University of Twente

Master Thesis Technical Medicine

Improving the SPES protocol by automating ER and DR detection and evaluation of the spatial relation between ERs and DRs

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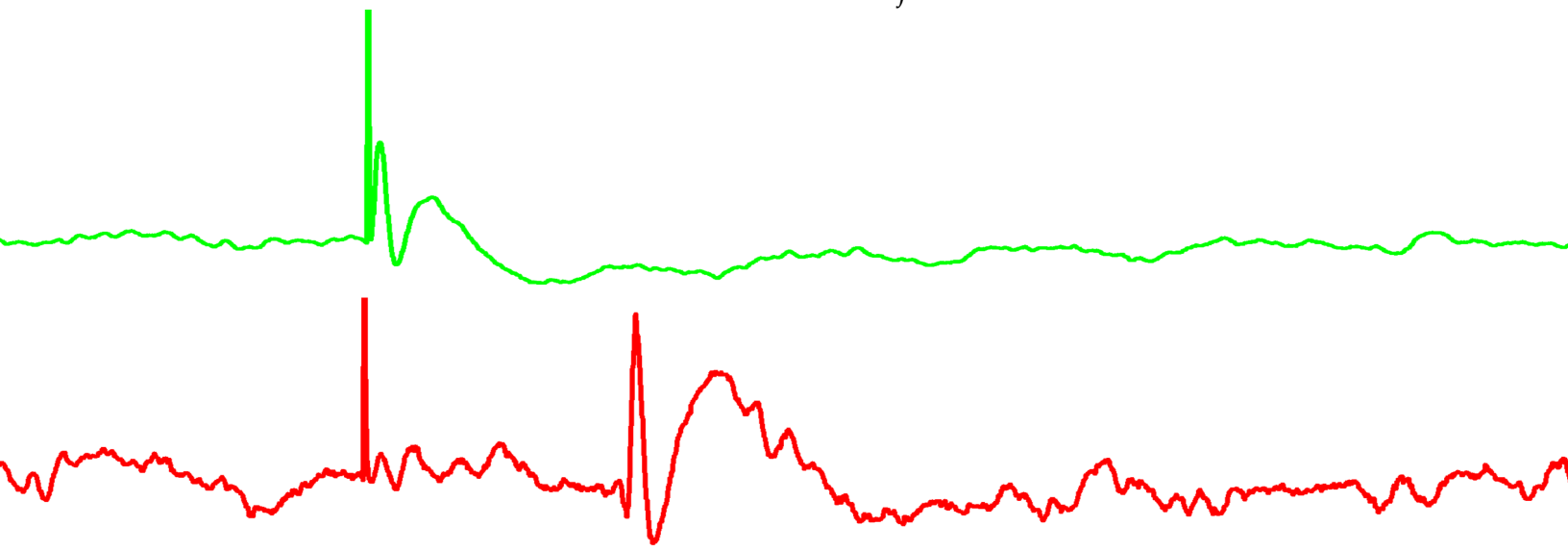

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Abstract

Introduction Epilepsy is one of the main causes of brain health disability. In 70% of the patients, seizure freedom is provided with medication. Patients with focal epilepsy, who have not responded to intensive medical treatment, are screened for the possibility of epilepsy surgery. When a visible lesion on an MRI image is found, and the lesion does not overlap with a functional area, the patient undergoes epilepsy surgery with acute electrocorticograpy (ECoG). In more complicated situations, when no visible lesion on an MRI image is found, or the lesion overlaps with functional areas, ECoG is performed with chronic grid implantation for maximally ten days. During this long-term ECoG recording, the seizure onset zone (SOZ) is delineated based on the seizures occurring during the chronic ECoG monitoring, and the resection area is determined based on the SOZ. The urge of having to have a seizure can be very stressful for the patient, and risks for complications are increased when chronic ECoG monitoring takes longer. Therefore, methods are investigated to delineate the SOZ faster and which are not based on seizures. In the future, such methods might replace chronic grid implantations by being performed during surgery to delineate both functional and resection areas.
With Single Pulse Electrical Stimulation (SPES), ten short electrical pulses are applied to stimulus pairs, which are neighboring electrodes on the electrode grid. SPES evokes early responses (ERs) and delayed responses (DRs). ERs are characterized as spikes or slow waves, starting within 100 ms after the stimulation artifact. ERs are proposed to reflect the physiological network. DRs are characterized as spikes or sharp waves occurring between 100 ms and 1 s after the stimulation artifact. DRs are proposed to be pathogenic and to correlate with the SOZ. Currently, the duration of the SPES protocol is at least one hour and the duration of visual analysis of the ERs and DRs takes almost one day. As a consequence, both the duration of SPES itself and the analysis are too long for usage during surgery. In this study, we have developed an automatic detection algorithm for ERs and DRs. We have also investigated the spatial relation between ERs and DRs. When we can discard stimulus pairs which will not evoke any DRs based on the physiological network from ERs, we can make the SPES protocol more time efficient.

Method For both ERs and DRs, two detectors were developed and compared. The first detector was based on an amplitude threshold. The peak from a spike or wave was considered as ER or DR when the peak exceeded a specific amplitude threshold. In the second detector, the standard deviation (SD) was calculated to distinguish an ER or DR from continuous spontaneous activity. A peak was considered as ER or DR when the ratio between the amplitude of a detected peak and the SD exceeded a specified SD factor.
In the ER detector, several parameters were varied: the amplitude threshold or SD factor, the minimal SD and sel, a parameter in the Matlab function *peakfinder*, which detected a peak when the peak was higher than the rest of the signal. The amplitude threshold was the value a peak had to exceed to be considered as ER or DR. The SD factor was a value, which the ratio between a the amplitude of a peak and the SD had to exceed, to be considered as ER or DR. The minimal SD was important in signals with very little spontaneous activity. In signals with a low SD, small peaks were easily considered as ER or DR. To prevent detection of these small peaks, a minimal SD had to be set.
In the DR detector, several parameters were varied as well. These parameters were the amplitude threshold or SD factor, sel and the cut off frequency (fc). The fc was implemented to prevent detection of high amplitude slow waves which were part of the ER, but present in the time window where DRs occurred.
Detections were compared with visual annotation. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated and ROC curves were made. The setting of parameters with the best performance was used in validation of the detectors.
For investigation of the spatial relation between ERs and DRs, the automatic detectors were used to find ERs and DRs. Detected DRs were visually checked. Per electrode, the number of stimulus pairs that

resulted in an ER or DR in this electrode were counted (sER/sDR). We distinguished the sERs and sDRs into stimulus pairs which only resulted in ERs or DRs (soER/soDR) or both ERs and DRs (sERDR). Per stimulus pair, the number of electrodes with an evoked ER or DR were counted (eER/eDR). We distinguished the groups of eERs and eDRs into electrodes in which only ERs or DRs were evoked (eoER/eoDR) or both ERs and DRs were evoked (eERDR). We tested with a two sided t-test (α = 0.05) whether the ratios of eERDR/eDR, eERDR/eER, sERDR/sER and sERDR/sDR differed significantly from 0.5. When ERs occurred equally in both signals with DRs and in signals without DRs, the ratio was 0.5. We tested whether ERs occurred significantly more often in signals with or without DRs. We also tested with a Mann Whitney U test whether more ERs were evoked in the electrodes in which DRs were evoked, than in the electrodes in which no DRs were evoked (α=0.05). A similar analysis was used for the stimulus pairs.

At last, we were allowed to execute a second SPES session. We investigated whether detected ERs are reproducible when the protocol is repeated, and we investigated the stimulation settings by comparing responses to pulses according to the standard SPES protocol and responses to pulses according to an alternative SPES protocol. In this alternative SPES protocol, we varied one parameter per situation. We varied the pulse form, the current intensity, and we applied a biphasic pulse instead of a monophasic pulse. To investigate reproducibility, we averaged the first three responses to an applied pulse per electrode for each trial from the first SPES session in six patients ($epoch1_3$). We averaged the first three responses to an applied pulse per electrode for each trial from the second SPES session in six patients ($epoch2_3$). Both $epoch1_3$ and $epoch2_3$ had the same settings (monophasic, positive pulse, 0.2 Hz, 8 mA, 1ms). We detected ERs in all averaged $epochs1_3$ and $epochs2_3$. We calculated the total, positive and negative agreement. When agreement was high, ERs are reproducible when the standard protocol is repeated.

To investigate whether neural stimulation is the greatest at the cathode, the anode or whether both electrodes contribute equal, we averaged the first three responses and the second three responses to a pulse per electrode for each trial of the second SPES session in four patients ($epochs2_3$ and $epochs2_6$ respectively). We used the automatic ER detector to detect ERs in both $epochs2_3$ and $epochs2_6$. To investigate whether both electrodes contribute equally, we compared $epochs2_3$ and $epochs2_6$ from the same stimulus pair. $epochs2_3$ had a positive pulse form, $epochs2_6$ had a negative pulse form. To investigate whether the anode contributed most, we compared $epochs2_3$ and $epochs2_6$ from neighboring electrodes in which one electrode is the anode. To investigate whether the cathode contributed most, we compared epochs from neighboring electrodes, in which one electrode is the cathode. We calculated the total, positive and negative agreement for each situation. The Mann Whitney U test (α=0.05) was used to determine whether one situation had a significantly better agreement than another test.

