, while only four patients also had function inside the SOZ; in two patients (partial) resection of this functional SOZ region took place. We found no significant differences in percentages of spontaneous and evoked HFOs with regard to functional eloquent areas outside the SOZ (table 8.2). We did find a higher proportion of electrodes with evoked ripples than those with spontaneous ripples, both in functional eloquent areas and in 'silent' areas (p=0.028 and p=0.011). These percentages and proportions did not differ for FRs (figure 8.3; table 8.2).

Figure 8.4 shows a patient example.

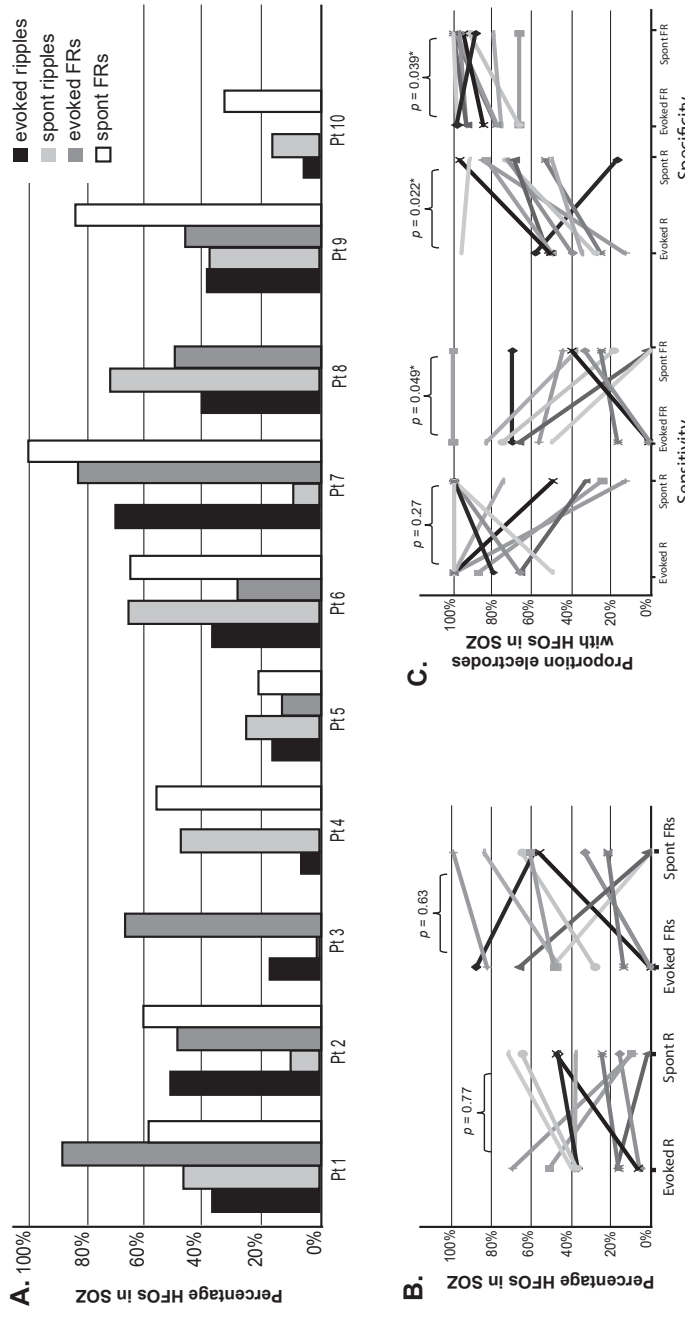

FIGURE 8.1
Comparison of percentage evoked and spontaneous (spont) HFOs in the seizure onset zone (SOZ). A.) Comparison of percentage evoked and spontaneous ripples and fast ripples (FRs) at the individual level. Note that in two patients (pt 3 and 8) in whom spontaneous FRs were absent, SPES did evoke a high percentage of FRs inside the SOZ. B.) Percentage evoked versus spontaneous ripples and FRs at group level. We found no significant differences between the percentage evoked and spontaneous ripples in the SOZ, nor for FRs. C.) Sensitivity and specificity of electrodes with HFOs for the SOZ. We found a significantly higher sensitivity for evoked compared to spontaneous FRs. On the other hand, the specificity for the SOZ of spontaneous HFOs was significantly higher than that of evoked HFOs, both ripples and FRs(*). Note that overall the sensitivity of FRs is lower than that of ripples, due to their low occurrence rates, while their specificity is higher than that of ripples.

TABLE 8.2
Statistics for SOZ, the resection area and functional areas

	Evoked R	Spont R	p-value	Evoked FRs	Spont FRs	p-value
SOZ:						
% HFOs in SOZ (mean(SD))	31.9 (20.6)	33.0 (24.6)	0.77	42.5 (31.8)	48.1 (33.6)	***0.63***
Sensitivity (mean(SD))	85.1(18.4)	69.7 (35.8)	0.27	51.8 (35.0)	36.9 (30.5)	***0.049****
Specificity (mean(SD))	44.8 (22.8)	69.2 (23.8)	***0.022****	83.1 (12.0)	91.6 (10.9)	0.039*
PPV (mean (SD))	21.7 (16.1)	24.8 (11.5)	0.66	35.8 (30.3)	42.1 (31.7)	0.38
NPV (Mean (SD))	97.7 (2.9)	82.3 (32.8)	0.20	92.8 (10.2)	85.6 (16.1)	0.17
% removed HFOs & outcome:						
Engel I (N=6)	35.3	57.4	0.38¥	48.1	45.6	0.91¥
Engel II-IV (N=4)	24.7	21.0		36.7	53.0	
Functional areas outside SOZ:						
% HFOs in functional areas	24.3 (20.6)	32.7 (38.1)	0.14	24.2 (27.2)	29.2(38.8)	0.37
Proportion electrodes with HFOs in functional areas (%) (mean(SD))	53.6 (30.4)	25.9(19.1)	***0.028****	17.5 (20.6)	9.1 (17.1)	0.12
Proportion electrodes with HFOs in 'silent' areas (%) (mean(SD))	56.8 (23.1)	26.9 (21.0)	***0.011****	8.6 (12.5)	6.2 (6.1)	0.85

SOZ=seizure onset zone, R=ripples (80-250Hz), FRs= fast ripples (250-500Hz), Spont=spontaneous, SD=standard deviation. PPV=positive predictive value, NPV=negative predictive value. SD=standard deviation. *A p-value ≤0.05 was considered significant using a Wilcoxon signed rank test for paired samples. ¥ a Kruskall-Wallis test was performed to test for difference between the four groups.

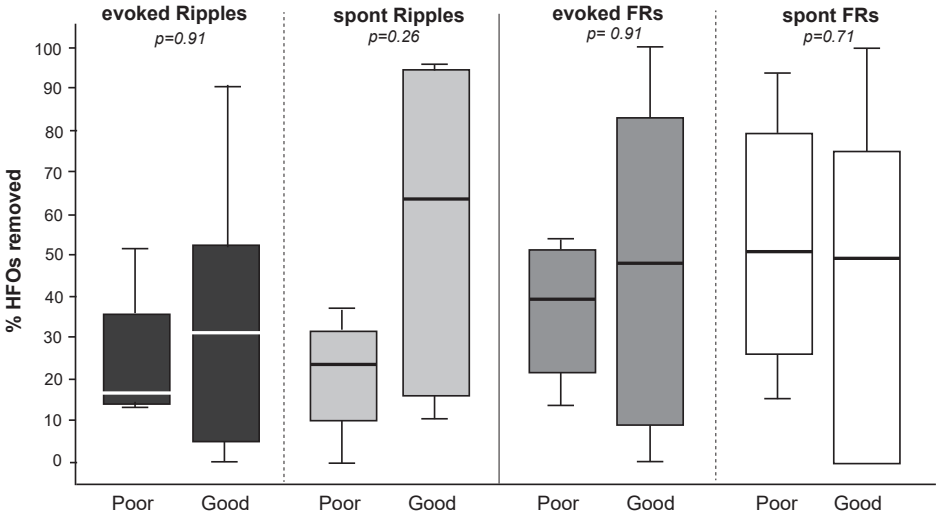

FIGURE 8.2
Percentage HFOs that was surgically removed in relation to post-surgical outcome after one year. All four HFO types showed a non-significant difference in their removed percentage HFOs between poor (Engel II-IV, N=4) and good (Engel I, N=6) outcome (Mann Whitney U test).

DISCUSSION

Evoked and spontaneous HFOs occurred in almost all patients. Two patients without spontaneous FRs did show evoked FRs in the SOZ. We found no difference between the percentage of evoked and spontaneous ripples in the SOZ. In comparison to spontaneous ripples, evoked ripples overall showed to be non-specific, as they occurred in more electrodes outside the SOZ. This was unrelated to whether the cortex underneath the electrodes was functionally eloquent or not. Evoked HFOs had a lower specificity for the SOZ than spontaneous HFOs and a somewhat higher sensitivity. We found no differences in the percentages of removed HFOs, evoked nor spontaneous, between good and poor post-surgical outcome. SPES elicits on-demand HFOs, especially FRs that are SOZ-specific, but also activates HFOs outside the epileptogenic zone. SPES is thus not able to distinguish pathological from physiological ripples.

COMPARISON WITH LITERATURE

That we can provoke seizures, epileptic activity and map function of the brain by applying electrical currents directly to the cortex is known since the late nineteen century[75, 148, 149]. SPES can evoke responses that mirror inter-ictal discharges and HFOs[124]. These delayed responses to SPES should not be confused with early responses that occur within 100 ms after stimulation. The latter are frequently described in cortico-cortical evoked potentials (CCEPs) studies[122, 130, 150].
Spontaneous HFOs have been associated with tissue that is responsive to direct electrical stimulation[151]. Their number can be reduced by 50-60 Hz electrical stimulation[152], and their

amplitude can be augmented by paired pulse stimulation[153]. We are the first to demonstrate the similarities and differences between SPES evoked HFOs and spontaneous HFOs in identifying the SOZ. This study showed that SPES can be valuable in patients in whom spontaneous HFOs, especially FRs are rare or absent. We were not able to reproduce in our small population the finding that incomplete removal of HFOs, evoked nor spontaneous, is associated with poor outcome[18, 19].

Patients who undergo long-term ECoG usually present complex clinical cases, in which the presumed lesion is difficult to localize or is entangled with functional eloquent areas. In this and a previous study SPES was performed in different anatomical lobes and evoked HFOs were found in all of them[124]. There seems no different behavior of SPES in different anatomical lobes as initially was described[21-23]. The pioneers of SPES never discussed the influence of SPES on functionally eloquent tissue. Our results indicate that outside the SOZ, functional eloquent areas are not more prone to react to direct electrical stimulation than other areas. Apart from direct cortical pulses, it has been reported that external stimuli, e.g. sensory stimulation, can also evoke fast cortical oscillations (200–500 Hz) in the somatosensory cortex in rats[154]. In humans, cortical responses with high frequency content in the central motor cortex can also be evoked with external somatosensory evoked potentials (SSEPs)[155]. Accumulating evidence suggests that spontaneous HFOs, even FRs, consist of both pathological and physiological subtypes[79]. Their features may be different and analysis requires expertise.

METHODOLOGICAL CONSIDERATIONS

We analyzed one minute of spontaneous HFO data, as visual analysis is cumbersome when done for a high number of channels. Remarkably, the amount of spontaneous HFOs resembled that of the evoked HFOs from a complete SPES protocol, as well as the ratio between ripples and FRs. In order to compare different types of HFOs from data epochs of different lengths we normalized for the total number of HFOs found per patient. Longer epochs might have resulted in more robust results[45]. We determined the traditional functional eloquent areas based on 50 Hz stimulation. Brain areas covered by electrodes that did not respond to 50 Hz stimulation tests, we named 'silent'. It is unlikely that these areas are truly without function. The SOZ is considered to represent the epileptogenic zone, but its correctness is only proved by complete seizure freedom after resection. We strictly divided HFOs or electrodes with HFOs as occurring in- or outside the SOZ. However, HFOs neighboring the SOZ still might be of importance, whereas HFOs remote from the SOZ are probably not related to the epilepsy and are valid to reject. We have to study larger populations to draw stronger conclusions on interactions between the SOZ, anatomical lobes, functional eloquent cortex and their relation to outcome. Our study was limited by the selection of the 64 electrodes that was made because of the required high sampling rate for HFOs. This might have given an incomplete scope on the preferential occurrence of HFOs with respect to the sampled lobes as well as the potentially missed epileptogenic tissue. Currently available hardware will enable recording of all implanted electrodes at a high sampling rate.

A strong point is the wavelet based time-frequency analysis method for evoked responses. Visual classification of the ERSP plots, in which 10 stimuli are averaged, leads to equal or better inter-

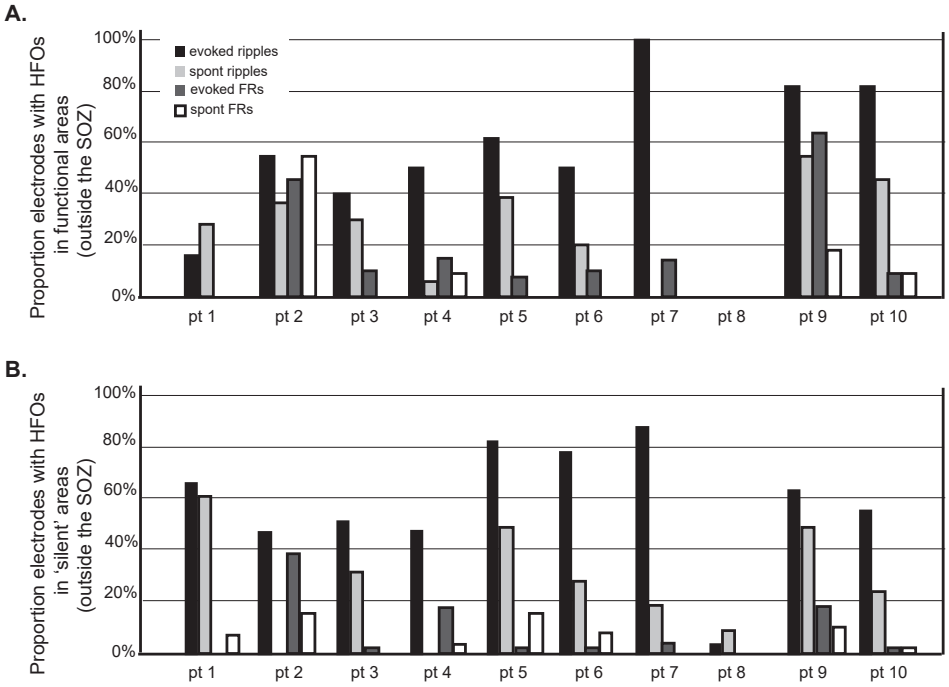

FIGURE 8.3
Comparison between evoked and spontaneous HFOs with respect to functional eloquent areas outside the seizure onset zone (SOZ). A.) Proportion of electrodes with HFOs in functional areas outside the SOZ. All ten patients had ≥2 electrode covering functional areas outside the SOZ. B.) Proportion of electrodes with HFOs in 'silent' areas outside the SOZ. Significant more electrodes showed evoked ripples than spontaneous ripples for both functional as 'silent' areas (p=0.028 and p=0.011). No significant differences for fast ripples (FRs) were found (table 8.2).

observer agreement and is faster than visual analysis in the time domain of the evoked HFOs. The duration of visual analysis of the time-frequency plots of a total stimulation protocol – execution time is approximately 1.5 hours - was equivalent to that of HFOs in one minute of inter-ictal ECoG data. Automated HFO analysis methods, in both time and time-frequency domain, are under development[25, 32, 101] and some of the time-frequency domain codes might enable automatic evaluation of our SPES time-frequency plots as well[156, 157].