To investigate whether other ERs were evoked when a lower current intensity (4 mA) was used instead of the standard current intensity (8 mA), we averaged $epochs2_3$ and $epochs2_6$ in four patients. We detected ERs with the automatic detector in both $epochs2_3$ and $epochs2_6$. We calculated the total, positive and negative agreement in each patients.

To investigate whether the same ERs are evoked when a biphasic pulse was applied instead of a monophasic pulse, we averaged $epochs2_3$ and $epochs2_6$ in two patients. We detected ERs with the automatic ER detector. We calculated the total, positive, and negative agreement in both patients.

Results The ER detector based on SD factor performed best with sensitivity, specificity, PPV and NPV of respectively 0.78, 0.91, 0.75, 0.92 when SD factor = 2.5, sel = 20 μV, minimal SD = 50 μV.

The performance of the DR detector based on SD factor performed best with a sensitivity, specificity, PPV and NPV of respectively 0.68, 0.92, 0.10 and 1.00 when SD factor = 4, minimal SD = 40 μV, fc = 1 Hz.

In seven out of twenty patients, we did not detect any DRs with the DR detector. In thirteen patients, we found that the ratio of eERDR/eER and sERDR/sER was significantly lower than 0.5. In three patients, we found that the ratio of eERDR/eDR and sERDR/sDR was significantly higher than 0.5. This suggests

that most ERs were evoked without a DR, but that most DRs are evoked with an ER. We found in one patient that in electrodes with evoked DRs, significantly more ERs were evoked. We found in six patients that stimulus pairs which resulted in DRs resulted in significantly more ERs.
When investigating the reproducibility of ERs, we found that the total, positive, negative agreement were respectively 0.85, 0.67, 0.90 on average in three out of six patients. The other three patients had a total, positive and negative agreement of respectively 0.67, 0.52, 0.75 on average. In the first three patients, the agreement is high. In the last three patients, the agreement is much lower. In the first three patients, the first SPES session was executed at least one day after surgery. In the last three patients, the first SPES session was executed on the same day as the grid implantation.
When investigating whether the anode, the cathode or both electrodes contribute equal to neural stimulation, we found a trend towards significance when we compared the total agreement and negative agreement between the situation in which both electrodes contribute equally and the situations in which the anode or cathode contributed more.
When investigating whether the same ERs are evoked with lower current intensity, we found that the total, positive and negative agreement between 4 and 8 mA were respectively 0.82, 0.52, 0.89 on average.
When investigating whether a biphasic pulse evokes the same ERs, we found a total, positive and negative agreement of respectively 0.85, 0.63, 0.89 on average.

Conclusion The performance of the ER detector is sufficient for further usage in this study. The sensitivity and PPV of the DR detector are not very high since the number of annotated DRs is not very high. This means that a few false positive or false negative detections have a large effect on the sensitivity and the PPV. Due to the high number of false positive detections, the detected DRs were visually revised when we investigated the spatial relation between ERs and DRs.
We found that electrodes with DRs had more ERs and that stimulation pairs which evoked DRs evoked more ERs. This research is a first step in automatic detection of ERs and DRs and in gaining insight in the relation between ERs and DRs. In further research, it is recommended to investigate indirect connections in the network to gain more insight in the relation between ERs and DRs. The current ER and DR detector does not work well in detecting Stable Responses (SRs) and Repetitive Responses (RRs). Both detectors can be improved to enable detection of SRs and RRs.
When investigating the stimulus settings, we found a difference in reproducibility of ERs when the first SPES session was executed on the same day as the implantation surgery. These patients were still sleepy, and possibly anesthetics had some effect on the physiological networks in the brain.
We also found that it is assumable that both electrodes contributes equally in neural stimulation. At last, we found that other ERs were evoked when a pulse with a lower current intensity or a bipolar pulse form was applied.

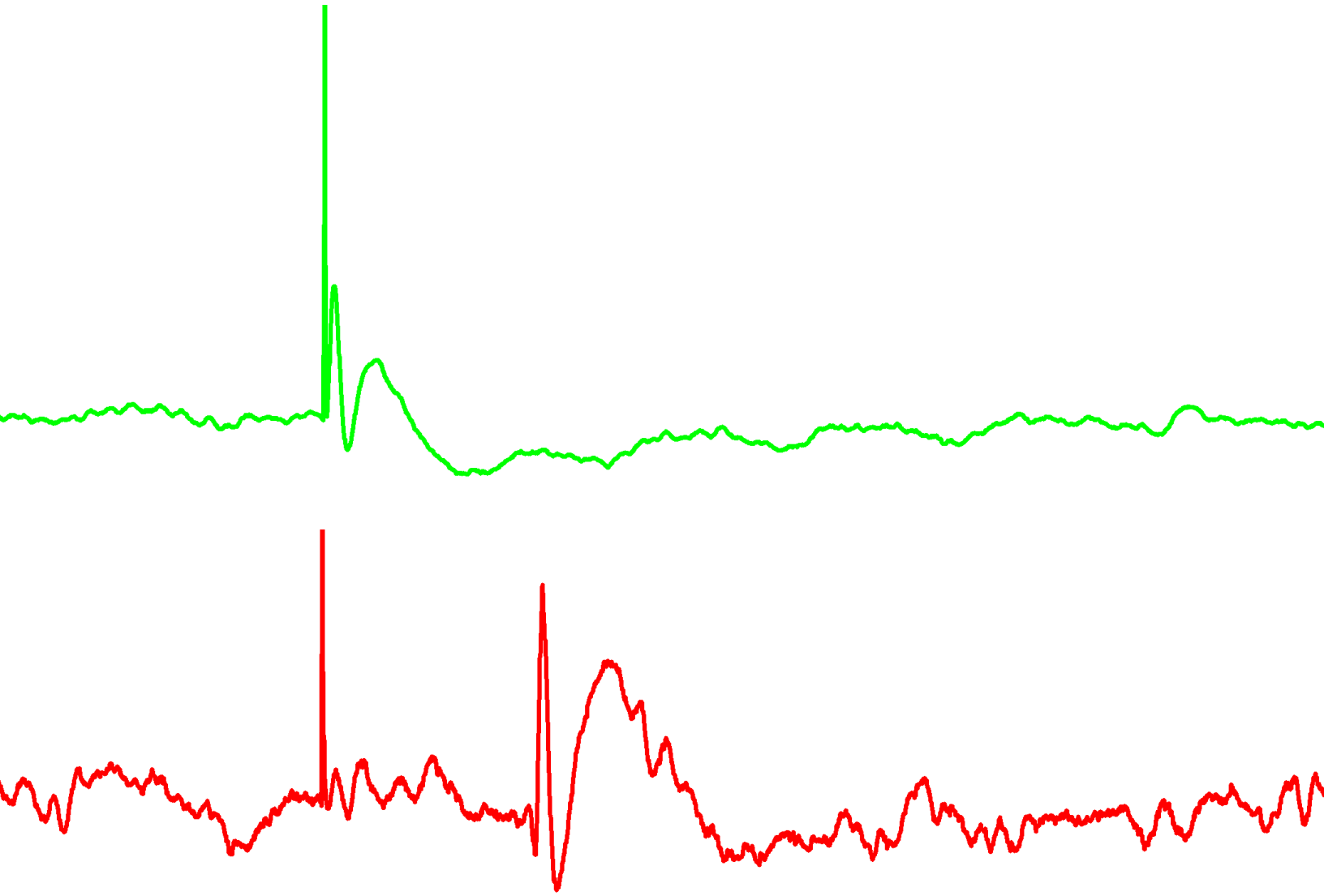
Contents

Abbreviation	Description
AUC	Area Under the Curve
BACI	Basic and Clinical Multimodal Imaging
C	Central
CCEP	Cortico-cortico evoked potential
DR	Delayed Response
DTI	Diffusion tensor imaging
ECoG	Electrocorticography
ER	Early Response
F	Frontal
fc	Cut off frequency
FCD	Focal cortical dysplasia
FN	False Negative
FP	False Positive
GUI	Graphical User Interface
IEC	International Epilepsy Congress
NA	Not applicable
NPV	Negative Predictive Value
MCA	Middle cerebral artery
METC	Medical Research Ethics Committee (MREC); in Dutch: medisch ethische toetsings commissie (METC)
MTS	Mesiotemporal sclerosis
O	Occipital
P	Parietal
PPV	Positive Predictive Value
ROC	Receiver Operating Characteristic
RR	Repetitive Response
SEEG	stereo-encephalogram
SD	Standard Deviation
SOZ	Seizure Onset Zone
SPES	Single Pulse Electrical Stimulation
SR	Stable Response
TN	True Negative
TSC	Tuberous sclerosis complex
TP	True Positive
UMCU	University Medical Center Utrecht

Definitions	Description
stimulus/stimulation	Each single pulse
trial	A batch of several identical single pulses applied to the same pair of electrodes, every 5 s

Chapter 1

General introduction



1.1 Epilepsy

Epilepsy is one of the main causes of brain health disability, especially among young adults, and accounts for a worldwide burden of illness similar to that of breast cancer in women and lung cancer in men [1, 2]. The incidence of epilepsy in the general population is approximately 45/100000 per year [3]; prevalence is 5.8 per 1000 [3] and the life-time probability of an epileptic seizure is approximately 3% [4]. In the Netherlands, there are approximately 84.000 patients with epilepsy [3].
Epilepsy is a disturbance in brain function, presenting itself with seizures with a variable frequency and duration [5]. Seizures are characterized by excessive or over-synchronized discharges of cerebral neurons [4]. Epilepsies are divided into two groups [6]: focal and generalized epilepsies [7, 8, 9]. The first group are epilepsies known variously as focal, partial or local [6]. Focal seizures arise from a focal or localized area of the brain [8] due to a tumor, ischemia, trauma, vascular malformation or other abnormalities of brain tissue [5], and the interictal EEG may show focal spikes and or sharp waves [7]. The second group contains epilepsies with seizures which arise from bilateral diffuse discharges in both hemispheres [8] and the EEG may show generalized, bilateral spike and wave discharges [7]. This group does not have an identifiable site of onset [6]. Generalized seizures involve deep structures such as the thalami to provide widespread synchronization. Both groups of epilepsies differ substantially in their pharmacology, cellular physiology and their clinical manifestations [6].
Medication is usually effective and provides seizure freedom in 70-80% of the patients [10]. Thus, about 20-30% of the patients with epilepsy have seizures that are not controlled by available anti-epileptic drugs [5, 11, 12]. When seizures persist after two consecutive years of medical treatment in which two or three first-line anti-epileptic drugs have failed, the odds are low (<5%) that they will ever be seizure free with drugs alone. Patients with focal epilepsy are then screened for epilepsy surgery [10, 11, 9, 3].

1.2 Surgery

The purpose of surgical treatment is to achieve seizure freedom in patients with intractable focal epilepsy that has a serious impact on their quality of life [5]. Results are good. With careful selection, only about 10% of patients obtain no improvement at all from epilepsy surgery and less than 5% worsen after surgery [8].
Using mainly non-invasive diagnostic techniques, the epilepsy focus is lateralized and localized at lobar or sublobar level. In the University Medical Center Utrecht (UMCU), most patients then undergo 'tailored' epilepsy surgery with acute, i.e. intraoperative, electrocorticography (ECoG) in which signals from the brain are measured with electrode grids during surgery to precisely determine which area of brain tissue has to be resected.
In patients with no abnormalities seen from imaging, when overlap of the lesion with functional areas is expected, or with complexity in interpretation of the epileptic area, chronic grid implantation with ECoG is performed for maximally ten days [13]. During this procedure, grids with silicone-embedded electrodes are placed subdurally on the brain surface, enabling registration of cortical signals at a high resolution, without interference from skull and in the absence of extracranial artifacts [5]. An MRI rendering of the brain with an electrode grid is displayed in Figure 1.1. During ECoG monitoring, spontaneous seizures are analyzed to find the area of the cortex from which seizures are generated: the seizure onset zone (SOZ) [14]. One, or preferably more, spontaneous seizures are necessary to enable delineation of the SOZ and to make a surgery plan [13]. The urge of having to have a seizure can be very stressful for the patient. Risks for complications, bleedings and infections are also increased in a time dependent manner during this period of chronic ECoG monitoring [13]. Therefore, finding a method in which the epileptic cortex can be identified during the interictal state is of high priority. With Single Pulse Electrical Stimulation (SPES), the epileptic cortex can be determined by stimulating electrodes and analysis of the responses in other electrodes on the grid [13, 15, 16].

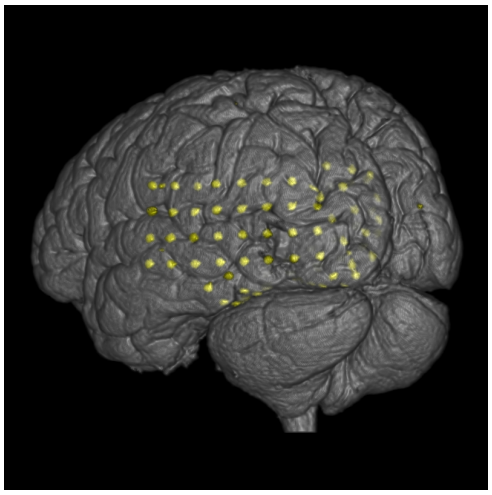


Figure 1.1: *This is a reconstruction of the brain in which an MRI scan is combined with a CT scan. This combination enables visualization of the location of the grid on the brain of a patient. This grid is placed for maximally ten days and records the brain signals.*

1.3 Single Pulse Electrical Stimulation

The idea behind SPES is that the balance between excitability and inhibition of the brain is studied with brief single pulses. This activates only a limited and localized population of neurons instead of activating widespread cortex due to long trains of current pulses (1-5s) as has been previously used to induce habitual seizures [15, 16, 17]. During a SPES measurement in the UMCU, all neighboring electrode pairs on a grid, irrespective of particular placement of these electrodes across the gyri, are stimulated ten times [18]. The responses to these stimulations in other electrodes give information on the epileptogenicity of the tissue.
Valentin et al. saw two main types of responses: early responses and late responses [15, 16, 17]. An early response (ER) is characterized as a spike and/or slow wave starting within 100 ms after the stimulation artifact [15, 19]. It consists of a sharp deflection immediately following the stimulus artifact or occasionally merging with it [15, 16, 20]. This initial deflection is followed by one or two slow waves of alternating polarity [15]. These responses are seen in most regions in all patients and seem to be a normal response of the cortex to the stimulation [15, 16]. They may be viewed as cortico-cortical evoked potentials. ERs are recorded in areas around the stimulated cortex but sometimes also at a distance, providing evidence of functional connections between stimulated cortex and the regions where early responses are recorded [20, 15]. These responses represent the underlying physiological network.
Late responses can be divided into delayed and repetitive responses [16]. Delayed responses (DRs) are spikes, sharp waves or spike-and-slow-wave complexes [19] occurring between 100 ms and 1 s after the stimulation artifact [15, 16, 19]. These responses were not seen after each stimulus (occurrence rates varied between 10-90%, depending on the patient and stimulation site), only in some regions and not in all patients and they are significantly associated with the SOZ [15]. Valentin et al. concluded that the presence of DRs can identify regions of hyper-excitable cortex [15]. The long latency between the stimulus and the DR may indicate multi synaptic connections [21].
A third type or response is the repetitive response (RR). This response arises exclusively when stimulating the frontal lobe of some patients with frontal epilepsy [16]. It consists of two or more consecutive waves, each resembling the initial early response [19]. This response will not be considered further here.
Valentin et al. defines both the electrodes showing DRs and RRs as reflecting the abnormal SPES area [16, 21]. Removal of this abnormal SPES area is associated with favorable outcome [16].
A different kind of response, called stable response (SR), was identified in a later study by Flanagan et

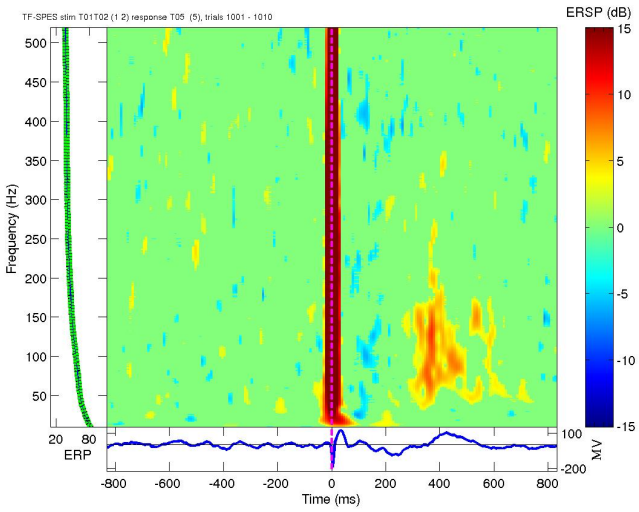


Figure 1.2: *An example of an averaged time frequency decomposition. At 0 ms, the stimulation artifact is visible. At 400 ms, high power is present between 50 and 150 Hz.*

al. [21]. SRs consist of a small spike or low amplitude sharp wave, most often superimposed on the slow wave of an ER. SRs had a latency of more than 100 ms. In contrast to DRs, SRs have a fixed latency (typically with a variation of less than 20 ms) [21]. Furthermore, when present during the stimulation of a particular site, SRs arise after most or all stimuli [21]. In this respect, they resemble ERs, except that the latency is typically more than 100 ms [21]. SRs were most common in the frontal and parietal lobes, especially around the area of the central sulcus [21]. Flanagan et al. [21] found that for each patient showing SRs, the location of the SRs was consistent, and SRs could be elicited by stimulation of a variety of contacts. There was no clear relationship between the location of the SRs and the location of the stimulating electrodes and between the location of SRs and the lesion location [21]. Therefore, Flanagan et al. assumes that SRs are comparable with ERs and not pathogenic. Identification of SRs is important because they may be misclassified as DRs, which have different clinical significance [21].