FUTURE RESEARCH & CONCLUSION

Investigation of other properties of the elicited responses could aid in understanding the mechanistic differences between the generators of physiological and pathological HFOs. For example by studying responses in relation to morphology[24, 111], the stimulus site[158], or directionality[143]. Network structure can be derived from SPES as well[158, 159]. Further research will focus on integration of functional network analysis with SPES and HFOs. To accelerate the SPES protocol, as well as increase the sensitivity of evoked HFOs, we could include the information of early responses evoked by SPES. Early responses occur more frequently than delayed responses,

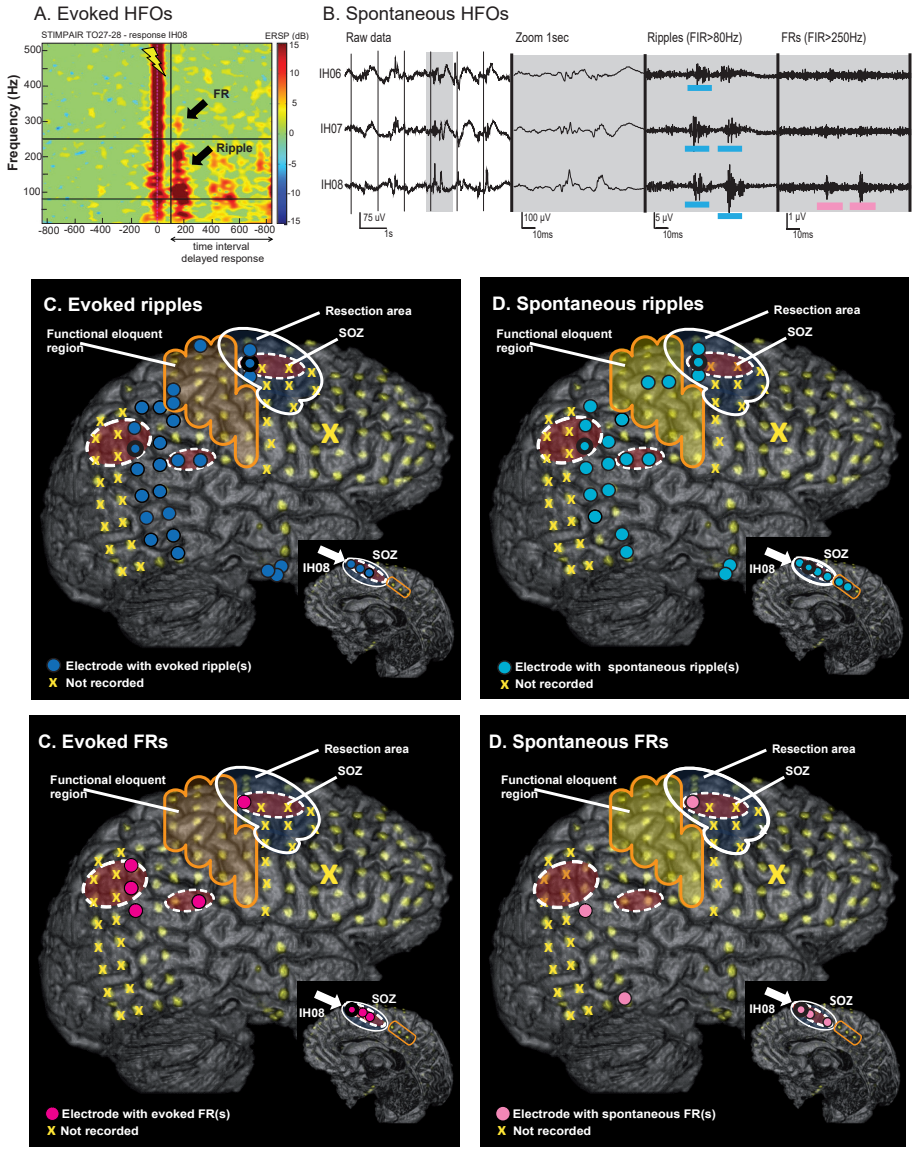

FIGURE 8.4
Example of a patient (pt 1) with a lesion near functional eloquent cortex, multiple seizure onset zones (SOZ) (red) could be identified. After a lesionectomy of the assumed primary SOZ the frontal lobe the patient did not become seizure free (Engel III). Pathology revealed malformation of cortical development. A.) Example of an ERSP image with evoked ripples and fast ripple (FRs) response in electrode IH08, when stimulating elsewhere. B.) Example of spontaneous ripples and FRs on electrode IH08 and neighboring electrodes IH07 and IH08. C.) Electrodes with evoked ripples by SPES. The distribution of electrodes with HFOs is shown (colored dots, dot with thick black line represents electrode with most HFOs). Note that the evoked ripples are present in- and outside the SOZ, and in- and outside the functional eloquent cortex (orange). D.) In this patient, similar results were found for electrodes with spontaneous ripples, they are also present in all areas. E.) Electrodes with evoked FRs by SPES show a high specificity for all SOZ regions. F.) In this patient, the electrodes with spontaneous FRs seem somewhat less specific for the SOZ than the ones with evoked FRs.

and high numbers of these early response are shown to be indicative for the SOZ[158]. SPES is reported to evoke SOZ-specific delayed responses under general anesthesia too[160]. Spontaneous HFOs are, however, reduced by anesthetic agents[43]. Further studies should investigate if HFOs can be evoked intra-operatively, with or without tapering of propofol, and if their characteristics are similar under those conditions. Together this could lead to a fast stimulation and detection protocol for the SOZ that could aid targeted subdural electrode placement, shorten the monitoring period or improve intra-operative tailoring to guide the surgeon in resection.

To conclude, SPES elicits on-demand HFOs, especially FRs, that are specific for the SOZ, but is also prone to activate HFOs outside the epileptogenic zone. Part of these HFOs are probably physiological. Careful interpretation of all HFOs and especially evoked ripples is advised. From a clinical point of view, it is important to realize that events remote from the epileptogenic lesion, whether these are spikes or HFOs, spontaneous or evoked, always should be considered carefully in relation to anatomy and etiology of the epilepsy. Usually these areas are not resected[90]. SPES provides an alternative to waiting for spontaneous HFOs, but does not warrant exclusively pathological ripples or FRs.

CHAPTER 9

NON-HARMONICITY TO DELINEATE EPILEPTOGENIC TISSUE IN THE INTRA-OPERATIVE CORTICOGRAM DURING SURGERY

Submitted.

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*Authors contributed equally

ABSTRACT

INTRODUCTION

We aimed to test the potential of ARRm, an artefact-insensitive method based on non-harmonicity of the high-frequency signal, to identify epileptogenic tissue during surgery.

METHODS

Intra-operative electrocorticography (ECoG) of 54 patients with refractory focal epilepsy were recorded pre- and post-resection at 2048 Hz. The ARRm was calculated in one-minute epochs in which high-frequency oscillations (HFOs; fast ripples, 250-500 Hz; ripples, 80-250 Hz) and spikes were marked. We investigated the pre-resection fraction of HFOs and spikes explained by the ARRm (h^2-index). A general ARRm threshold was set and used to compare the ARRm to surgical outcome in post-resection ECoG (Pearson X^2).

RESULTS

ARRm was associated strongest with the number of fast ripples in pre-resection ECoG (h^2=0.80, P<0.01), but also with ripples and spikes. An ARRm threshold of 0.47 yielded high specificity (95%) with 52% sensitivity for channels with fast ripples. ARRm values >0.47 were associated with poor outcome at channel and patient level (both P<0.01) in post-resection ECoG.

CONCLUSIONS

Brain tissue producing signals with high ARRm correspond to areas producing spikes and HFOs, especially fast ripples, and relate to outcome. The ARRm algorithm can intra-operatively delineate epileptogenic tissue that should be removed to optimize the chance of seizure freedom.

INTRODUCTION

Epilepsy is a dynamic condition as seizures occur intermittently and in most cases unexpectedly. This suggests that the underlying processes responsible for the generation and cessation of seizures are non-harmonic, i.e. non-linear and non-stationary. A high level of non-linearity in the system is needed to initiate seizures[161]. The epileptiform EEG contains several biomarkers which all have a strong non-linear component[162, 163]. This holds true for epileptiform inter-ictal spikes, for the recently discovered high frequency oscillations (HFOs; >80 Hz) and for seizures themselves. All these events appear without notice[18, 164, 165].

During epilepsy surgery there is need for biomarkers to delineate the epileptogenic tissue that should be resected to assure seizure freedom after surgery. Even in the presence of an epileptogenic lesion on MRI the resection boundaries are not always clear, e.g. in cases of neuronal tumors, in which the epileptogenic zone may extend beyond the lesion, or when the hippocampus is secondarily affected[166]. Epilepsy surgery can be tailored by identifying spikes in the intra-operative electrocorticogram (ECoG), but this method is controversial[14, 79]. Surgical removal of cortex showing fast ripples, between 250 and 500 Hz, has been associated with post-operative seizure freedom[19, 79]. HFOs probably result from hyper synchronous, out-of-phase firing of groups of principal neurons[80], and might be more local to the epileptogenic tissue than spikes[80, 84]. It may thus be better for the tailoring of epilepsy surgery with ECoG to rely on HFOs, especially fast ripples, rather than on the currently used spikes[79].

Visual analysis of HFOs is time consuming and requires the presence of expert reviewers during surgery[45]. The time available to record and analyze HFOs is limited, and HFO occurrence is infrequent, so they can easily be missed[67, 79]. Automatic detectors have been developed, but most still require extensive computing time[25, 44, 53, 84, 91-93, 167], making these techniques unsuitable for use in the time frame of surgery. Additionally, it can be difficult to distinguish pathological HFOs from physiological high frequent activity. High frequent physiological activity probably shows a harmonic pattern[87]. It can also be difficult to distinguish HFOs from artifacts, while artifact sources, such as the diathermy and the surgical microscope, are ubiquitous in the surgical theatre[32]. Analysis of non-harmonicity in high-frequency components of the EEG signal, recorded at a high sampling rate >2000 Hz, may provide an automated and objective solution that bypasses these issues.

The variance of the auto-regressive model residual modulation (ARRm) can be used to predict which intracranial EEG channels are within the presumed epileptogenic area[94]. We developed the ARRm algorithm to identify 'bad' channels. The ARRm reflects the amount of non-harmonicity in the high-frequency components of the signal, in terms of high residual signal variation after autoregressive modeling. The ARRm can be computed rapidly and only requires short epochs of EEG and thereby seems to lend itself to online analysis during surgery[94]. We adjusted the ARRm algorithm to reduce the influence of typical intra-operative ECoG artifacts; artifacts may produce spuriously large residual signal variations, resulting in false positive high ARRm values unrelated to the epileptic tissue. We focused on the rejection of subtle short-lasting electrode and movement artifacts, as these are the most difficult to identify.

We aimed to test the potential of the new ARRm algorithm to identify epileptogenic tissue during surgery. Therefore we compared the ARRm to the occurrence of fast ripples, ripples and spikes in pre-resection intra-operative ECoG, and studied the relationship between ARRm results in post-resection ECoG and post- surgical outcome.

METHODS

ECOG DATABASE

Patients

The database consisted of intra-operative ECoG recordings sampled at 2048 Hz, before and after resection, of patients with refractory focal epilepsy who underwent tailored epilepsy surgery between 2008 and 2012 at the UMC Utrecht. The surgical strategy was based on the results of pre-surgical diagnostics (e.g. MRI, PET, MEG, video-EEG) together with intra-operative tailoring based on inter-ictal spikes and spike patterns, using conventional EEG settings (70 Hz low pass filter, 10 seconds/page) in a common average reference montage. HFOs were never analyzed during surgery. The database was collected according to the guidelines of the institutional ethical committee of the UMC Utrecht, The Netherlands.

The database was constructed based on the inclusion and exclusion criteria as described in a previous study[79]. We included 54 patients (median age 15.5 years, 29 male, 30 right-sided surgeries) with a median follow-up after surgery of 25 months (range 17.0-35.8 months). The resection area was temporal in thirty-three patients, frontal in twelve, peri-central in seven, and parietal and occipital each in one patient. Pathology was classified in four groups; mesial temporal sclerosis (MTS, N=8), tumors with glial components (including gangliomas and dysembryoplastic neuroepithelial tumors (DNET); N=23), malformations of cortical development (e.g. focal cortical dysplasia (FCD) and tuberous sclerosis; N=15) and other pathologies (e.g. cavernomas and gliosis; N=8). Thirty patients (56%) became seizure free after surgery (Engel 1A)[5]. This post-surgical outcome was determined for the most recent follow-up with a minimum of one year.

ECoG recordings

Intra-operative ECoG recordings were made with a 64-channel EEG system (MicroMed, Veneto, Italy) at 2048 Hz sampling rate using an anti-aliasing filter at 538 Hz. We used 4x5 or 4x8 electrode grids and 1x6 or 1x8 electrode strips (Ad-Tech, Racine, WI). The platinum electrodes are embedded in silicone with a 4.2 mm^2 contact surface and 1 cm inter-electrode distance. Grids and strips were placed in multiple configurations before (pre-resection ECoG), during extension of the resection, and after resection (post-resection ECoG). Propofol anesthesia was stopped during ECoG registration until a continuous ECoG background pattern was achieved. To minimize propofol effects we selected the last minute of each pre- and post-resection recording for analysis; on average this was 11 minutes after propofol was stopped. Analysis was performed on bipolar electrode pairs lengthwise on the grids. Bipolar channels with continuous artifacts that were visible in the raw ECoG signal were excluded.

MARKING OF HFOS AND SPIKES

We used an automated detector previously developed, adapted for our intra-operative ECoG, to detect HFOs[44]. The detector uses a high pass finite impulse response (FIR) filter >80 Hz for ripples and >250 Hz for fast ripples[44, 67]. Subsequently, visual post-processing of the data and the HFO detections in Stellate Harmonie Reviewer (v7.0, Montreal, QC, Canada) was done in consensus by two of three reviewers (MvtK/MZ/NvK) to correct for artifacts, falsely identified and missed HFOs. A split screen was used to visualize ripples (gain 5 μV/mm) and fast ripples (gain 1 μV/mm) simultaneously at an elongated time interval of 0.4 s/page and the same filter settings as mentioned above[18]. Spikes were visually marked (MvtK) in the same bipolar montage, using conventional infinite impulse response (IIR) filter settings of 0.5-70Hz at a gain of 75-200 μV/mm and 10 s/page. Marked spikes were checked by a clinical neurophysiologist (FL). Spikes were defined as paroxysmal sharp transients, with a maximum duration of 80 ms[49] and minimum amplitude of twice the baseline. Sharp waves co-occurring with spikes on other bipolar channels were also marked. HFOs were marked independently and blinded for spikes and vice versa. All events were marked blinded for outcome.

AUTOREGRESSIVE RESIDUAL MODULATION

Original autoregressive residual modulation

We used the original AutoRegressive model Residual modulation ($ARRm_{orig}$) algorithm, as previously described[94]. The $ARRm_{orig}$ algorithm quantifies the variance of residual signal variation (r) after modeling short windows of ECoG data using autoregressive models with model order 3. High ARRm values represent the intermittent occurrence of non-harmonicity in the ECoG signal. The window length was 40 samples, approximately 20 ms of ECoG data, and consecutive windows with 50% overlap were chosen. The algorithm quantifies the high-frequency components of the EEG signal, as frequencies below 50 Hz cannot be modeled within a 40 sample window. These signal components therefore do not influence the residual signal variation.