1.4 Current situation

SPES has proven to be a safe and reliable diagnostic tool to identify epileptic cortex [17]. In the UMCU, SPES is used as a confirmation of the resection area when the SOZ is determined based on a spontaneous seizure. However, the role of SPES could be expanded when both the acquisition protocol and analysis of the responses is improved. In the future, the aim is to use SPES in delineating the epileptic cortex during surgery itself, which means that it should become less time consuming.

1.4.1 Acquisition of the SPES protocol

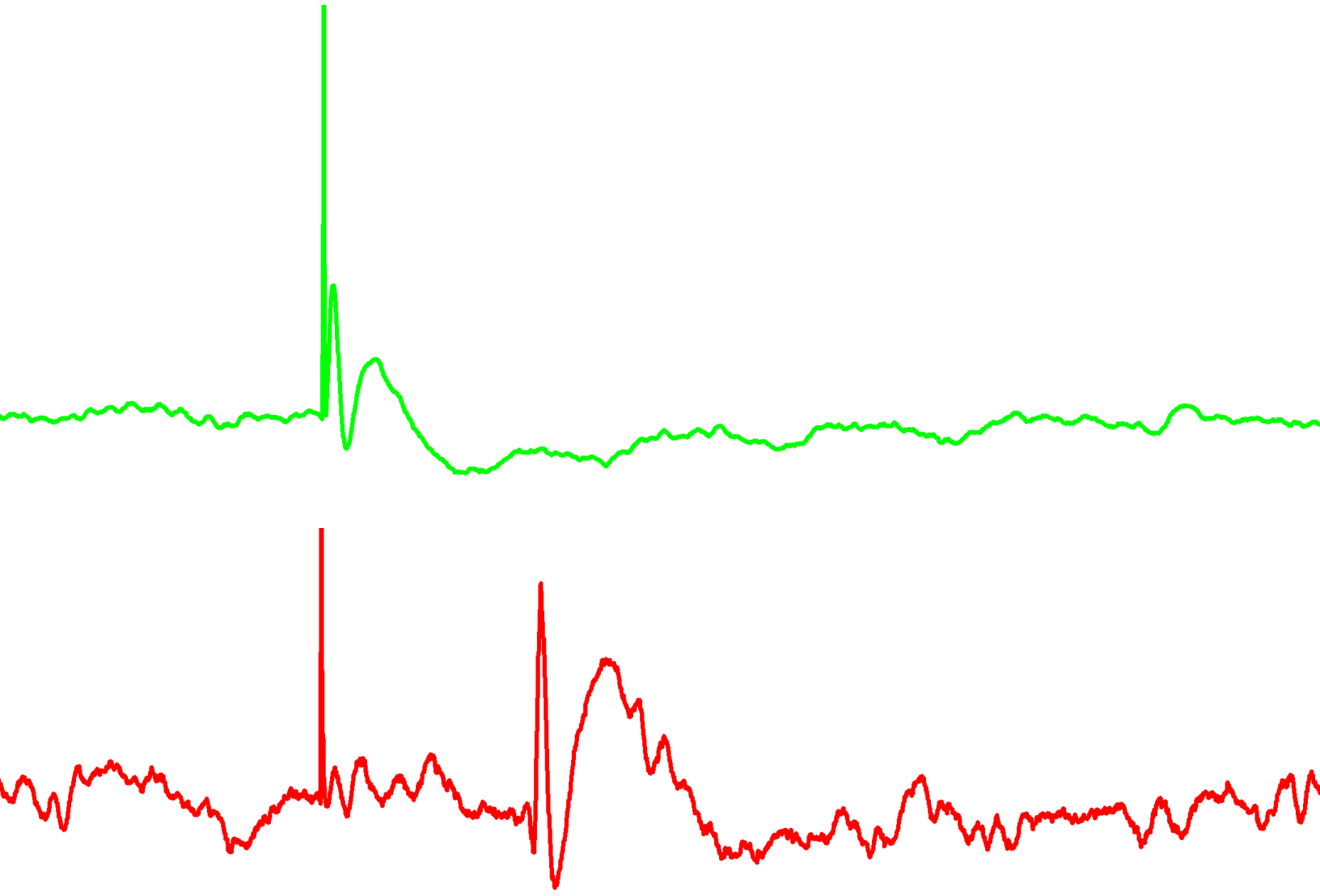
In the current SPES protocol, ten pulses are applied to each electrode pair with 5 s between two pulses. Therefore, the current duration of stimulating one electrode pair is (5*10=) 50 s. On average 72 electrodes (8*9) are implanted in a patient. In each row, 7 stimulus pairs are located. So, with on average 72 electrodes on a grid, 63 electrode pairs (7*9) are stimulated. The total SPES measurement takes at least ((50*63)/60=) 52.5 minutes. This duration is too long for use during surgery in which a maximum of 20 minutes is allowed.

1.4.2 Analysis of responses

Currently, SPES responses are analyzed using a time frequency decomposition of the average of ten pulses (Figure 1.2) [18]. Especially time frequency decompositions with high power in the fast ripple frequency band help in delineating epileptic cortex [18]. However, obtaining all averaged time frequency decompositions takes hours, and the visual annotation of DRs in the ripple and fast ripple frequency band is very time consuming. Maryse van 't Klooster and David Keizer attempted without success to automate detection of DRs in these time frequency decompositions during previous traineeships [22, 23]. Instead of detecting DRs in these time frequency decomposition, an alternative method for detection of DRs is required.
Besides, ERs merge with the stimulation artifact in these decompositions due to a low time resolution and cannot be differentiated from the stimulation artifact. Also, an alternative method for detection of the ERs is required.

Chapter 2

Research questions



Current issues in the field, as outlined above, can be addressed by one main research question and two sub questions.

- Can we make the SPES protocol time-efficient?
  - Can we improve the analysis of early and delayed responses?
  - Can we improve the acquisition of the SPES protocol?

Both sub questions are investigated in different chapters.

2.1 Analysis of responses

2.1.1 Pre-processing

Before we can analyze responses, we have to pre-process the ECoG recordings towards signals we can investigate in Matlab, first. In **Appendix A**, we describe the characteristics of the stimulator and the process of recording the data using SystemPlus Micromed towards epochs in Matlab.
David Keizer averaged ten responses to each stimulus. In this averaged signal, he detected ERs. In **Appendix B**, we investigate whether ERs can be detected in this averaged signal as good as in single responses to pulses in which ERs are observed.

2.1.2 Early responses

An ER is characterized as a spike or slow wave. Therefore, in the time domain, these responses are visible as deflections with a higher amplitude than the surrounding samples in the time domain. Therefore, it may be possible to detect these ERs in the time domain. This information can be investigated with this question:

- Can we improve the analysis of SPES by automating the detection of ERs in the time domain?

In **Chapter 3**, we investigate this question. In some electrodes, a lot of spontaneous activity is present. This obscures differentiation between ERs and peaks from spontaneous activity. Therefore, we investigate whether setting a factor based on de standard deviation (SD) is helpful in differentiating between ERs and peaks from spontaneous activity. When the ratio between the amplitude of a detected peak and the SD exceeds the set SD factor, the peak is considered to be an ER.
We know that at the moment when a pulse is applied to two electrodes, it is not possible to detect ERs. The potential difference between the ground and the reference electrode is recorded instead of the potential difference between the electrode and the reference. In **Appendix C**, we investigate the time window after an applied pulse before we are able to record the potential difference between an electrode and the reference again.

2.1.3 Delayed responses

As with ERs, DRs are visible in the time domain as spikes or sharp waves. This can be addressed by the following question:

- Can we improve the analysis of SPES by automating the detection of DRs in the time domain?

The same kind of detector used for ERs may automatically detect DRs. This is investigated in **Chapter 4**. Since a slow wave following the initial sharp peak of an ER overlaps with DRs in the time window, we investigate whether we may improve detection of DRs when we shift the time window for detecting DRs from 100 ms-1s after the pulse to 200 ms-1s after the pulse. This is described in **Appendix D**.

2.2 Acquisition of the SPES protocol

ERs are deterministic and are supposed to occur each time when observed during stimulating a stimulus pair. First, we will try to investigate whether ten stimuli are needed to establish a reliable ER or whether fewer stimuli is enough to establish a reliable ER and to reconstruct a network based on ERs. The requirement of only a few stimuli instead of ten to detect an ER would already speed up the protocol considerably. In **Chapter 5**, we investigate how many pulses are required to establish a reliable ER with this question:

- How many stimuli are needed to establish ERs?

Secondly, we assume that many electrodes do not have to be stimulated to detect DRs, when it may be predicted that no DRs would be evoked when applying pulses to some stimulus pairs. This may depend on the presence of ERs. Enatsu et al. [24] stimulated with repetitive 1 Hz bipolar electrical stimuli. This is called a cortico-cortical evoked potential (CCEP). He found that the CCEP amplitudes were significantly larger in the ictal propagation area than out of the propagation area. Boido et al. [25] found that more bidirectional connections were present in the epileptic zone and the epileptic propagation zone. Both studies suggest that physiological connection may be changed in the area involved with the seizure.
Similarly, we assume that some electrodes do not have to be analyzed for DRs since no DRs would be evoked there. Therefore, we will try to predict the occurrence of DRs through construction of a network based on evoked ERs. ERs are supposed to describe the normal neuronal network, but that does not imply that pathological information is absent from their occurrence or characteristics. When a relation between ERs and DRs can be found, candidate locations of DRs may be predicted. This would result in a faster SPES protocol. These issues can be addressed by several questions:

- What is the relation between evoked ERs and DRs and can this relation be exploited to make the acquisition protocol more efficient?

  - Are ERs stimulated in the same population of stimulus pairs as DRs?

  - Are ERs evoked in the same population of electrodes as DRs?

  - When DRs are stimulated by a stimulus pair, are significantly more ERs stimulated by this stimulus pair as well?

  - When DRs are evoked in an electrode, are significantly more ERs evoked in this electrode as well?

These questions are investigated in **Chapter 6**. In **Appendix E**, the grid configurations are displayed for the patients, in which the spatial relation between ERs and DRs is investigated. We also constructed a General User Interface (GUI) in Matlab to obtain more insight in the ERs and DRs evoked after applying a stimulus to different stimulus pairs. This GUI is described in **Appendix F**.

2.3 Stimulation settings of the SPES protocol

The settings of SPES (monophasic, duration of 1 ms, current intensity of 4-8 mA) have been empirically determined. No evidence is available that these settings are the best to obtain all reliable responses. We do not know whether the cathode, anode or both contributes the most during stimulation. We also do not know whether a second SPES would result in the same ERs. At last, we do not know whether the current intensity of 8 mA is suitable or whether a lower current intensity is more suitable. Between November 2014 and June 2015, we executed a second SPES protocol, in which different settings were used. These patient specific settings are described in **Appendix G**. In **Chapter 7**, the following questions are investigated:

- Are ERs reproducible when the standard protocol is repeated?
- Is neural stimulation the greatest at the cathode, the anode or do both contribute equal?
- Are the same ERs evoked when a lower current intensity is used?
- Are the same ERs evoked when a bipolar stimulus is used instead of a monophasic positive pulse?

2.4 Other work

2.4.1 Abstracts

For the International Epilepsy Congress (IEC2015) in Istanbul, the first abstract in **Appendix H** was submitted. A poster was presented during the congress. The second abstract was submitted to the International Conference on Basic and Clinical Multimodal Imaging (BACI) in Utrecht. During the conference, a poster was presented.

2.4.2 METC

When only a single stimulus may be required per stimulus pair to determine the physiological network based on ERs, the SPES protocol would be sped up with a factor 10, resulting in a SPES protocol with a duration of less than 6 minutes. When we may discard 30% of the stimulus pairs, because we assume that these would not evoke any DRs, the duration of the second part of the SPES protocol would be 33 minutes. The total SPES protocol would take 39 minutes, which is 13 minutes less than the duration of the current protocol. A duration of 39 minutes is still too long for usage of SPES during surgery. Therefore, we wrote a request to ask the Medical Research Ethics Committee permission for execution of a third SPES session [26]. Currently, this request is approved. In a neural mass model based on the patient specific network from ERs, an optimal SPES protocol is constructed. This protocol may be tested in a third session. An overview of the strategies towards a contemplated future 'fast' SPES protocol and the current 'slow' SPES protocol is displayed in Figure 2.1.

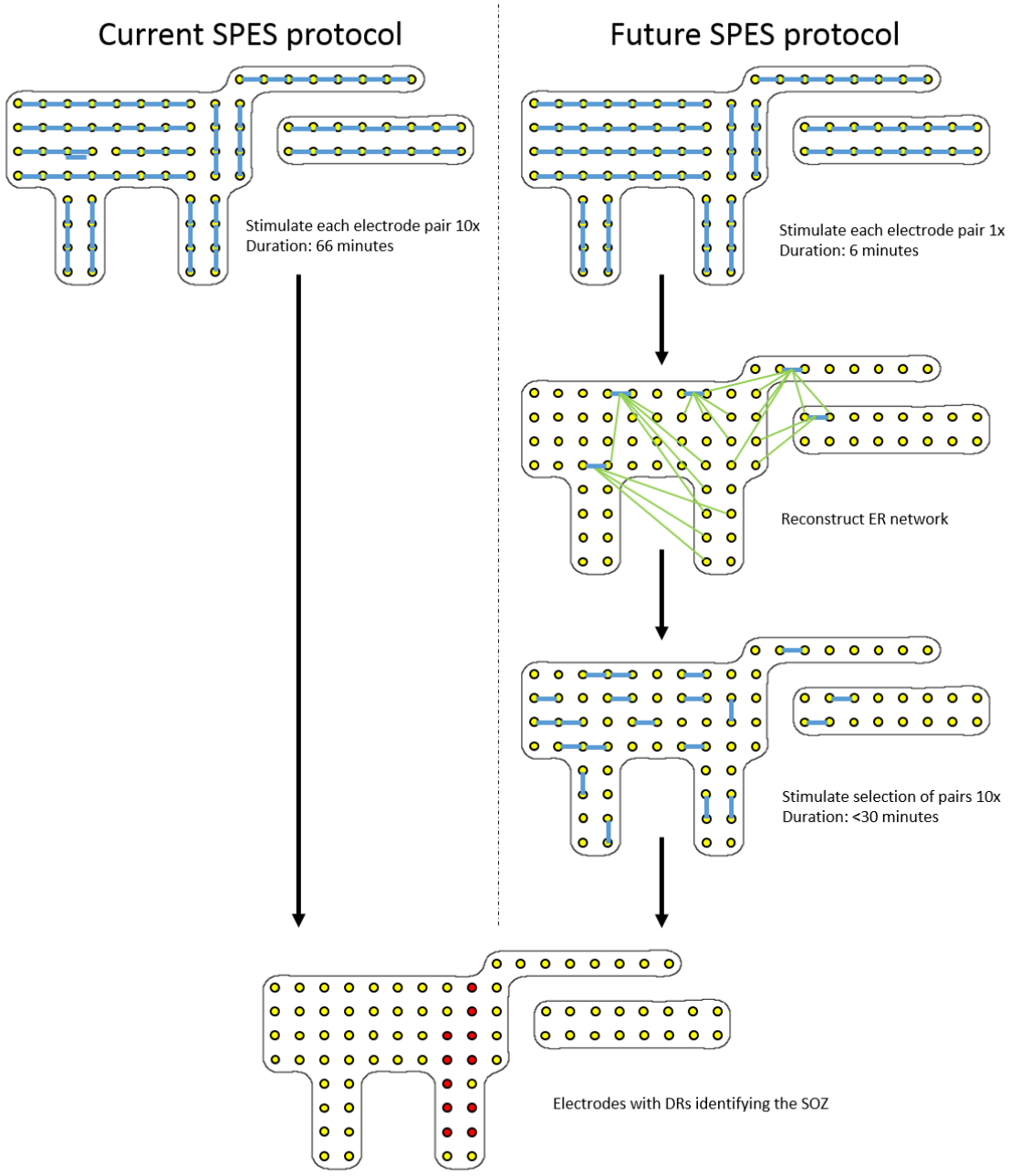
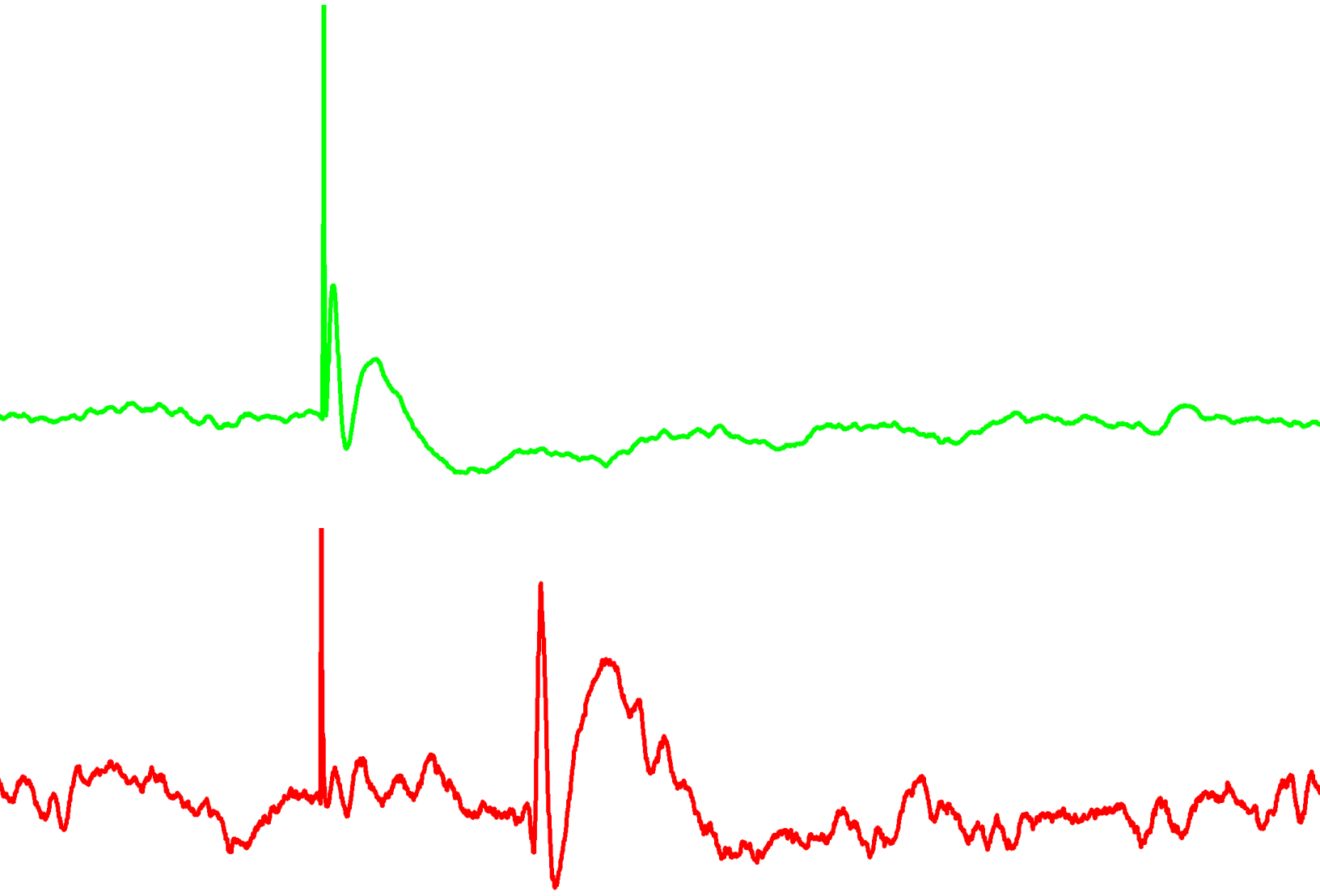