Improved autoregressive residual modulation

Artifacts may produce spuriously large residual signal variations, which result in false positive high ARRm values unrelated to the epileptic tissue. We modified the ARRm algorithm to be less sensitive to subtle artifacts than the original $ARRm_{orig}$ algorithm. Artifacts can be roughly classified into two groups; 1.) Gross artifacts, which can be observed in the unfiltered ECoG, e.g. from electrode movement, or lack of contact with tissue. Such artifacts are easy to recognize and may be prevented by careful recording. 2.) Subtle short-lasting artifacts may result from electrical interference, touching the electrode or short electrode problems and have a very small amplitude. These artifacts can be short and sharp, resulting in a strong non-linear component, and thus greatly influence the ARRm.

A preliminary study on a learning set showed that these artifacts differ from HFOs when looking at residuals from different autoregressive model orders. The learning set consisted of twelve 5-second ECoG epochs from seven patients chosen from the pre-resection ECoG dataset. We included examples of artifacts, spikes and HFOs, and data without such events, but cases were

otherwise chosen randomly. We observed that r-values stayed high for increasing model orders in windows containing artifacts. Windows containing true events, however, showed r-values declining much faster for increasing model orders. The difference between artifacts and HFOs was largest when looking at the steepness of r decline from the first to the second order (r_1 to r_2). The decline of r over an increasing model order was used to reject high r_3-values resulting from artifacts. Using the same learning set, an appropriate decline threshold for the ARRm algorithm was chosen. Figure 9.1 illustrates the artifact rejection method used in the ARRm algorithm.
ECoG epochs are evaluated per bipolar channel to provide an ARRm value. First, second and third order AR models were estimated, thereby obtaining residual signal variance time series (r_1, r_2 and r_3) with values for each 40-sample window. Residual decline (D) over order one and two was calculated for each window (w), as follows:

$$D(w)=\frac{2(r_1(w)-r_2(w))}{r_1(w)+r_2(w)} \qquad \text{(Eq 9.1)}$$

Outliers in r_3, i.e. exceeding the 95th percentile, and its two circumjacent r_3 samples, were removed from the r_3 time series if the outlier window meets the condition $D<0.9$. This provides us with a collection of 'cleaned' windows (w^c).
Subsequently, the improved autoregressive residual modulation ARRm can be calculated, using only the clean windows:

$$ARRm=\frac{\sigma_{w^c}(r_3)}{\mu_{w^c}(r_3)} \qquad \text{(Eq 9.2)}$$

where σ is the standard deviation and μ is the mean.

STATISTICAL ANALYSIS

$ARRm_{orig}$ and ARRm validation in pre-resection data

The $ARRm_{orig}$ and ARRm results from the ECoG data recorded pre-resection were compared on a channel level with spike, ripple, and fast ripple counts. For this comparison the h^2 index was used. The h^2 index is a measure of the association between pairs of data in general (linear or non-linear) [168]. We obtained the h^2 index to test the fraction of variation of $ARRm_{orig}$ and ARRm that can be explained by the number of spikes, ripples and fast ripples. This results in a value between 0 and 1, where $h^2(A,B) = 1$ implies that all variation of A is explained by B. The h^2 findings are significant if h^2 exceeds the critical value, or significance margin, in a bootstrapped test with $\alpha = 0.05$.
We evaluated the ability of $ARRm_{orig}$ and ARRm to correctly classify channels with (+) and without (-) spikes, ripples or fast ripples. ROC curves for all event types were obtained using the sensitivity and specificity for various $ARRm_{orig}$ or ARRm thresholds at channel level. The ROC curve for fast ripples - the most specific biomarker for epileptogenic tissue - provided a clinical threshold. This threshold was used for comparison of ARRm results in post-resection ECoG and surgical outcome. For this analysis we dichotomized the ARRm results into channels with values above (ARRm+) and below (ARRm-) the determined threshold.

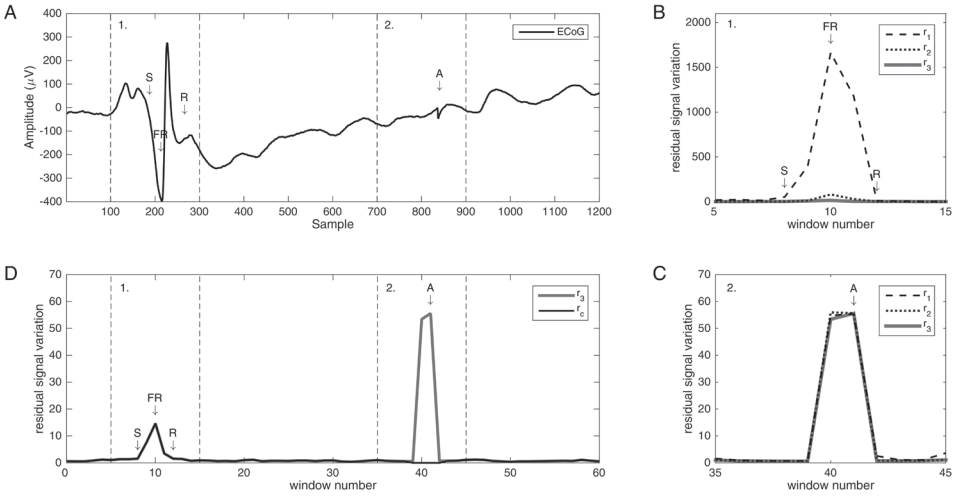


FIGURE 9.1

Illustration of the artifact rejection method in the ARRm algorithm. A.) ECoG raw signal (1200 samples = 0.6 seconds). The segments indicated with the dotted-line boxes hold events. Box 1 holds a spike (S), a ripple (R) and a fast ripple (FR), whereas box 2 holds an artifact (A). Arrows indicate the timing of these events. B.) Residual signal variations (r) calculated using autoregressive model order 1, 2 and 3, from the ECoG segment in box 1, containing S, R and FR events. The r-values are high for events for all three orders, but the r-value declines steeply when the model order increases. C.) r-values for model order 1, 2 and 3 from the ECoG segment including the artifact in box 2. During this artifact, the r-values are high for all three orders; there is no steep decline of r-values when the model order increases. D.) r_3-values that exceed the 95th-percentile of the epoch, and have a small r_1-to-r_2 decline, or modulation, are rejected. Shown are the r-values from model order 3 (r_3) and the resulting clean r_3 time series (r_c). For visualization purposes, the rejected values are shown here as zeros; in reality they are removed from the r_3 time series.

9

Relationship of ARRm to outcome in post-resection data

In contrast to the rejected subtle artifacts, gross artifacts could be prevented during registration and can be easily observed in the unfiltered ECoG. We, therefore, visually checked the data for an explanation of the ARRm+ value in that specific channel. We removed channels with gross artifacts causing ARRm+ results from the post-resection data analysis.

We investigated the spatial distribution of fast ripples and ARRm values in relation to resection and outcome in patients who had a.) fast ripples and ARRm+, in order to confirm overlap of the channels that identified the epileptogenic area, b.) fast ripples but ARRm-, to see whether ARRm is less susceptible to false positives due to physiological fast ripples and c.) no fast ripples but ARRm+, to see whether the ARRm identifies new patients with poor prognosis who were missed by identification with fast ripples.

Outcome prediction based on ARRm

We performed statistical analysis of the ARRm results at channel level and maximal ARRm value at patient level in comparison to outcome (Mann-Whitney U test). We did this for the dichotomized ARRm results based on the clinical threshold as determined on pre-resection ECoG data (Pearson X^2-test or Fisher exact-test). We stratified for the presence and absence of fast ripples, ripples and

spikes both at patient and channel level. We calculated the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the maximal ARRm value for poor outcome.

Computation of the ARRm results and h^2 association index was performed in Matlab (version 8.5.0; The MathWorks Inc., Natick, MA, USA). Statistical analysis was performed in IBM SPSS Statistics 21 (IBM Corp, Armonk, NY, USA). We considered a p-value ≤0.05 significant.

RESULTS

DATA

All 54 patients in our database of intra-operative ECoG recordings sampled at 2048 Hz were included in the analysis. We included all 2744 bipolar channels of the pre-resection recordings, resulting in a median (inter quartile range, IQR) of 41.0 (36.0-63.5) channels per patient for pre-ECoG. For the post-resection ECoG we included 1291 of 1325 bipolar channels, resulting in a median (IQR) of 18.5 (range: 15.0-29.0) channels per patient after resection.

ARTIFACT REJECTION

Figure 9.2 shows the effect of several artifact types on $ARRm_{orig}$ and ARRm. Glitches, which are commonly appearing subtle artifacts that have a substantial effect on $ARRm_{orig}$, are successfully rejected (figure 9.2A). Several other gross artifact types are also shown, which are not rejected by ARRm. Figure 9.3 illustrates the effect of artifact rejection on channel level, considering the presence of fast ripples.

COMPARISON OF $ARRM_{ORIG}$ AND ARRM TO HFOS AND SPIKES IN PRE-RESECTION DATA

The $ARRm_{orig}$ and ARRm results from the ECoG data recorded pre-resection were compared on a channel level with spike, ripple, and fast ripple counts. In figure 9.4, the results of the six comparisons are shown, together with the resulting h^2 values reflecting the amount of association. All h^2 associations between $ARRm_{orig}$ and spikes, ripples, or fast ripples were significant ($p<0.01$), as well as for ARRm ($p<0.01$). Thus $ARRm_{orig}$ and ARRm variation may be explained by the quantity of events, where associations were strongest for fast ripples. ARRm was better associated with fast ripples than $ARRm_{orig}$ ($h^2(FR,ARRm)=0.80$, $h^2(FR, ARRm_{orig})=0.31$, both $p<0.01$).

$ARRM_{ORIG}$ AND ARRM AS EVENT CHANNEL CLASSIFIERS

We investigated the ability of $ARRm_{orig}$ and ARRm to correctly classify channels with and without spikes, ripples or fast ripples. The boxplot and ROC curve illustrating the FR+/- channel classification ability of $ARRm_{orig}$ and ARRm are shown in Figure 9.5. Best performance is found for classification of FR+ and FR- channels with a ROC area under the curve (AUC) of 0.83 for $ARRm_{orig}$ and 0.88 for ARRm. For spikes the AUC values were 0.70 and 0.76, and for ripples 0.70 and 0.78 for $ARRm_{orig}$ and ARRm, respectively. ARRm performance exceeds $ARRm_{orig}$ performance for all event types, which implies successful artifact reduction. 100% specificity for FR- channels could not be obtained with any threshold for $ARRm_{orig}$ - there were many FR- channels with high $ARRm_{orig}$

values - but was possible for ARRm (see figure 9.4C and F). Since the ARRm outperforms the $ARRm_{orig}$, we used the ARRm algorithm throughout the rest of the study.

ARRM CLINICAL THRESHOLD

A threshold is needed to use ARRm as a classifier to find channels located in epileptogenic tissue. We chose this threshold favoring specificity; if a low ARRm value is measured, non-epileptogenicity of the tissue at that location should be concluded with as much certainty as possible. Therefore the 95% specificity point, ARRm>0.47, was chosen as the ARRm threshold to define a channel as suspect, i.e. likely to be located on epileptogenic tissue.

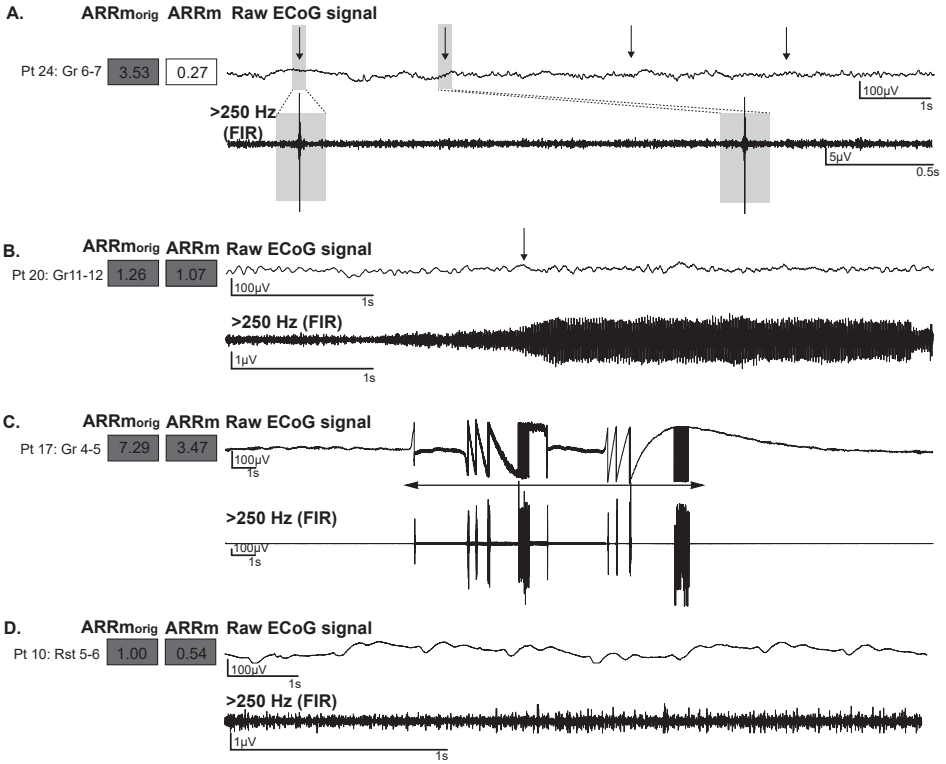


FIGURE 9.2

Example of common subtle and gross artifacts in the intra-operative ECoG and accompanying $ARRm_{orig}$ and ARRm value. A.) Subtle intermittent glitches are one of the most common artifacts in intra-operative ECoG data and are barely visible in the raw data signal. They don't hamper visual analysis of spikes, but may cause spurious artifacts in the higher frequency ranges, and can be easily mistaken for HFOs. The ARRm algorithm, unlike the $ARRm_{orig}$, was successfully designed to reject this type of artifact. B.) Sudden onset or waxing and waning high frequency noise on individual channels can cause undesirably high $ARRm_{orig}$ as well as ARRm values. This subtle artifact is also difficult to spot in the raw signal. C.) Typical gross artifact on one electrode due to interference of the surgeon touching the grid. D.) Example of a floating channel that does not touch the brain surface and picks up noise, especially visible in the higher frequency ranges. This often occurs for electrodes on corner of grids or the last electrode of a strip. Note that in B, C and D the ARRm value was substantially lower compared to the $ARRm_{orig}$ value, as some artifacts in these signals might have fulfilled the rejection criteria. None of the here depicted channels showed fast ripples.

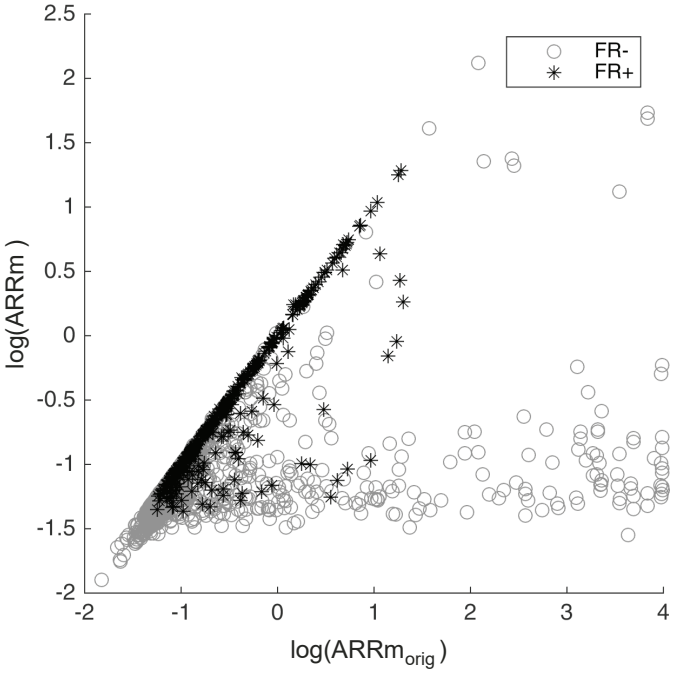


FIGURE 9.3
Scatterplot of ARRm$_{orig}$ values vs. ARRm values, where each data point corresponds to one channel. Grey data points correspond to fast ripple negative channels (FR-), where no fast ripples were scored visually. Black data points correspond to fast ripple positive channels (FR+), where one or more fast ripples were scored visually. Note that both axes are scaled logarithmically. It can be seen that the majority of FR- channels get lower ARRm values than their corresponding ARRm$_{orig}$ values, while FR+ channels keep similar values. This indicates successful rejection of high-impact artifacts.

ARRM COMPARED TO POST-SURGICAL OUTCOME

ARRm in relation to fast ripples in post-ECoG and clinical decisions

We investigated the spatial extent of residual fast ripples and the ARRm results in post-resection ECoG in relation to outcome (table 9.1). An illustrative patient example with overlapping fast ripple and ARRm results is shown in figure 9.6. We dichotomized the ARRm results at channel level based on the above mentioned threshold. We found six patients who had both fast ripples and ARRm+ channels (group A) in the same locations, all had recurrent seizures. Five patients had fast ripples but did not show ARRm+ channels (group B). This group contains three seizure-free patients with fast ripples after resection. For example one patient showed fast ripples in Broca's area and the central motor area, which we believe represent physiological fast ripples. This suggests the ARRm might be insensitive to physiological fast ripples. On the other hand, there were three patients with fast ripples and poor outcome but without ARRm+ results.

Although the channels with fast ripples exhibited the highest ARRm values when ranked, these values did not exceed the predefined threshold. Three patients did have ARRm+ channels but did not show residual fast ripples (group C). Two of these patients had poor outcome. In one patient with recurrent seizures the ARRm+ channels overlapped with an event that was incorrectly discarded as artifact during visual analysis (see figure 9.7).

Outcome prediction with ARRm

The ARRm at channel level and the maximal ARRm value at patient level are both indicators of poor outcome if the value is above 0.47 (both $p<0.01$, Person X^2 test; table 9.1). A maximal ARRm >0.47 predicted poor outcome with a sensitivity of 33.3%, a specificity of 96.7% and had a PPV of 88.9 % and a corresponding NPV=64.4 %. When stratified for the presence or absence of fast ripples, ripples and spikes, the ARRm performs better at channel than at patient level. Remarkably, even in the absence of fast ripples (FR-) at channel level, the ARRm >0.47 is an indicator of poor post-surgical outcome (p=0.02, Fisher exact) (table 9.1).

TABLE 9.1
Performance of ARRm in predicting surgical outcome

	Channel level (N=1290)			**Patient level (N=54)**		
	ARRm			**max ARRm**		
	>0.47	**<0.47**	**p-value**	**>0.47**	**<0.47**	**p-value**
# Counts	27	1264		9	45	
# Outcome (Poor/Good)	26/1	518/746	<0.01[a,c]	8/1	16/29	<0.01[a,c]
Events in ECoG:						
FRs+ (Poor/Good)	20 (20/0)	32 (18/14)	<0.01[b,c]	6 (6/0)	6 (3/3)	0.18[b]
FRs- (Poor/Good)	7 (6/1)	1232 (500/732)	0.02[b,c]	3 (2/1)	39 (13/26)	0.29[b]
Ripples+ (Poor/Good)	27 (26/1)	751 (324/427)	<0.01[a,c]	9 (8/1)	42 (16/26)	<0.01[b,c]
Ripples- (Poor/Good)	0 (0/0)	513 (194/319)	n.a.	0 (0/0)	3 (0/3)	n.a.
Spikes+ (Poor/Good)	26 (25/1)	468 (225/243)	<0.01[a,c]	8 (7/1)	30 (19/11)	<0.01[b,c]
Spikes- (Poor/Good)	1 (1/0)	796 (293/503)	0.37[b]	1 (1/0)	15 (5/10)	0.38[b]

Abbreviations: Poor= recurrent seizures (Engel Ic-IV), Good= seizure freedom (Engel Ia,b),[a]=Pearson X2-test, [b]=Fisher exact-test, n.a.=not available, [c]=p-value <0.05 considered significant. Note that the FR- group contains channels or patients without marked fast ripples while the FR+ group contains channels or patients in which one or more fast ripples were marked, and similar for ripples and spikes + and- .

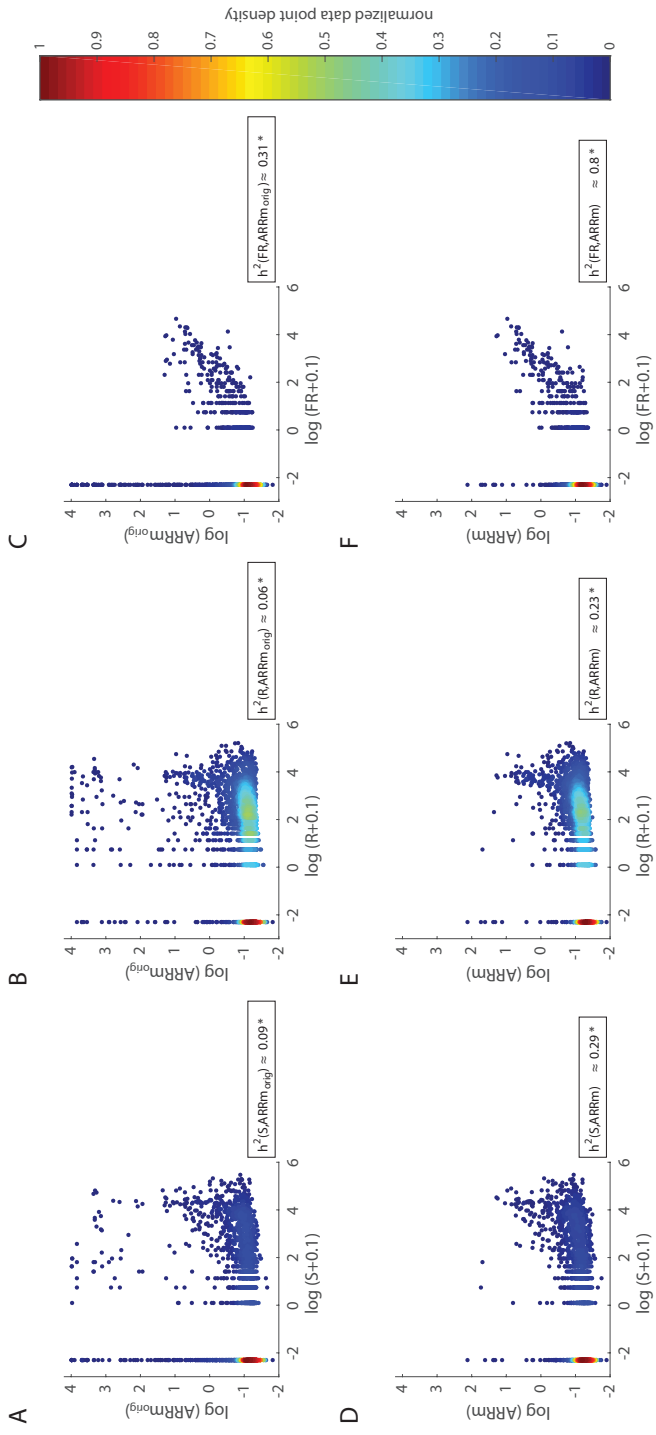


FIGURE 9.4
Scatter density plots of ARRm$_{orig}$ (panels A, B and C) and ARRm (panels D, E and F) results from the ECoG data recorded pre-resection, compared on a channel level with spike (S), ripple (R), and fast ripple (FR) counts. Both axes are scaled logarithmically. Note that 0.1 is added to all event counts (x-axis) for visualization purposes, for log(0)=∞. Thus, a data point at -2.3 on the x-axis corresponds to zero events to have been scored on a channel. h^2 association between the compared results is shown in the boxes next to the figures (*p<0.01 was considered significant). ARRm showed particularly low values for channels which had very high ARRm$_{orig}$ values but no fast ripples, showing that the artifact rejection was successful (see figure 9.4C and F, and figure 9.3).

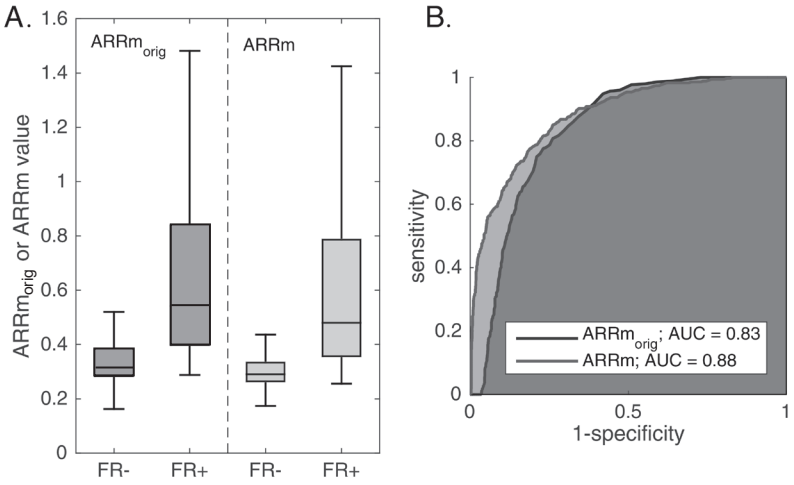


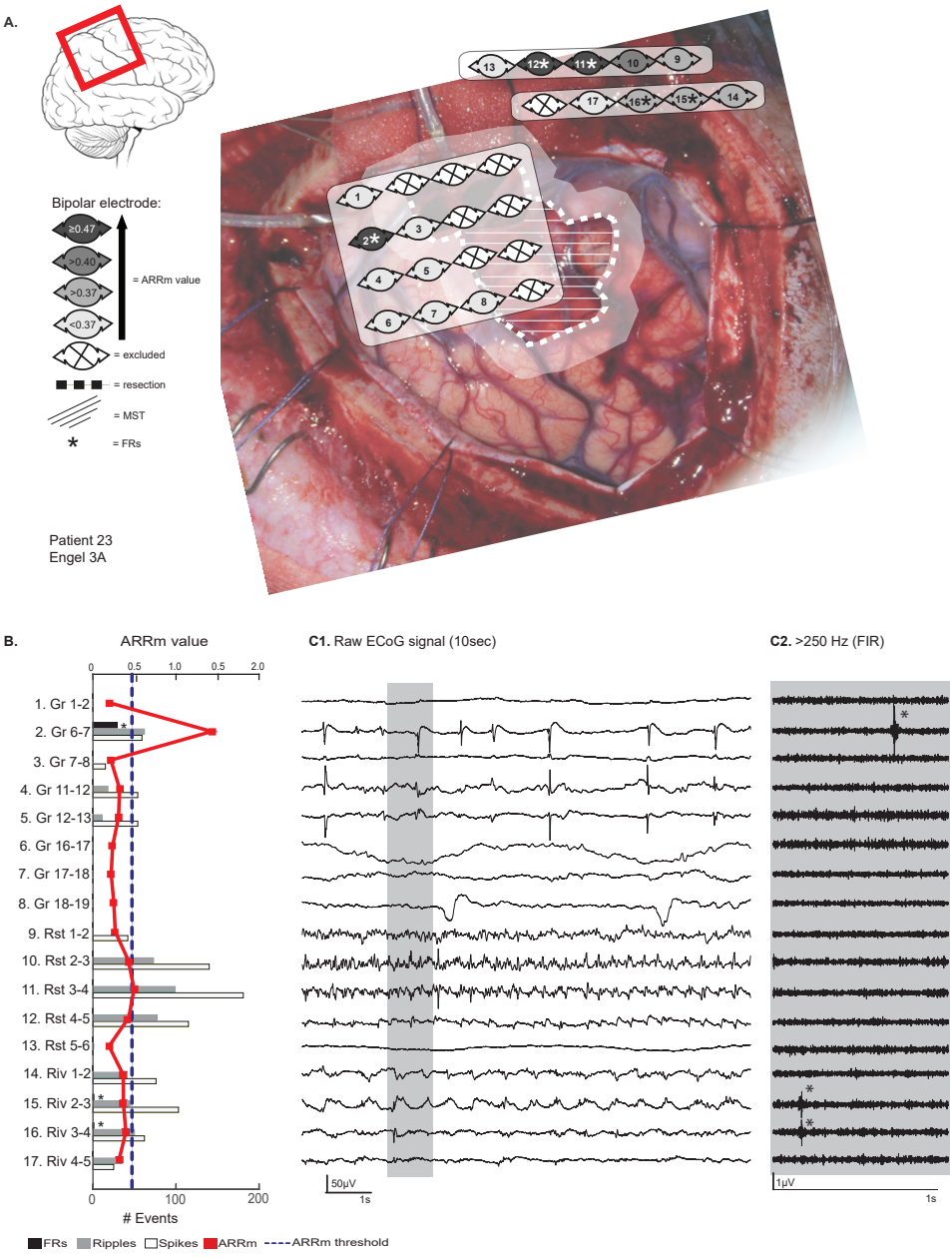
FIGURE 9.5
Performance $ARRm_{orig}$ and ARRm in pre-resection ECoG. Boxplot A.) and receiver operating characteristic (ROC) curve B.) illustrating the ability of $ARRm_{orig}$ (dark gray) and ARRm (light gray) to classify channels negative or positive for fast ripples. The FR- group contains channels without marked fast ripples and the FR+ group contains channels in which one or more fast ripples were marked. Outliers are not shown in the boxplots. The calculated ROC area under the curve (AUC) values for $ARRm_{orig}$ and ARRm are indicated in textboxes in the figure.

DISCUSSION

The AutoRegressive model Residual modulation (ARRm) algorithm provides a meaningful tool for intra-operative delineation of the epileptogenic zone. The algorithm is inspired by the most characteristic aspect of epilepsy; its unpredictable nature. In the ECoG ARRm is a 'bad' channel identifier rather than an event detector such as HFO detectors. We adjusted the ARRm to become less sensitive to subtle but influential artifacts in the intra-operative ECoG. We showed that brain tissue producing signals with high non-harmonicity corresponds to areas producing fast ripples, and to a lesser extent to ripples and spikes. High ARRm results had a positive predictive value of 88.9 % for poor outcome, including in patients without fast ripples. The ARRm calculated from inter-ictal post-resection ECoG could be an equally good or even better indicator of post-surgical outcome than residual fast ripples, provided that gross peri-surgical artifacts are prevented during recording. We believe that the ARRm algorithm has the potential to replace visual HFO analysis for tailoring of epilepsy surgery, as it can be quickly calculated and thus used in real-time during surgery.