Figure 2.1: *An overview of the difference between the current and future SPES protocol. The blue lines represent the electrode pairs which are stimulated. The green lines represent the connections between stimulated electrode pairs and electrodes in which an evoked ER occur. In the red electrodes, DRs are evoked when other electrode pairs are stimulated.*

Chapter 3

Automatic detection of early responses



This chapter contains three sections. In the first section, two algorithms to automatically detect ERs are constructed. The first detector is based on an amplitude threshold. The amplitude of a detected peak must exceed a set amplitude threshold to be considered as an ER. The second detector is based on an SD factor. The ratio between the amplitude of a detected peak and the standard deviation (SD) must exceed a set SD factor to be considered as ER. It is determined whether the two detectors are able to differentiate between electrodes with ERs and electrodes without an ER. The detectors are evaluated and possible improvements are implemented in the second section. The performance of the detector based on the amplitude threshold and the detector based on the SD factor are determined. The detector with the best performance is validated in the third section.

3.1 Constructing the ER detector

3.1.1 Introduction

Valentin et al. [15] defined early responses (ERs) as sharp deflections immediately following the stimulus artifact or occasionally merging with it (Figure 3.1). ERs were observed in most regions in all patients and these responses were therefore considered to be a normal response of the cortex to stimulation [15] and to expose the underlying physiological network.

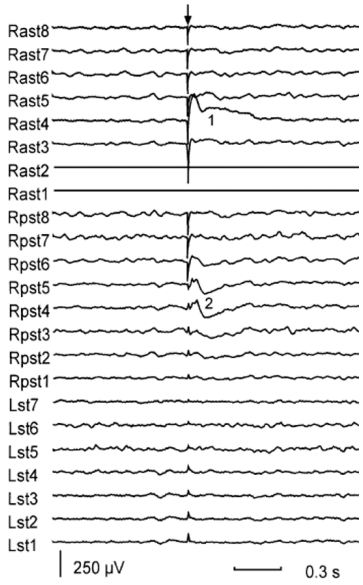


Figure 3.1: *The example of an ER according to Valentin et al. [15]. To the electrodes with the flat lines, a pulse is applied. 1: an ER located in an electrode within 3cm to the stimulated site. 2: an ER located in an electrode more than 3 cm away from the stimulated site.*

For automatic detection, a peak detected as an ER should have the following properties:

1. it appears within 100 ms after the stimulation artifact
2. it is a local extreme in the time domain.

In the University Medical Center Utrecht (UMCU), David Keizer constructed an ER detector in which the stimulation artifact was removed using Wiener filtering [23] before detection of ERs was enabled. This ER detector was optimized for use of an old stimulator. This stimulator has not been used

any more since December 2010. In the new stimulator, the potential difference is recorded between the ground and the reference instead of the reference and the electrodes during an applied pulse. Therefore, removal of the stimulation artifact using Wiener filtering does not make sense, since it is shown that no brain signals are recorded within 9 ms after the stimulation artifact (Appendix C). This was different in the old stimulator in which removal of the stimulation artifact was required to enable detection of ERs. We decided to look at an alternative method for detecting ERs in the time domain, since ERs were not detectable in the time frequency decompositions used for DRs due to the temporal resolution. Lacruz et al [20] identified ERs visually. The ERs were considered significant if their amplitude after averaging was at least twice the amplitude of the background activity. Background activity was considered during the 400 ms previous to the stimulus artifact. Other studies [27, 28] constructed a detector based on the standard deviation (SD). David et al. [29] detected significant responses when the averaged CCEP amplitude increase is above twice the standard deviation of the background activity.
In this section, we try to differentiate responses to stimulations in each stimulus pair with and without an ER by setting an amplitude threshold and an SD factor. In line with Valentin et al. [15], the term "stimulus" or "stimulation" refers to each single pulse and the term "trial" will be used to designate a batch of several identical single pulses applied to the same pair of electrodes, every 5 s. The term "epoch" refers to the response in an electrode to a pulse. In the detector based on an amplitude threshold, a peak is detected as ER when it exceeds a specific amplitude threshold.
In the detector based on an SD factor, a peak is detected as an ER when the ratio of the amplitude and the SD of the signal exceeds the set SD factor. When a signal shows a lot of spontaneous activity, obscuring detection of a peak as an ER, the SD will be larger. The ratio between the amplitude of a peak and the SD is calculated for each electrode for each stimulus. This ratio is called "the SD ratio".
We hypothesize that the amplitude of epochs with an ER is much higher than in epochs in which an ER is absent. With the SD factor, epochs with ERs and without ERs can be differentiated as well. Due to spontaneous activity, we hypothesize that the detector based on SD factor enables generation of a general threshold for differentiation between epochs with and without ER, due to correction for spontaneous activity.

3.1.2 Method

Visual annotation In one patient, ERs are annotated within 100 ms after the stimulation artifact by Dorien van Blooijs (DvB) using Micromed, SystemPlus Evolution with 5 s/page, no additional software filtering, and a variable scaling (usually 1200 μV/cm), depending on the amplitude of the signals and the number of electrodes shown in the display.

Preparation In Appendix A, it is explained how the raw data is processed towards an averaged epoch. In Appendix B, we concluded that ten epochs can be averaged without obscuring ERs which were present in the individual epochs.

Detectors For each trial, the following procedure is performed for each electrode (Figure 3.2). Ten epochs are averaged. The mean amplitude of the averaged signal is calculated in a time window of 2s before and 3s after the stimulation artifact. This value is subtracted from the averaged signal resulting in a signal fluctuating around zero. In this new signal, the Matlab function *peakfinder* (settings: sel = 10, thresh = not defined) is used to detect a positive and/or negative peak in a time range of 9 ms - 100 ms after the stimulation. In the detector based on amplitude threshold, the amplitude of a peak in each electrode for each trial is calculated. In the detector based on SD factor, the SD is calculated in the signal during 2s before the pulse. The amplitudes of the detected peaks are divided by the SD to obtain the SD ratio.