AUTOMATED EPILEPTOGENIC ZONE LOCALIZATION IN LITERATURE

For decades, researchers have focused on the challenge of identifying automatically the precise location of the epileptogenic zone, by quantifying various signal aspects in the intracranial EEG[169-173]. The recent discovery of HFOs has inspired researchers to develop new algorithms for


A.
Bipolar electrode:
≥0.47
>0.40
>0.37
≤0.37
= ARRm value
= excluded
= resection
= MST
= FRs
Patient 23
Engel 3A
B.
ARRm value
1. Gr 1-2
2. Gr 6-7
3. Gr 7-8
4. Gr 11-12
5. Gr 12-13
6. Gr 16-17
7. Gr 17-18
8. Gr 18-19
9. Rst 1-2
10. Rst 2-3
11. Rst 3-4
12. Rst 4-5
13. Rst 5-6
14. Riv 1-2
15. Riv 2-3
16. Riv 3-4
17. Riv 4-5
Events
FRs
Ripples
Spikes
ARRm
ARRm threshold
C1. Raw ECoG signal (10sec)
50µV
1s
C2. >250 Hz (FIR)
1µV
1s

localization[55, 92, 167, 174]. The overall reported sensitivity of HFO detection algorithms varies between 50% and 100%, and the specificity ranges between 36% and 90% [174]. A drawback of the majority of HFO detectors is false positive detections, due to artifacts that are mistaken for HFOs or physiological high frequent activity, with false discovery rates reported as low as 13% but as high as 75%[93, 174]. The ability of non-linearity quantifiers to localize the epileptogenic zone in brain signals has been extensively studied[139, 173, 175-177]. Despite promising correlations between resection areas identified by such quantifiers and post-surgical seizure freedom[171, 173], many of these algorithms are unsuitable for automated online analysis during surgery, as measurements with an extensive duration are needed for reliable calculations. One of the strengths of the ARRm algorithm is that calculations are very fast with virtually no delay, as the relatively simple calculations are performed on short unfiltered segments of the raw ECoG signal instead of detection of separate events. Therefore, the algorithm could be used real-time during surgery.

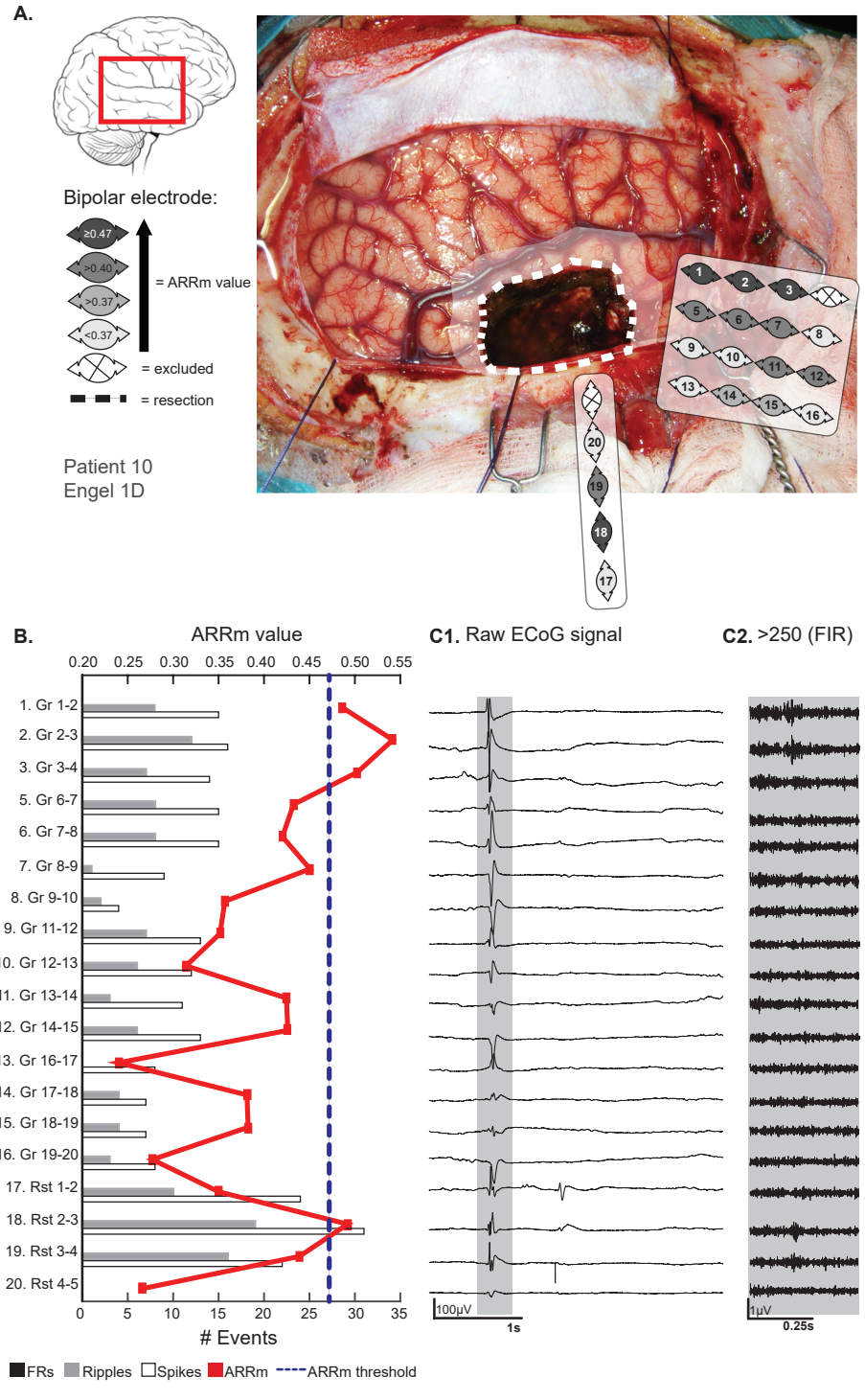
Non-harmonicity

Traditionally, residual signal variance after an autoregressive fit is regarded as noise, but in our application it indicates non-harmonic characteristics, i.e. the nonlinear or non-stationary dynamics of the underlying system generating the signal. The main assumption underlying ARRm methodology is that epileptic HFOs are, in contrast to physiological high frequent activity, of the non-harmonic type. In other words, they cannot be well described as solutions of a linear differential equation of a certain order[94]. Quantification of the variance of residual signal variation in the higher frequencies is thus used to delineate epileptogenic tissue. Previously autoregressive fitting was used to quantify inter-ictal spiking[169, 178]. One might argue that focusing on high values of the residuals in the ECoG data, which is essentially what the ARR algorithm does, is just another way of detecting inter-ictal spikes, with or without HFOs on top. Our results suggest that the ARRm is more a reflection of liability towards, rather than an indicator of, epileptiform transients measured on the electrodes. We see that not every electrode with spikes has a high ARRm value. Spikes are not necessarily associated with nonlinear quantifiers, but nonlinear quantifiers coincide quite well with the epileptogenic zone[179].

Our results give rise to the question of whether the ARRm algorithm would give the same results on 'clean' epochs without inter-ictal events. This was suggested by a study in which the authors found tissue epileptogenicity by studying signal properties of very short (1-2 second) epochs that did not exhibit inter-ictal events[180]. This should be studied further.

◀ **FIGURE 9.6**

Patient example of ARRm results. A.) A patient with neocortical epileptogenic lesion in the right central cortex that was resected and additionally multi subpial transections were performed (MST) (poor outcome). Pathology revealed a mild organizational disorder of the cortex. B.) Post-resection recordings showed multiple channels with spikes, but since this was an eloquent area, it was decided not to resect more tissue. However, in only few channels ARRm was elevated, similar to channels with fast ripples (*). C1.) Ten seconds of raw unfiltered intra-operative ECoG as recorded. Note that the ARRm results are calculated on this raw signal, while for marking of HFOs the signal has to be filtered. C2.) A close-up of the ECoG high-pass filtered at 250 Hz high-pass, showing fast ripples (*).


A.
Bipolar electrode:
≥0.47
>0.40
>0.37
<0.37
= ARRm value
= excluded
= resection
Patient 10
Engel 1D
1
2
3
5
6
7
8
9
10
11
12
13
14
15
16
20
19
18
17
B.
ARRm value
0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55
1. Gr 1-2
2. Gr 2-3
3. Gr 3-4
5. Gr 6-7
6. Gr 7-8
7. Gr 8-9
8. Gr 9-10
9. Gr 11-12
10. Gr 12-13
11. Gr 13-14
12. Gr 14-15
13. Gr 16-17
14. Gr 17-18
15. Gr 18-19
16. Gr 19-20
17. Rst 1-2
18. Rst 2-3
19. Rst 3-4
20. Rst 4-5
0 5 10 15 20 25 30 35
Events
FRs Ripples Spikes ARRm ARRm threshold
C1. Raw ECoG signal
100µV
1s
C2. >250 (FIR)
1µV
0.25s

Pathophysiology

A common difficulty in working with HFOs is the inability to distinguish physiological from pathological HFOs. Physiological HFOs may arise as a consequence of synchronous interneuron-mediated inhibitory postsynaptic potentials (IPSPs) that coordinate principal cells to fire sparsely [181]. In contrast, pathological ripples are thought to arise due to reduced IPSPs that give rise to brief bursts of population spikes[182]. Pathological fast ripples may result from slightly out-of-phase firing of independent clusters of these pathologically interconnected neurons. Proposed mechanisms for generating these fast pathological oscillations are ephaptic interactions, electrotonic coupling via gap junctions, or fast synaptic transmission[32, 80]. It is known that physiological HFOs can occur in functionally eloquent regions such as the sensorimotor [67] and visual cortex[37] and in the hippocampus, where ripples may be involved in memory processing[146]. In a previous study we described a patient who became completely seizure free (and stopped using medication) but showed residual spikes and fast ripples in Broca's area and the central motor area after surgery[79]. We found normal ARRm values in these regions in the same patient, suggesting that the ARRm might be insensitive for what is probably physiological oscillatory brain activity. This finding also confirms that pathological HFOs may have a more non-harmonic signature than physiological HFOs. This potential insensitivity of ARRm to physiological oscillations is also reflected in a better poor outcome prediction compared to fast ripples, as previously described[79]; the PPV was 88.9% for ARRm and 75.0% for fast ripples, while they have an equal NPV; 64.4% and 64.3% respectively[79].

Analogously, while the exact mechanism behind the non-linear behavior quantified by the ARRm remains unclear, we might be able to find some explanatory links between our method and its clinical results. The observation that modulation of the autoregressive residual variation localizes epileptogenic tissue, suggests that the processes causing high ARRm values are related to relatively short bursts of highly non-harmonic signal features interrupting the background activity. This is supported by the findings by Dumpelmann et al. (2012) who distinguished epileptogenic HFO activity on the basis of its non-stationary appearance[53]. The neuronal processes accountable for such bursts of exceptional activity might be avalanche dischargers as shown in computational models[183, 184]. An even more challenging question is how to relate those processes to the epileptic properties of neural circuits. Recently it was demonstrated that both epileptic transitions and action potential avalanches could be also be generated by excessive amounts of gap junctions[161].

◀ **FIGURE 9.7**

Patient example with ARRm results. A.) A patient with a right neocortical temporal epileptogenic lesion that was resected (poor outcome). Pathology revealed a ganglioglioma WHO grade II. (B) Post-resection recordings showed spikes and ripples, in the anterior temporal area, but no fast ripples (FR). The ARRm was elevated in 4 channels. C1.) Segment of raw unfiltered intra-operative ECoG as recorded. In the grey box, an event can be observed. This event was initially marked as an artifact. C2.) 250 Hz high-pass filtered ECoG data from the grey box in panel C1, showing fast activity that could be classified as fast ripples. If this is the case, the four channels with high ARRm values should be associated with paroxysmal events. These channels were then wrongly discarded during visual HFO marking, and falsely classified as fast ripple negative channels.

These discharges also generated high values of residual variance after auto-regressive model fitting. In resemblance to pathological HFOs, we speculate that a possible cause of high ARRm values is the presence of high electric conductance between the axonal compartments in the brain tissue picked up by the electrode.

STUDY LIMITATIONS

We implemented a threshold for the ARRm, based on results from pre-surgical ECoG recordings and the ability of ARRm to predict channels with fast ripples, with a specificity of 95%. Such a general threshold may not fit all individual patients, and could induce false negative results as shown by the results in group B (fast ripples but no ARRm+ channels). With respect to surgical outcome, only 8 of 24 patients with seizure recurrence showed an elevated ARRm in post-resection ECoG. The absence of epileptic biomarkers in the post-resection ECoG may be due to a too strict threshold definition, inadequate sampling of the cortex or ongoing epileptogenesis after surgery. A strength from a clinical perspective is that our unsupervised ARRm algorithm is a channel identifier rather than an event identifier. Surgeons require information about the channels, representing cm^2 of cortical tissue, that need to be resected or not. However, a more gradual scale could be preferred over this black-and-white decision offered by a thresholded quantifier. This way, ARRm values could indicate tissue epileptogenicity risk.

We incorporated artifact rejection designed to reject subtle but influential artifacts. This method did not work for all types of artifacts (figure 9.2). Intra-operative ECoG recordings are sensitive to artifacts and noise due to the electrical equipment and surgical manipulation. These types of artifacts could all be actively prevented by a 'time-out' procedure, in which all interfering devices are switched off if possible, and all hands are clear from the patient. It then takes only one minute of recording to calculate accurately the ARRm results.

Due to the retrospective nature of this study and the limited amount of data, it was not possible to investigate the stationarity of our results. It was recently suggested that the performance of automatic HFO detection depends on the type of resting state, and benefits from sleep rather than awake data[185]. This fits the findings that slow wave (non-REM) sleep gives the best HFOs signals[18, 186]. Intra-operative recordings are a hybrid form, where sedation induced by propofol is subsequently tapered until a continuous EEG pattern is reached. We know that propofol, also used as an anti-epileptic agent in status epilepticus, suppresses the amount of inter-ictal HFOs in the signal, but not their location[43]. If pathological HFOs and non-harmonicity are indeed linked, anesthetics and anti-epileptic drugs would influence non-harmonicity in the signal as well. This would be in line with evidence found for attenuation of non-linear EEG quantifiers by such drugs[177, 187, 188].

CLINICAL SIGNIFICANCE AND FUTURE DIRECTIONS

We showed that the ARRm predicts poor post-surgical outcome when measured after resection. It performs as well as, or maybe even better than, residual fast ripples[79]. There is an increasing need for implementation of automated algorithms to find the epileptogenic zone in clinical EEG software. Detection software requirements for clinical use in the operating theatre differ from long-term recordings, since time is precious and prolonged surgery is associated with increased

complication risks[189]. The ARRm is the first unsupervised real-time analysis that could be used intra-operatively to provide an 'on demand' interpretation per electrode about whether or not the underlying tissue needs to be removed. Future research should focus on further optimizing the ARRm algorithm and on finding the minimum recording time and the optimal threshold. Meanwhile, we should aim to enable artifact-free recordings in the operating theatre and know the influence of propofol on the non-harmonicity of the ECoG signal.

SUMMARY & DISCUSSION

CHAPTER 10

SUMMARY, DISCUSSION AND FUTURE PERSPECTIVES

SUMMARY

Epilepsy surgery is the sole treatment to cure patients with focal epilepsy. How well we can pinpoint the epileptogenic zone and delineate the margins of the diseased tissue for resection determines the chance on success: seizure freedom. Non-invasive pre-surgical diagnostic techniques such as MRI and scalp EEG help to lateralize and localize the region of interest. Long-term and intra-operative electrocorticography (ECoG) can be performed to exactly localize the epileptogenic tissue to guide the surgeon during the resection. Recording ECoG at a high sampling rate (>2000 Hz) enables recording of newly discovered high frequency oscillations (HFOs, >80-500 Hz) that seem to be a more precise biomarker of epileptogenic tissue than epileptiform spikes. The goal of intra-operative ECoG - so called tailoring - is threefold: 1.) to identify the exact location of the epileptogenic zone (pre-resection recordings), 2.) to guide the extent of resection (per-resection recordings), and 3.) to assess its completeness (final post-resection recordings).
The aim of this thesis was to lay a foundation for the clinical use of HFOs in the intra-operative ECoG for tailoring of the resection to increase the chance of seizure freedom after surgery.

PART I - INTERPRETATION OF HFOS
A mandatory first step for clinical use is to know how to interpret these HFOs in the light of the current clinical gold standard, inter-ictal spikes, in a retrospectively collected dataset. **Chapter 2** explores the differences in behavior of the 'new' HFOs, subdivided into fast ripples (250-500 Hz) and ripples (80-250 Hz), and 'old' spikes (<80 Hz) in recordings before and after the surgical resection. As expected the amount of fast ripples, ripples and spikes decreased after resection in most non-resected brain areas. More importantly the resection did not seem to cause new HFOs on the edge of the resection, an undesired behavior reported for spikes. We also found an increase in ripples independent of spikes in the sensorimotor cortex after successful surgery, suggesting that removing the epileptogenic focus leads to an increase in physiological ripples.
Chapter 3 shows that fast ripples that remained in the post-resection ECoG were the only prognostic marker for seizure recurrence. This finding highlights the importance of repeated recordings during surgery and a final post-resection ECoG, as this provides a prognosis for outcome. Tailoring could rely on fast ripples, but requires careful assessment of the signal in relation to the anatomy, as fast ripples in functionally eloquent areas might not be pathological.
In **chapter 4** we provide a strategy on how to tailor surgery based on fast ripples and spikes in combined pre- and post-resection ECoG recordings. In addition to the findings in chapter 3, especially fast ripples that persist after resection predicted seizure recurrence. The absence of spikes altogether seemed to predict good surgical outcome. Incomplete resection of spikes, ripples or fast ripples present in pre-resection recordings alone did not show any predictive value for outcome.

PART II - INTERVENTION WITH HFOS
The benefit of tailoring based on HFOs in the intra-operative ECoG could be a smaller resection, with an accompanying reduced chance of neurological deficits, which – together with an equal

or increased seizure freedom rate – may lead to an overall better quality of life. Randomized controlled trials (RCT) are considered the gold standard to evaluate therapeutic interventions, but are not trivial in the field of epilepsy surgery. Important considerations are feasibility, influenced by standardized diagnostic and treatment procedures, as well as recruitment speed. Dutch epileptologists have gained extensive experience with intra-operative ECoG and established a standardized surgical protocol over the years which makes the Netherlands the perfect testing ground for a first RCT initiative. **Chapter 5** describes the details of a single-blinded multi-center RCT to determine prospectively if intra-operative ECoG tailored surgery using HFOs, instead of inter-ictal spikes, is feasible and will lead to an equal or better post-operative seizure outcome (non-inferiority). An unique feature of the trial is the inclusion of children, as a shift in epilepsy surgery towards the pediatric population has been set in motion over the last years. "The HFO trial" was approved in 2014 by the medical ethical board of the University Medical Center Utrecht, and the first patient (9 years old) was enrolled in November that same year. At the moment of writing this summary, June 2016, in total 30 patients, out of 78 participants needed, are included. The results of this interventional trial are expected in 2018.

PART III - INNOVATION DRIVEN BY HFOS

A key issue for clinical implementation of HFOs for tailored surgery in the international epilepsy surgery community is simplified and automated analysis of HFOs. **Chapter 6** describes time-frequency analysis of single pulse electrical stimulation (SPES) that evoked pathological delayed responses (>100 ms after stimulation) in the frequency range of spikes, ripples and fast ripples. These evoked HFOs in long-term ECoG, especially fast ripples, are highly specific for the seizure onset zone (SOZ) and thus can serve as a tool for delineation of the epileptogenic cortex. SPES not only results in delayed pathological responses but also early physiological time-locked responses (<100 ms after stimulation). In **chapter 7** we show that electrodes with many early responses appear more frequently in- than outside the SOZ. Stimulating the SOZ evoked early responses that were associated with the seizure propagation area. In other words, early responses evoked by SPES can add information for identification of epileptic cortex. As they occur more frequently than delayed responses they could be used to prune the duration of the stimulation protocol. Can we simply replace inter-ictal spontaneous HFOs by evoked HFOs? Yes we can, as we showed that evoked and spontaneous HFOs behave similarly with respect to identification of the seizure onset zone (**chapter 8**). SPES can elicit SOZ- specific FRs in patients without spontaneous FRs, but activates ripples in all regions which is unrelated to functional eloquent cortex. Unfortunately, SPES could not aid in the differentiation between physiological and pathological HFOs. In **chapter 9** we took an alternative approach. Exploration of non-harmonicity in the ECoG signal reveals a measure to identify electrodes covering epileptogenic tissue, rather than a method for detecting each individual HFO. Brain tissue producing signals with a high auto-regressive residual modulation value corresponds to areas producing epileptiform biomarkers, but more importantly relates to post-surgical outcome. This measure might provide a tool for intra-operative delineation of epileptogenic tissue as it can be rapidly computed, is insensitive to short artifacts and less sensitive for physiological fast ripples.

In conclusion, intra-operative tailoring based on HFOs - fast ripples in particular - next to spikes could improve the prognosis for the seizure freedom after surgery. The use of HFOs proofs to be feasible in clinical practice, although caution is advised with respect to the occurrence of physiological HFOs. The presented innovative signal analytical approaches to automate, standardize and optimize the yield of HFOs detection procedures in the intra-operative recordings strengthens the basis of clinical use of HFOs for epilepsy surgery.

DISCUSSION & FUTURE PERSPECTIVES

The studies described in this thesis lay a foundation for the clinical use of HFOs during epilepsy surgery in order to improve the overall success rate of this curative treatment for focal epilepsy. This was done by investigating how to interpret HFOs, by testing intervention based on HFOs in a clinical setting and by developing innovative signal analysis methods. This discussion provides a synthesis of this process, what we learned and what challenges are yet to overcome.

CONSIDERATIONS ON METHODOLOGY

The use of HFOs in epilepsy surgery can improve the chance of seizure freedom (chapter 3). But can we now consider spikes obsolete? Probably not, as long as we use visual analysis of EEG signals we need spikes in order to judge the HFOs, as they usually co-occur, and to discard artifacts. The choice for spikes or HFOs might depend on the tailoring strategy for the individual patient (chapter 4). Studied patient groups in epilepsy research are usually small and heterogeneous. In order to avoid fragmentation, we need to join forces internationally to enable targeted large data studies. Information from detailed studies might enable us to personalize tailoring strategies in the near future.

Second, it is not trivial to define surgical success. The perspective of a patient on success might differ from that of scientists or doctors. This is reflected by the Engel classification that distinguishes between (almost) seizure free, worthwhile improvement and no improvement[2, 5]. Currently more attention is given to the effect of surgery on cognitive improvement, especially in children. Reversibility of cognitive problems can be considered as an equally important gain for the patient's quality of life, as absolute seizure freedom[11]. Ongoing study of our database shows that the removal of a large amount of fast ripples predicts better cognitive outcome (unpublished). We hypothesize that the increase in ripples in the central region after resection in patients that became seizure free might be an early indicator of healthy network reorganization reflecting the plasticity of the brain (chapter 2). Instead of writing off physiological HFOs as unwanted confounders, we might be able to use them as an additional biomarker to monitor surgical success in terms of cognitive improvement.

The true success of surgery can only be determined after discontinuation of anti-epileptic medication, requiring long-term follow-up. In part I we differentiate between complete seizure freedom (Engel 1A) and seizure recurrence including auras (all other classes) in order to evaluate the predictive value of HFOs on seizure outcome. Auras can be considered as simple partial seizures and thus an indicator that some epileptic tissue is still present[73]. Since we consider fast ripples as a very specific biomarker for epileptogenic tissue, we still might come across remaining fast ripples post-resection in those patients (chapter 3). Although complete removal of fast ripples is necessary to achieve true long-term seizure freedom, removing the majority of fast ripples might be enough to achieve improvement in disease severity, which still might be considered a surgical success in individual cases.

Third, we do not find residual fast ripples in all patients with poor outcome (chapter 2, 3, 4). Fast ripples are considered a very focal phenomenon, so they are easily missed. Drawback of intra-operative ECoG recordings is the short data segments that can be recorded combined with a

sparse sampling of the cortex (chapter 3, 9). The use of high density grids - smaller and more electrodes per square centimeter - would improve the resolution and thereby increase the sensitivity for fast ripples.

Finally, the focus of this thesis lies on pathological HFOs, but we came across physiological HFOs in functionally eloquent regions, both spontaneous (part I) as well as evoked (chapter 8). Questions that remain unanswered are 'How do we distinguish these physiological HFOs from pathological HFOs?' and 'How do we deal with them?' Some will advocate that until these questions are answered it is still too early to start using HFOs for clinical tailoring. Although tailoring based on spikes is considered the gold standard it is not an evidence based method. The discussion on harmless 'green' and harmful 'red' epileptic spikes is still ongoing. It is important to realize that the same restrictions for tailoring are valid for HFOs as for spikes; The ECoG findings are used to either confirm or adapt the surgical plan. Reduction or extension of the planned resection is restricted to non-eloquent cortex. Cortex with spikes occurring remote from the resection site are usually not removed[90]. Altogether, this is why we deemed it ethical to initiate the first RCT on HFOs compared to spikes for surgical tailoring as described in chapter 5. In this randomized controlled trial precautions were taken by excluding certain areas, e.g. occipital or central motor regions, from HFO analysis and ensured careful assessment of HFOs in the light of the clinical hypothesis of the individual patient. Decision making is primarily based on fast ripples, but in the absence of fast ripples distinct high amplitude ripples on top of spikes are considered.

IMPLICATIONS FOR FUTURE HFO RESEARCH

Pathological versus physiological HFOs

Still little is known about the generating mechanisms of HFOs. The thought that fast ripples are exclusively pathological might be wrong. Cumulating evidence from animal, cell and computer models shows that gap junctions are responsible for the synchronous coupling of neurons[80, 161, 182] that generates pathological fast ripples, but other candidate mechanisms may be held responsible for physiological fast ripples. Healthy ripples are thought to occur due to inhibitory mechanisms, whereas pathological ripples are thought to arise due to excitatory mechanisms[190, 191]. This might reflect a protective rather than harmful nature of physiological ripples, similar as is suggested for spikes[192, 193]. Increasing knowledge on the generating mechanism will help to develop automated analytical methods to differentiate between physiological and pathological HFOs which will simplify clinical decision making.

The relation between HFOs and spikes

Another missing link is the relation between HFOs and spikes. HFOs and spikes often occur simultaneously, but not every spike coincides with an HFO and vice versa. It is unknown if the spike triggers the HFO or the HFO the spike or if their co-occurrence is a coincidence. Combined invasive EEG with micro-electrodes **-** very small wires that measure signals at the individual neuron level - could give us more insight in the generating mechanisms underlying spikes and HFOs in the (invasive) EEG signal. Micro-wires can sample the local field potential of a very small patch of tissue. In this local field potential we can study spikes and HFOs, and in

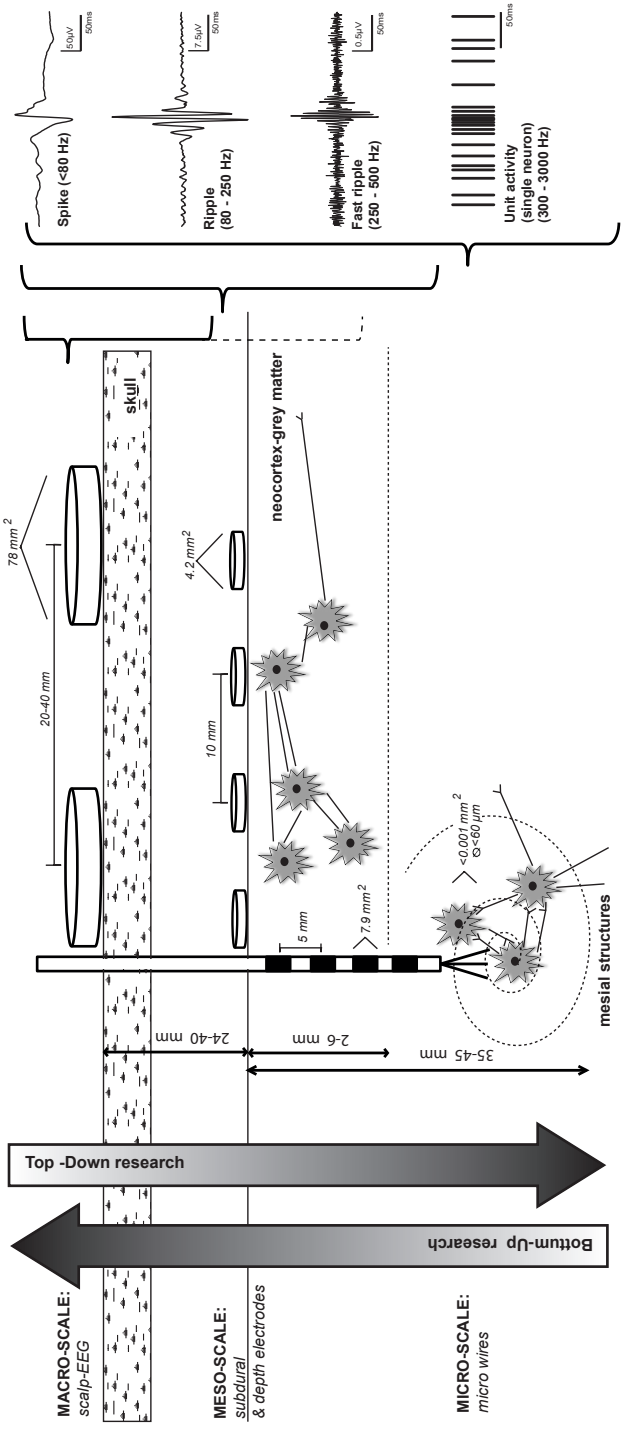


FIGURE 10.1
Schematic overview of the different EEG scales to illustrate the importance of HFO research at combined micro-, meso-, and macro scale to unravel all characteristics of HFOs and their ultimate potential for diagnosis and treatment of epilepsy patients. Note that the here depicted specifications of electrode size and contact surfaces were based on the currently used equipment in the UMC Utrecht.

addition we can analyze action potentials of individual neurons. Clustering analysis of these action potentials enables differentiation between inhibitory and excitatory potentials. The occurrence of HFOs and spikes correlates to changes in neuronal firing[194-196], and should be further studied.

We should study simultaneous EEG recordings at different scales to fully understand the mechanisms underlying HFOs, their relation to spikes and their clinical impact; at micro-scale using micro wires, at meso-scale in invasive depth and subdural electrodes, and at macro-scale in non-invasive scalp EEG[191]. Research in the near future should contain both top-down and bottom-up research over these scales, to bridge the gap between patient-based clinical research, focused on macro- and meso-level, and more fundamental research, focused on meso- and micro-level (figure 10.1). The interplay between clinical and fundamental research is essential to fill the gaps in our knowledge about HFOs.