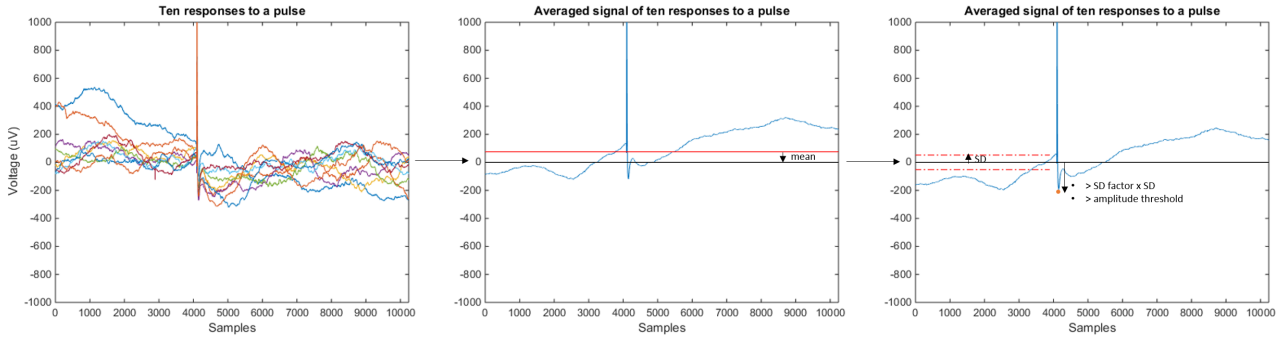


Figure 3.2: *The procedure in the ER detector. First, ten responses to applying a pulse to one an electrode pair in one electrode are averaged. In this averaged signal, the mean amplitude is calculated. This value is subtracted from the averaged signal, resulting in a signal fluctuating around 0. In the new signal, the SD is calculated in 2s before the stimulation artifact. A peak is detected in a time window of 9ms-100 ms after the stimulation artifact. In the detector based on an amplitude threshold, the peak is considered as an ER when the amplitude of the peak exceeds a set amplitude threshold. In the detector based on an SD factor, a peak is considered as an ER when the ratio between the amplitude of the peak and the calculated SD exceeds a set SD factor.*

Boxplots For each trial, the detected peaks are divided into two groups. The first group contains the electrodes in which an early responses (ER) was visually annotated. The second group contains the electrodes in which visually no ER was observed (non ER). First, for each trial, a boxplot is made in which the distribution of the amplitude values for the ER-group and the non ERs-group are displayed. Secondly, for each trial, a boxplot is made in which the distribution of the SD ratios are displayed for both the electrodes in the ER group and the electrodes in the non ERs-group. For both boxplots, it is assessed whether it is possible to differentiate between ERs and non ERs based on amplitudes or SD ratios.

Varying thresholds Two detectors are compared: a detector based on amplitude thresholds, and a detector based on SD factors. These are called "Setting A" and "Setting B" respectively. In setting A, the amplitude threshold is varied from 0 μV to 300 μV with an interval of 10 μV in the detector based on the amplitude threshold. In setting B, the SD factor is varied between 1 and 15 with an interval of 0.5 in the detector based on SD factor. ERs are found with both detectors, for the varying thresholds. For each threshold, the detections are divided into false negative (FN), false positive (FP), true positive (TP) and true negative (TN) detections based on the visual annotation. A detected ER is indicated as true positive (TP) when it is annotated as an ER, false positive (FP) when it is not annotated as an ER. An averaged epoch is true negative (TN) when both visually and with the detector no ER is found, and false negative (FN) when an ER is visually annotated but not detected. For each threshold, the sensitivity, specificity, PPV and NPV (respectively Formula 3.1, 3.2, 3.3 and 3.4) are calculated. An ROC curve is made for both settings. The amplitude threshold and SD factor resulting in the sensitivity and specificity with the shortest distance to the upper left corner of the ROC curve (Formula 3.5) is the optimal threshold. The FP and FN detections are investigated in detail. Possible improvements are evaluated in the next section.

$$Sensitivity = \frac{TP}{TP + FN} \tag{3.1}$$

$$Specificity = \frac{TN}{TN + FP} \tag{3.2}$$

$$PPV = \frac{TP}{TP + FP} \tag{3.3}$$

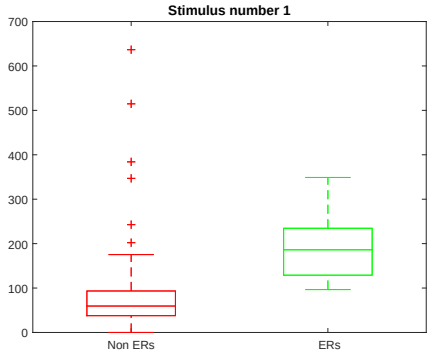
$$NPV = \frac{TN}{TN + FN} \tag{3.4}$$

$$Distance = \sqrt{(1 - Sensitivity)^2 + (1 - Specificity)^2} \tag{3.5}$$

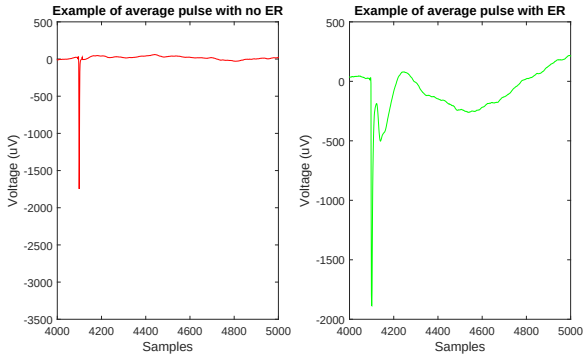
3.1.3 Results

Patient specification Patient 81 (male, 11 years) was implanted with 99 electrodes in December 2014. In 74 trials, 1336 ERs were annotated (per trial: median: 15.5, range: 0-66).

Boxplots of detector based on amplitude threshold In Figure 3.3a, a boxplot for one trial is displayed. This figure shows that the amplitude values for the ERs-group (25th and 75th percentile: 129-235 μV) are higher compared to the amplitude values for the non ERs-group (25th and 75th percentile: 37-93 μV). An example of an averaged epoch with an ER and an epoch without ER is displayed in Figure 3.3b. It shows that an averaged epoch with an ER has a peak with a higher amplitude than an averaged epoch without an ER.



(a) *Distribution of amplitudes for trial 1. A pulse is applied to electrodes 1-2. The amplitude values of peaks detected in the electrodes in the group with annotated ERs (ERs) are displayed in the first boxplot and the amplitude values of peaks detected in the electrodes which were not visually annotated as ERs (non ERs) for this trial are displayed in the second boxplot. The 75th percentile of the non ERs-group is 93 μV. The 25th percentile of the ERs-group is 129 μV.*



(b) *For one trial, two signals are displayed. These signals are the average of ten responses to stimulation in the same trial. In the left figure, the epoch has a peak with an amplitude of 32 μV. In the right figure, the epoch has a peak with an amplitude of -515 μV.*

Figure 3.3: *One stimulation with distribution of amplitudes and two examples of responses to stimulating electrodes 1-2.*

In Figure 3.4, two boxplots are displayed for 74 trials in patient 81. In the first boxplot (red) for each trial, the distribution of amplitude values of detected peaks in electrodes, in which no ER was visually annotated, are displayed. In the second boxplot (green) for each trial, the distribution of amplitude values of detected peaks in electrodes, in which an ER was visually annotated, are displayed. It can be observed that the distribution of amplitude values for the non ERs-group is lower than the distribution of amplitude values for the ERs-group in most of the trials. In trial 28, 44, 47, the distribution of the