Automated HFO analysis

Visual marking of HFOs is tedious, which makes quantitative EEG analysis of HFOs the Holy Grail. HFOs are considered better suited for computerized detection than spikes. From a signal analytical point of view the shape of HFOs seems promising, they are short successive oscillations with rapid increasing then decreasing amplitude that clearly stand out from the background activity[25]. Frequently applied measures that can describe such irregularities in time are the signal strength expressed as variance of energy or power of the signal in a certain frequency band[84, 98, 197, 198] and the variability of information characterized by entropy[44, 55, 91]. Detectors that identify HFOs based on time frequency plots, similar to the techniques described in chapter 6, 7 and 8, seem more robust and better equipped to handle artifacts, but are computationally time consuming[40, 89, 92, 174, 185]. Drawback of the majority of developed HFOs detection algorithms is that they detect more events than there actually are, therefore often visual post-processing is required. Generalizability of developed HFO detectors on different types of data from different centers is not trivial, but essential to compare their performance[44].

The ideal HFO detection algorithm for intra-operative ECoG works unsupervised, analyses the data real-time, and identifies electrodes covering tissue that needs to be resected. Preferably, it includes information about the temporal and spatial relation between each identified HFO and simultaneously occurring HFOs in other electrodes[47]. In a recent collaboration the results of chapter 3 – residual fast ripples in post-resection data predict outcome - were reproduced using an unsupervised time-frequency based HFO detector instead of visual analysis. As this detector is reasonably fast and accurate it could be used intra-operatively[156]. In chapter 9 we took a completely different approach; Instead of counting each individual HFO, the algorithm calculates per electrode the amount of unexplained non-linear residual information in the signal. Results described in chapter 9 suggest that it is worthwhile to look beyond individual HFOs and spikes. There are studies that indicate that apart from those paroxysmal epileptiform events, information about the epileptogenic network is intrinsically present in the background signal. These studies, based on functional network analysis or non-linearity of the signal, can find the epileptogenic focus in 'clean' segments of EEG without events[180, 199]. This would also solve the question about the duration of ECoG recordings required to capture enough HFOs,

as they occur less frequent than spikes[43, 79]. We recently started studying high frequency functional network analysis in ECoG and hope that in combination with HFOs we can further improve delineation of the epileptogenic tissue. This direction of research converges with the hypothesis that epilepsy is a network disease rather than a focal disease of the brain as defined by the hypothesized epileptogenic zone by Luders (chapter 1). Removal of the main core of the network, instead of all its outward extended connections, could be sufficient to gain seizure freedom. The results described in chapter 4 that incomplete removal of tissue that shows fast ripples before resection, which abolish after resection, is associated with good outcome, might support this hypothesis.

HFOs on demand

In chapter 6, 7 and 8 we propose a method to increase the yield of HFO-detection by means of electrical stimulation. We can elicit seizure onset zone specific fast ripples by single pulse electrical stimulation (SPES). This means we do not have to wait for seizures nor for spontaneous HFOs to occur (chapter 6, 8). We might be able to accelerate this stimulation protocol by means of a feedback loop based on early and delayed responses (chapter 6 and 7). Such a feedback algorithm that provides HFOs on-demand will enable us to rapidly zoom in on the epileptogenic zone during surgery. Its benefits are twofold, as it can be used for targeted placement of grids for long-term ECoG monitoring and improve the accuracy of intra-operative ECoG based tailoring of resections in a larger group of patients. Instead of looking for responses only, we can investigate the direction of responses on SPES. Recently bi-directionality of connections after stimulation were described as a prerequisite of epileptogenic tissue[143]. Further study on the effect of electrical stimulation can help understand the (patho-) physiology of HFOs and the connectivity in the epileptogenic network.

The additional value of non-invasive HFOs

Invasive EEG recordings will always be limited by the amount of electrodes that can be implanted and the surface that can be covered. Scalp EEG and MEG give a complete overview of the brain surface, so HFOs in scalp EEG or MEG could help pre-surgical targeting of depth-EEG electrodes or ECoG grids[59, 200]. Non-invasive HFO analysis could aid monitoring after tailored surgery; 1.) In patients with recurrent seizures in whom residual HFOs in the post-resection ECoG were absent, it could help to identify missed HFO areas and thereby help to build a case for a second surgery. 2.) In patients with recurrent seizures in whom residual HFOs were seen, it could facilitate individualized anti-epileptic drug policies, as it is known that HFOs are reduced by anti-epileptic medication mirroring the suppression of seizures[43, 69]. 3.) In patients that seem seizure free it can confirm the post-resection ECoG prognosis, and could indicate the safety of early withdrawal of anti-epileptic drugs.

EEG combined with transcranial magnetic stimulation (TMS-EEG) might provide a non-invasive alternative to single pulse electrical stimulation, as it could elicit delayed responses, and perhaps HFOs as well, in patients with focal epilepsy[201].

RECOMMENDATIONS FOR THE FUTURE OF EPILEPSY SURGERY

The increase in surgical expertise and experience over the years causes a shift in the candidates considered eligible for epilepsy surgery. Nowadays, epilepsy surgery is increasingly performed in young children and in patients with neocortical extra-temporal epilepsies, whereas mesio-temporal lobe surgery in adults is decreasing in frequency[11, 105, 202]. This shift is driven by new technological advances, such as the 7 Tesla MRI to identify previously invisible lesions[203], as well as the increased knowledge about the effect of surgery on social and cognitive development and quality of life of patients[10, 106]. The earlier the surgery, the more the patient has to gain. The perception that surgery is offered as a last resort might not hold for very long. In all patients with focal epilepsy with presumed structural etiology, drug resistant or not, pros and cons of surgical treatment should be considered at an early stage, as it is an attractive alternative for life-long use of anti-epileptic medication with all its side-effects.

Although not all questions about the underlying mechanisms, pathological significance and predictive value of HFOs are answered yet, it is time to take a next step. Centers that deemed traditional tailoring based on spikes ineffective might rethink their strategy when it is proved that tailoring based on HFOs is feasible and leads to equal or improved outcome (chapter 5). For a sustained clinical acceptation of HFOs international collaboration and consensus is crucial. Evidence based medicine ultimately requires proof of superiority of HFOs over spikes, demanding a large international RCT in order to cover the large sample size needed - ±300 participants, based on the same assumptions as the non-inferiority trial in chapter 5 - . Feasibility of RCTs in epilepsy surgery is severely influenced by standardized diagnostics and treatment procedures and subjectivity of human clinical decision making. Crucial steps to create an international standard would be to 1.) share intra-operative ECoG data to develop an HFO detector that can be applied on all types of data, independent of the software and hardware used for recording, and 2.) implement this algorithm into clinical EEG software to give everybody access to the same signal analysis method. Hopefully research in the near future will lead to objective 'tailoring' tools that can assist the surgeon. In addition, if the surgeon can be in charge, this could propel use of HFOs in an even bigger field of neuro-oncology. Here the focus lies on the removal of the tumor to improve prognosis on life-expectancy, but the patients quality of life can be improved as well if surgeons are enabled to identify and remove the accompanying epilepsy generating tissue[204].

This digital era we live in, allows us to collect increasing amounts of patient data during pre-, per- and post-surgical work-up. Recent developments include multi-modal imaging platforms, in which EEG, ECoG, MRI, PET, and MEG data of each individual patient can be combined[205]. Such multi-modal platforms, linked with automated signal analysis, will have a significant impact on the success rate of epilepsy surgery at the individual patient level. Scientific research will evolve too, as it enables comparison of multiple diagnostics over different scales. These technological advances in medicine demand new professions in health care, such as the technical physician, that can assist clinicians and connect innovative research with patient care.

In conclusion, HFOs have the potential to improve surgical decision making, tailoring, and seizure outcome after epilepsy surgery in a large patient population. In order to better appreciate to what extent HFOs have added value over conventional EEG measures, how to optimize their yield and to create worldwide acceptance, three key ingredients are necessary; intensive international collaboration, multi-scale research, and a pioneer spirit in order to accelerate the transition from research to clinical care.

APPENDICES

References
Samenvatting | Dutch summary
Dankwoord | Acknowledgements
List of publications
Curriculum Vitae

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SAMENVATTING | DUTCH SUMMARY

Epilepsie chirurgie is de enige genezende behandeling voor patiënten met een duidelijke epilepsiehaard (lokalisatie-gebonden epilepsie). De succeskans van deze operatie, oftewel de kans op aanvalsvrijheid, wordt bepaald door de nauwkeurigheid waarmee het epileptogene gebied gelokaliseerd kan worden en de randen van het zieke weefsel afgegrensd kunnen worden. Niet-invasieve pre-chirurgische diagnostische technieken zoals MRI en oppervlakte EEG dragen bij aan het bepalen welke hersenhelft betrokken is en het lokaliseren van het epileptogene hersengebied. Het invasieve intra-operatieve electrocorticogram (ECoG) kan vervolgens worden gebruikt om het epileptogene weefsel zeer precies te lokaliseren. Het ECoG dient als leidraad voor de chirurg tijdens de operatie waarbij het afwijkende weefsel wordt verwijderd (de resectie). Registratie van het ECoG met een hoge sample rate (>2000 Hz) maakt het mogelijk om de recent ontdekte hoog-frequente oscillaties (HFOs, >80-500 Hz) op te vangen. Deze HFOs lijken preciezer het epileptische weefsel weer te geven dan de tot nu toe gebruikte inter-ictale (tussen aanvallen in) epileptiforme pieken. Het doel van het intra-operatieve ECoG, ook wel 'tailoring' genoemd, is drieledig: 1.) het identificeren van de exacte locatie van het epileptogene gebied (pre-resectie meting), 2.) het dirigeren van de uitbreiding van de resectie (per-resectie meting), en 3.) het bepalen van de volledigheid van de resectie (laatste post-resectie meting).
Dit proefschrift legt een basis voor het daadwerkelijke klinische gebruik van HFOs in het intra-operatieve ECoG ter afgrenzing van het epileptogene weefsel, om hiermee de kans op aanvalsvrijheid na epilepsie chirurgie te vergroten.

DEEL I – INTERPRETATIE VAN HFOS

Een vereiste eerste stap voor het klinische gebruik van HFOs is het opbouwen van kennis over hoe deze HFOs geïnterpreteerd dienen worden in vergelijking tot de huidige gouden standaard; de inter-ictale pieken. Dit is onderzocht in een retrospectief verzamelde database van intra-operatieve ECoG metingen. In **hoofdstuk 2** worden de verschillen in gedrag verkent van de 'nieuwe' HFOs, onderverdeeld in fast ripples (250-500 Hz) en ripples (80-250 Hz), in vergelijking tot de 'oude' pieken (<80 Hz) in metingen voor en na de resectie. Zoals verwacht, neemt na resectie het aantal fast ripples, ripples en pieken af in bijna alle niet verwijderde hersengebieden. Een belangrijke bevinding is dat chirurgische manipulatie niet leidt tot nieuwe HFOs aan de rand van de resectie.Dit gebeurt wel bij pieken en is ongewenst. Een toename van ripples die onafhankelijk van pieken voorkomen werd gevonden in de primaire sensore en motore cortex na succesvolle operaties. Dit suggereert dat het verwijderen van de epileptische haard leidt tot een *toename* van ripples die als gezonde activiteit, ook wel fysiologisch genoemd, kunnen worden beschouwd. **Hoofdstuk 3** laat zien dat het achterblijven van fast ripples in het post-resectie ECoG de enige voorspeller is voor blijvende aanvallen na een operatie. Dit onderstreept het belang van herhaalde metingen tijdens de operatie, en ook van een laatste post-resectie meting omdat deze een prognose geeft voor het succes van de operatie. De fysiologische variant van fast ripples kan echter ook voorkomen in functioneel onmisbare hersengebieden. Tailoring op basis van fast ripples is mogelijk, mits een zorgvuldige analyse van het signaal in relatie tot de anatomie plaats vindt. In **hoofdstuk 4** wordt de de beste strategie voor tailoring van de operatie onderzocht,

gebaseerd op HFOs en pieken in gecombineerde pre- en post-resectie ECoG metingen. Het incompleet verwijderen van spikes, ripples en fast ripples aanwezig in pre-resectie metingen is geen op zichzelf staande voorspeller voor de succeskans van de operatie. In aanvulling op de bevindingen van hoofdstuk 3, blijkt dat het voortbestaan van fast ripples na resectie, die ook al aanwezig waren vóór resectie, wel voorspellend is voor blijvende aanvallen. De áfwezigheid van pieken lijkt een voorspeller van aanvalsvrijheid.

DEEL II – INTERVENTIE OP BASIS VAN HFOS

Een voordeel van tailoring gebaseerd op HFOs in het intra-operatieve ECoG is een mogelijk kleinere resectie, met een bijkomende verkleinde kans op uitval van belangrijke hersenfuncties. Samen met een zelfde of betere kans op aanvalsvrijheid kan dit leiden tot een algehele betere kwaliteit van leven voor deze patiënten. Gerandomiseerde gecontroleerde klinische trials (RCTs) worden gezien als de beste methode om een nieuwe therapeutische behandeling of interventie te evalueren. Dit type studies zijn zeldzaam binnen het vakgebied van epilepsie chirurgie. Belangrijke overwegingen zijn de uitvoerbaarheid, die wordt beïnvloed door gestandaardiseerde diagnostiek en behandelprocedures, en de snelheid van de werving van deelnemers. Nederlandse epileptologen hebben uitgebreide ervaring met het intra-operatieve ECoG en hebben over de jaren heen een gestandaardiseerd chirurgisch protocol opgezet. Dit maakt Nederland de ideale bakermat voor de eerste RCT naar het klinische gebruik van HFOs. **Hoofdstuk 5** beschrijft de details van een enkelvoudig geblindeerde multi-center RCT om prospectief te bepalen of tailoring gebaseerd op HFOs, in plaats van op inter-ictale pieken, in het intra-operatieve ECoG mogelijk is en of het leidt tot eenzelfde of zelfs betere uitkomst na de operatie (non-inferioriteitsonderzoek). Uniek bij deze studie is de inclusie van kinderen, waarbij epilepsie chirurgie in toenemende mate plaats vindt. De HFO studie is goedgekeurd door de medisch ethische toetsingscommissie van het Universitair Medisch Centrum Utrecht. De eerste patiënt (9 jaar oud) werd geïncludeerd in november 2014. Op het moment van het schrijven van deze samenvatting (juni 2016) zijn in totaal 30 patiënten, van de benodigde 78, geïncludeerd. De resultaten van deze interventiestudie worden verwacht in 2018.

DEEL III – INNOVATIE GEDREVEN DOOR HFOS

Essentieel voor het wereldwijde klinische gebruik van HFOs tijdens operaties met tailoring is de ontwikkeling van simpele en geautomatiseerde analyse methoden. **Hoofdstuk 6** beschrijft tijd-frequentie analyse van single pulse elektrische stimulatie (SPES) waarbij late responsies (>100 ms na stimulatie) uitgelokt kunnen worden in de piek-, ripple- and fast ripple- frequentiebanden. In het langdurige ECoG blijken deze uitgelokte HFOs, en uitgelokte fast ripples in het bijzonder, zeer specifiek voor de locatie van het epileptische weefsel waar de aanval begint. SPES kan dus gebruikt worden bij het afgrenzen van het epileptogene hersenweefsel. Naast deze late responsies gerelateerd aan afwijkend hersenweefsel, lokt SPES ook vroege fysiologische responsies uit (<100 ms na stimulatie). **Hoofdstuk 7** laat zien dat elektroden met veel vroege responsies vaker voorkomen in het gebied met het aanvalsbegin dan daarbuiten. Het stimuleren van dit aanvalsgebied geeft vroege responsies in gebieden die overeenkomen met het gebied van de aanvalsspreiding. Vroege responsies uitgelokt door SPES kunnen dus ook een bijdrage leveren

aan de identificatie van het epileptogene hersenweefsel. Omdat vroege responsies vaker voorkomen dan late responsies, kunnen ze wellicht gebruikt worden om het stimulatie protocol te versnellen. Mogen de inter-ictale spontane HFOs simpelweg vervangen worden door uitgelokte HFOs? Ja, dat mag. Uitgelokte en spontane HFOs gedragen zich hetzelfde ten aanzien van het identificeren van het aanvalsbegin (**hoofstuk 8**). SPES lokt fast ripples uit die specifiek zijn voor het aanvalsbegin, maar activeert echter ook ripples in allerlei andere hersengebieden, waaronder onmisbare functionele hersengebieden. SPES kan daarom niet helpen om afwijkende oftewel pathologische HFOs van gezonde fysiologische HFOs te onderscheiden**.** In **hoofdstuk 9** wordt een innovatieve methode voor automatische analyse van HFOs in het ECoG signaal beschreven: een hoge mate van niet-harmoniciteit in het ECoG signaal identificeert elektrodes die over epileptogeen weefsel liggen. Dit is een heel andere benadering dan de tot nu toe ontwikkelde methoden die vooral gebaseerd zijn op detectie van individuele HFOs in het signaal. Hersenweefsel dat signalen produceert met een zogenoemde hoge 'auto-regressive residual modulation' waarde komt overeen met gebieden die epileptische biomarkers (spikes, HFOs) genereren en, belangrijker, is gerelateerd aan de succeskans na operatie. Deze methode lijkt zeer geschikt voor intra-operatieve afgrenzing van epileptogeen weefsel vanwege de korte benodigde rekentijd, de ongevoeligheid voor subtiele artefacten en een verminderde gevoeligheid voor fysiologische fast ripples.

Intra-operatieve tailoring gebaseerd op HFOs, en fast ripples in het bijzonder, als toevoeging op of ter vervangen van pieken zal de succeskans van epilepsie operaties doen toenemen. Het gebruik van HFOs in de klinische praktijk is mogelijk, hoewel men bedacht dient te zijn op het voorkomen van fysiologische HFOs. De gepresenteerde innovatieve signaal-analytische methoden om de opbrengst van HFO-detectie methoden te optimaliseren, standaardiseren en automatiseren versterkt de basis voor het gebruik van HFOs ten bate van epilepsie chirurgie.

DANKWOORD | ACKNOWLEDGEMENTS

Het lang verwachte moment is daar, mijn boekje is af! Zonder de steun en bijdrage van vele anderen was dit niet gelukt, mijn dank is groot. Op deze plek wil ik een aantal mensen persoonlijk bedanken:

Prof. dr. Braun, beste Kees, dank voor de vrijheid die je mij de afgelopen jaren hebt gegeven. Jouw frisse blik op mijn werk, enthousiasme voor de wetenschap en je kritische kanttekeningen heb ik altijd zeer gewaardeerd. Bijzonder en fijn vond ik de momenten waarop jij de, soms zo broodnodige, rol van afremmer in plaats van aanjager op je nam.

Dr. Zijlmans, beste Maeike, zonder jou was dit project en dit proefschrift er niet geweest. Bedankt dat je samen met mij het HFO avontuur wilde aangaan. Ik heb zo veel van je mogen leren; jouw neus voor interessante resultaten en schrijftalent zijn niet te evenaren. Fijn dat ik altijd met je kan sparren en kan lachen zowel in werk als privé sfeer. Ik kijk met veel plezier terug op de gedeelde hotelkamers, de etentjes en de dansjes tijdens de vele congressen en onze gesprekken op de fiets naar Utrecht CS. Ik bewonder hoe jij jouw eigen team met scientific ladies hebt opgebouwd en ze in toom houdt. Tegelijkertijd ben ik je dankbaar voor de ruimte die je mij biedt om mijn eigen pad te bewandelen. Zowel links- als rechtsom zijn we een goed team! Ik hoop dan ook dat we elkaar in de toekomst tegen blijven komen.

Dr. Huiskamp, beste Geertjan, je was mijn vaste kamergenoot de afgelopen jaren. Je bent van onschatbare waarde voor de KNF, voor mij en alle andere studenten; jij staat altijd klaar om te helpen, mee te denken en op te lossen. Jouw onuitputtelijke creatieve geest leidt niet alleen tot mooie wetenschappelijke hypothesen maar ook tot de leukste gesprekken. Ik ga onze dagelijkse onderonsjes over epic failures, de vuile was, aioli en andere Spaanse hapjes, de KNF-tour tussenstand, de kattenfotos en niet te vergeten de koffie missen.

Dr. Leijten, beste Frans, vanaf het allereerste moment dat ik als student binnenkwam bij de KNF ben je bij mijn onderzoek betrokken geweest. Ik wil je graag bedanken voor het feit dat je altijd op de bres sprong voor mij als Technisch Geneeskundige, voor de EEG lessen, voor de vaderlijke adviezen, en voor de etentjes in Hoofddorp over de jaren heen. Jouw liefde voor het vak is groots en inspirerend!

Prof. dr. Ramsey, Prof. dr. Joëls, Prof. dr. ir. Maurits, Prof. dr. Vonck and Prof. dr. Engel Jr., thank you for being part of the thesis committee. Prof. van Huffelen, ik voel me vereerd met uw aanwezigheid als voorzitter van mijn promotieplechtigheid.

I want to thank all co-authors of the publications in this thesis, as well as all international researchers with whom I collaborated over the past years.

I like to thank the all the people at the lab at UCLA, in particular Rick Staba, for giving me the opportunity to learn so much about micro-recordings during my short research visit. Shennan

Weiss, thanks for putting up with this brutally honest Dutchie; I have learned so much from you. Steph Moy, thanks for the sweets, the yarn expeditions, and above all being such fun company. I am confident you are going to be an amazing doctor and researcher.

Ook wil ik iedereen bedanken die mee heeft gewerkt aan de totstandkoming van de clinical trial "de HFO studie". Een spannende uitdaging die jullie toch aan durfden te gaan. In het bijzonder Peter van Rijen en Peter Gosselaar, Janine Ophorst, Monique van Schooneveld, Martine van Zandvoort en de KNF-ECoG laboranten (Bert, Jesper, Bea, Barbara en ook Linda), bedankt voor al het meedenken en de hulp bij de uitvoering! Ook de mensen bij de VUmc wil ik bedanken, in het bijzonder Hans Baaijen, Sander Idema , Hanneke Ronner, Peterjan Ris en Annette Hospes; jammer dat het er niet van heeft mogen komen. Eltje Bloemen dank voor al je positiviteit en praktische tips. Sophie Goemans dank voor de ondersteuning bij al het papierwerk.
Ook alle (ouders van) patienten die bereid zijn (geweest) mee te werken aan "de HFO studie" wil ik van harte bedanken. Zonder jullie medewerking kan dit onderzoek niet plaatsvinden. Ik kijk verwachtingsvol uit naar de resultaten.

Truus de Groot, ik heb je alle hoeken van het personeelsportaal laten verkennen. Dank dat jij het altijd wist op te lossen! Hélène Willems-van Paridon, ook jou wil ik bedanken voor jou vinger aan de pols met betrekking tot de agenda afspraken en andere formaliteiten.

Collega's en frequente bezoekers van de KNF (huis-)kamer F02.201; waar het allemaal begon! Dirk, Merel, Nico, Nizare, Willy en alle laboranten, dank voor de gesprekjes, (KNF tour) activiteiten en gezelligheid tussendoor. Tineke Gebbink, met jouw pragmatische 'hands-on' mentaliteit ben je een aanwinst voor het klinische team en bovendien een fijne en gezellige kamergenoot. Cyrille Ferrier, zonder jou geen SPES, bedankt voor de humor die jij brengt en je bijdrage aan mijn vliegende start! Imogene Pieters en Jurgen Hebbink, leuk dat jullie met veel plezier het SPES werk, vanuit verschillende disciplines, voortzetten.

Mede-epilepsie onderzoekers, onafhankelijk van de samenstelling waren congresbezoeken in het buitenland altijd een groot succes. Mede door de Airbnbs en de vele activiteiten die werden georganiseerd buiten het congresprogramma om. Hanna, Eric, Bart, Tim, Kim, Matea en Herm, dank voor het stellen van kritisch vragen, het meedenken, en de gezellige afleiding de afgelopen jaren. Wim Otte, dank voor je adviezen en relativisme in tijden van statistische nood. Lieve dames van de knutselfederatie, Judith en Jolien, dank voor alle goede gesprekken en gezelligheid tijdens en buiten werktijd!

Ik heb met veel plezier veel TG, SUMMA en geneeskunde studenten mogen begeleiden. Jullie zorgden er voor dat ik de grondbeginselen van epilepsie chirurgie en de wetenschap niet uit het oog verloor. Huidige lichting studenten Donqing, Hanneke en Marij, ik heb genoten van jullie nieuwschierigheid en gedrevenheid. Ik ben blij dat een groot aantal van de studenten bij de harde kern van de KNF/Epilepsie-club zijn gaan horen; Brian Mouthaan, als een van de weinige mannelijke studenten hield jij stand tussen alle dames bij de KNF. De lol die jij uitstraalt

tijdens je werk maakt het fijn om met je samen te werken. Evelien Geertsema, het begon met een korte M2 stage bij de KNF, om via de wetenschapcommissie bij de NVvTG, nu af te sluiten met een gezamenlijke SEIN-UMCU publicatie. Ik bewonder jouw ongedwongen manier van werken en wil je bedanken voor de vanzelfsprekendheid waarmee jij al de extra klusjes deed om mij te ontlasten. Dorien van Blooijs, jouw bruisende persoonlijkheid is een aanwinst voor het PhD team. Bedankt voor de koffie en muffins tijdens moeilijke momenten in de laatste maanden.

Lieve ladies van het HFO team, jullie mogen natuurlijk niet ontbreken. De laatste jaren hebben we veel lol, frustraties en wetenschap gedeeld. Zonder jullie bijdrage was de database en "de HFO studie" nooit van zulke hoge kwaliteit geweest. Nicole van Klink, ik bewonder jouw talent voor het stellen van prioriteiten, en wil je bedanken voor het feit dat ik echt altijd op je kan rekenen. Succes met het afronden van jouw, zeker weten prachtige, proefschrift! Willemiek Zweiphenning, ik geniet van jouw ongeremde enthousiasme en oprechtheid die ik graag nog langer had willen meemaken. Ik had me geen betere opvolger kunnen wensen! Anne Mooij, jouw gevoel voor accuratesse is een mooie aanvulling op het team. Dames, maak er met elkaar de komende jaren wat moois en gezelligs van!

Oud-bewoners van A Capella en Huize Herbie, jullie vormen de onuitwisbare roots van mijn studententijd. Nog altijd voelt het bij jullie als thuiskomen, hier ben ik dankbaar voor. Wiebe, dank voor alle muzikale hoogtepuntjes.

Lieve Lobke, dank voor jouw altijd oprechte interesse, de mooie gesprekken en alle grappige en lieve kaartjes de afgelopen jaren. Ik wens je een mooi avontuur en een behouden vaart!

Lieve Veronique, al meer dan 16 jaar duurt onze vriendschap. Ik hecht veel waarde aan de vanzelfsprekendheid dat we elkaar altijd weer vinden en het dan altijd goed en gezellig is.

Lieve Enschedese dames, over de (gezamenlijke PhD) jaren heen kwam geleidelijk het besef dat succes(jes) gevierd dienen te worden, en dit leidt tot meer en meer borrels, etentjes en activiteiten en bovenal verhoogde levensvreugd. Het is echt de hoogste tijd om die al te vaak besproken plannen voor een gezamelijk wintersportvakantie uit te voeren! Monique en Astrid, zo fijn dat jullie nog steeds zo lekker dichtbij wonen. Bedankt voor de spontane theetjes en borrels, de Edward Scissorhands experiences, het gebruik van de printer, en het oppassen op huis en kat. Aan alle mannen; bedankt voor het lijdzaam ondergaan van alle gesprekken die toch wel (te) vaak over promoveren gingen!

Lieve paranimf Willemijn, na 6 jaar samen studeren bracht de fascinatie voor het EEG ons beide naar het UMCU. Jij op de 6^{e} etage bij de IC en ik op de 3^{e} bij de KNF, inclusief een gezamenlijke publicatie! Dank voor al je support en advies, en bovenal de thee met bitterbal momenten, het shoppen, de dansjes, de concertjes en logeerpartijtjes.

Lieve paranimf Dagmar, met jouw positieve levensinstelling weet je mij altijd weer te motiveren en bovenal te inspireren. Bedank dat je 'my person' wilt zijn! Als jij nou ook 'ff' promoveert, dan kunnen we naar Japan!

Lieve schoonfamilie, Joke & Ivo, Thomas, Eske, dank voor jullie interesse in mijn bezigheden en de luxe van de weekendjes en schrijfretraitres in altijd heerlijk Zeeland.

Lieve Berit, swesje, wij zijn match & winnaars. Ik geniet van je verhalen over jouw dierlijke patiënten en hun baasjes. Ik hoop dat we onze disciplines ooit eens samen mogen verenigen. De Achterhoek wordt nog mooier met zo'n mooi stel als jij en Niek.

Lieve paps en mams, ik voel me gezegend met jullie als ouders. Dankjulliewel voor jullie onvoorwaardelijke steun en zorgzaamheid. Ontzettend fijn om een plek te hebben waar er altijd ruimte is voor een lach en traan, en tijd voor een luisterend oor, advies en bemoedigende woorden.

Allerliefste Bart, zonder jouw rotsvaste vertrouwen in mij, je zorgzaamheid, stabiliteit en relativisme was het me niet gelukt. Samen met jou is het leven mooier!

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THIS THESIS

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*These authors contributed equal to this work.

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# These authors share senior authorship

CURRICULUM VITAE

Maryse was born on April 11th, 1985 on an organic dairy farm named 'Ark'. Not 'cow' but 'why?' and 'how?' were among her first words. It became obvious early on in her life that she would not follow in her father's footsteps.

The combination of technology with a human touch made the newly started study Technical Medicine a good fit. She spends six years at the Twente University in Enschede, to pursue her Bachelor and Master degree, specialized in signal analysis. Her graduation internship brought her to the University Medical Center Utrecht in 2010. Here, at the Clinical Neurophysiology department, is where her fascination for the brain and it signals took hold.

After successfully completing her Master thesis in 2011, she continued working as a technical physician in the discipline of Clinical Neurophysiology at the department of Neurology & Neurosurgery, of which this PhD thesis is the result. Being a pioneer at heart, she enjoyed the challenge of initiating and coordinating a unique clinical trial to test the use of high frequency oscillations during surgery. Her first paper (Van 't Klooster et al. Brain 2011) was awarded with multiple prizes, e.g. the Storm van Leeuwen-Magnus prize 2013 (NVKNF) and the Rudolf Magnus Research prize 2012 (BCRM). Curious to learn more about the behavior of high frequency oscillations at the neuronal level, she spent three months studying micro-electrode recordings at the Seizure Disorder Centre of the David Geffen Medical School at the University of California Los Angeles. For this project she was awarded multiple scholarships, including the 'Ter Meulen Grant' by the Royal Netherlands Academy of Arts and Sciences (KNAW). She is an active member of the Dutch Association for Technical Physicians (NVvTG).

During the last four years she not only finished this PhD thesis, but visited fifteen countries on four continents - as tourist or conference speaker -, read almost all books of Haruki Murakami, and became a passionate knitter. She lives in Utrecht together with Bart and their cat Morris.

Brain Center Rudolf Magnus

Universiteit Utrecht

ISBN 978-90-393-6645-5