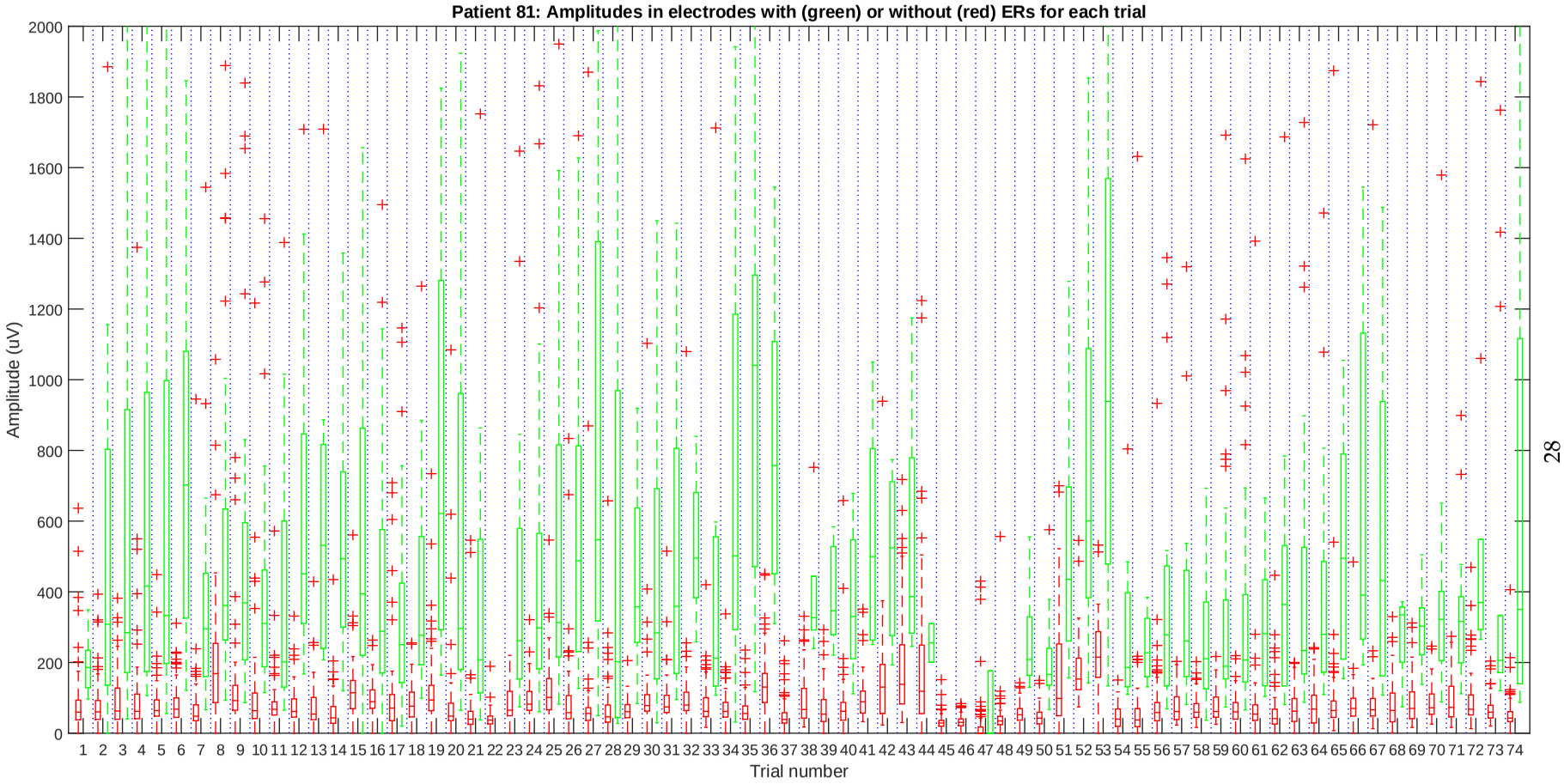


Figure 3.4: *The distribution of amplitude values for each trial in which the electrodes were categorized based on visual annotation. When an ER was observed in an electrode, the electrode was categorized in the ER-group (green). When no ER was observed in an electrode, the electrode was categorized in the non ER-group (red). The amplitude values of the peaks in the electrodes in both groups are distributed in two boxplots for each trial.*

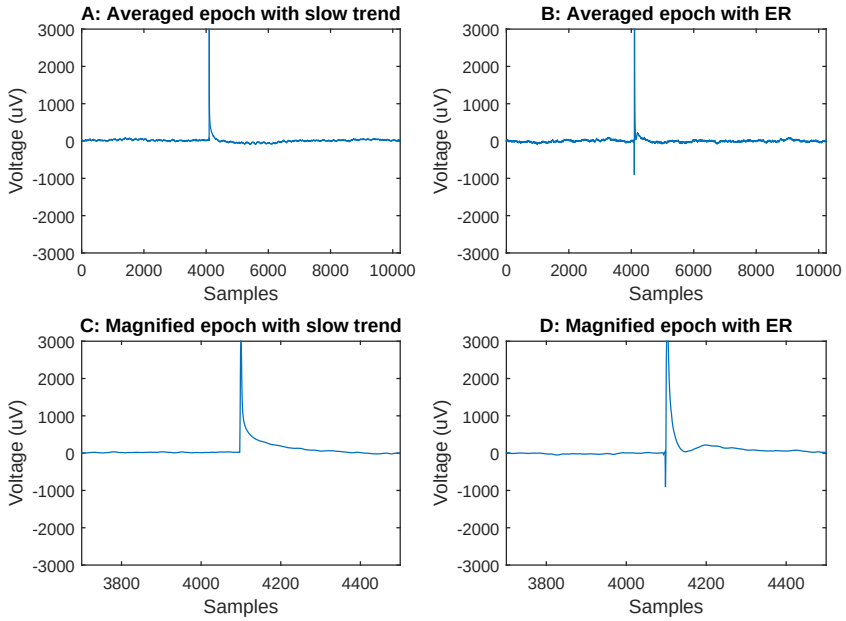
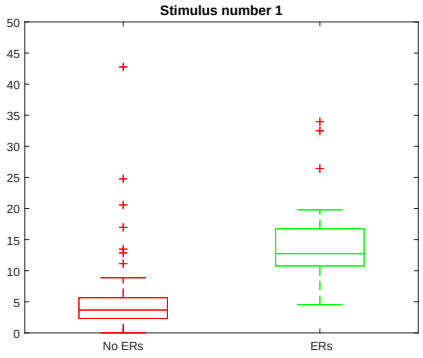


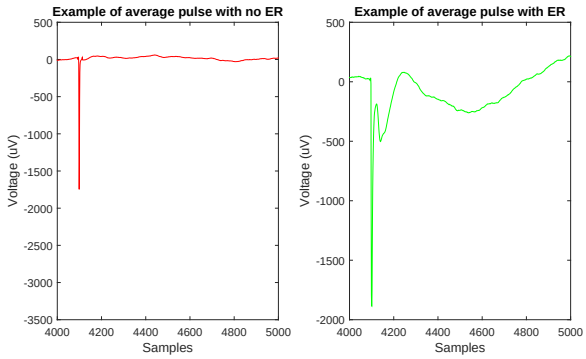
Figure 3.5: *Two averaged responses to trial 47 are displayed. Figure A does not show an ER. Figure B does show an ER. Without magnification, it is difficult to differentiate both situations. Figures C and D are the magnified figures of A and B. In these figures, the difference between an epoch with a slow trend and an epoch with an ER is much easier. During visual annotation in trial 47, many responses with a slow trend were wrongly annotated as ERs.*

amplitude values in the non ERs-group overlaps with the distribution of the amplitude values in the ERs-group. In trial 28 and 44, the median value of the distribution of amplitude values in the ERs-group is higher than the median value of the distribution of amplitude values in the non ERs-group. In trial 47, both the median value of the distribution of amplitude values in the non ERs-group and the median value of the distribution of amplitude values in the ERs-group is 0 μV. When the averaged epochs of several electrodes were investigated in trial 47, a slow trend was found, which was visually misinterpreted as an ER (example in Figure 3.5). This results in a low distribution of amplitude values for both the ERs-group and the non ERs-group.
In all other trials, the distribution of amplitude values of detected peaks in the ERs-group was higher than the distribution of amplitude values of detected peaks in the non ERs-group. This suggests that it was possible to differentiate between epochs with ERs and epochs without an ER. For example, in trial 51, an amplitude threshold between 253 and 261 μV (respectively 75th percentile of peak amplitudes in the non ERs-group and 25th percentile of amplitudes in the ERs-group) would enable differentiation between epochs with an ER and responses to stimuli without an ER. In trial 50, an amplitude threshold between 39 and 136 μV (respectively 75th percentile of amplitudes in epochs with no ER and 25th percentile of amplitude in epochs with ER) would enable differentiation.

Boxplot of detector based on SD ratio For trial 1, the distribution of SD ratio values for electrodes, which were visually annotated as electrodes with an ER (ERs-group), were displayed and the distribution of SD ratios for electrodes, which were visually not annotated as electrodes with an ER (non ERs-group) are displayed. It is observed that the distribution of SD ratio values in the ERs-group (respectively 25th percentile and 75th percentile: 10.8-16.7) is higher compared to the distribution of SD ratio values in the non ERs-group (respectively 25th percentile and 75th percentile: 2.3-5.7). An example of an averaged signal of ten epochs with an ER and an averaged signal of ten epochs with no ER is displayed in Figure



(a) *Distribution of SD ratios for trial 1. Electrodes 1-2 are stimulated. Based on visual annotation, the electrodes are divided into two groups. The ERs-group contains electrodes in which an ER was observed. The non ERs-group contains electrodes in which no ER was observed during visual annotation. The ratio between the SD and the detected peak in each electrode was calculated. For both the ERs-group (green) and the non ERs-group (red), distributions of these ratios are displayed. The 75th percentile of the SD ratios in the non ERs-group is 5.7. The 25th percentile of the SD ratios in the ERs-group is 10.8.*



(b) *For one stimulation pair, two averaged signals of ten epochs are displayed. In the left figure, the epoch has a peak with an absolute SD ratio of 2.1. In the right figure, the epoch has a peak with an absolute SD ratio of 24.7.*

Figure 3.6: *One trial with distribution of SD ratios and two examples of averaged responses to stimulating electrodes 1-2.*

Table 3.1: *Performance of the detectors*

Setting	Amplitude threshold (μV)	Sensitivity	Specificity	PPV	NPV	Distance
A	140	0.87	0.86	0.62	0.96	0.19

Setting	SD factor	Sensitivity	Specificity	PPV	NPV	Distance
B	7	0.86	0.83	0.57	0.96	0.2176

3.6b.

In Figure 3.7, the distribution of SD ratios for electrodes in the ERs-group (green) and the non ERs-group (red) are displayed for each trial. In each trial except trial 47, the SD ratios for electrodes in the ERs-group is higher compared to the electrodes in the non ERs-group. This suggests that it is possible to differentiate between epochs with an ER and epochs with no ER. In trial 4, epochs with ERs and epochs with no ERs may be distinguished with an SD factor between 7-13 (respectively 75th percentile of the SD ratios in epochs with no ER and 25th percentile of the SD ratios in epochs with ER). In trial 50, epochs with ERs and epochs with no ERs may be distinguished with an SD factor between 3-9 (respectively 75th percentile of the SD ratios in epochs with no ER and 25th percentile of the SD ratios in epochs with ER).

Varying thresholds In Table 3.1, the performance of the detector with setting A and B are displayed. An ROC-curve is displayed in Figure 3.8.

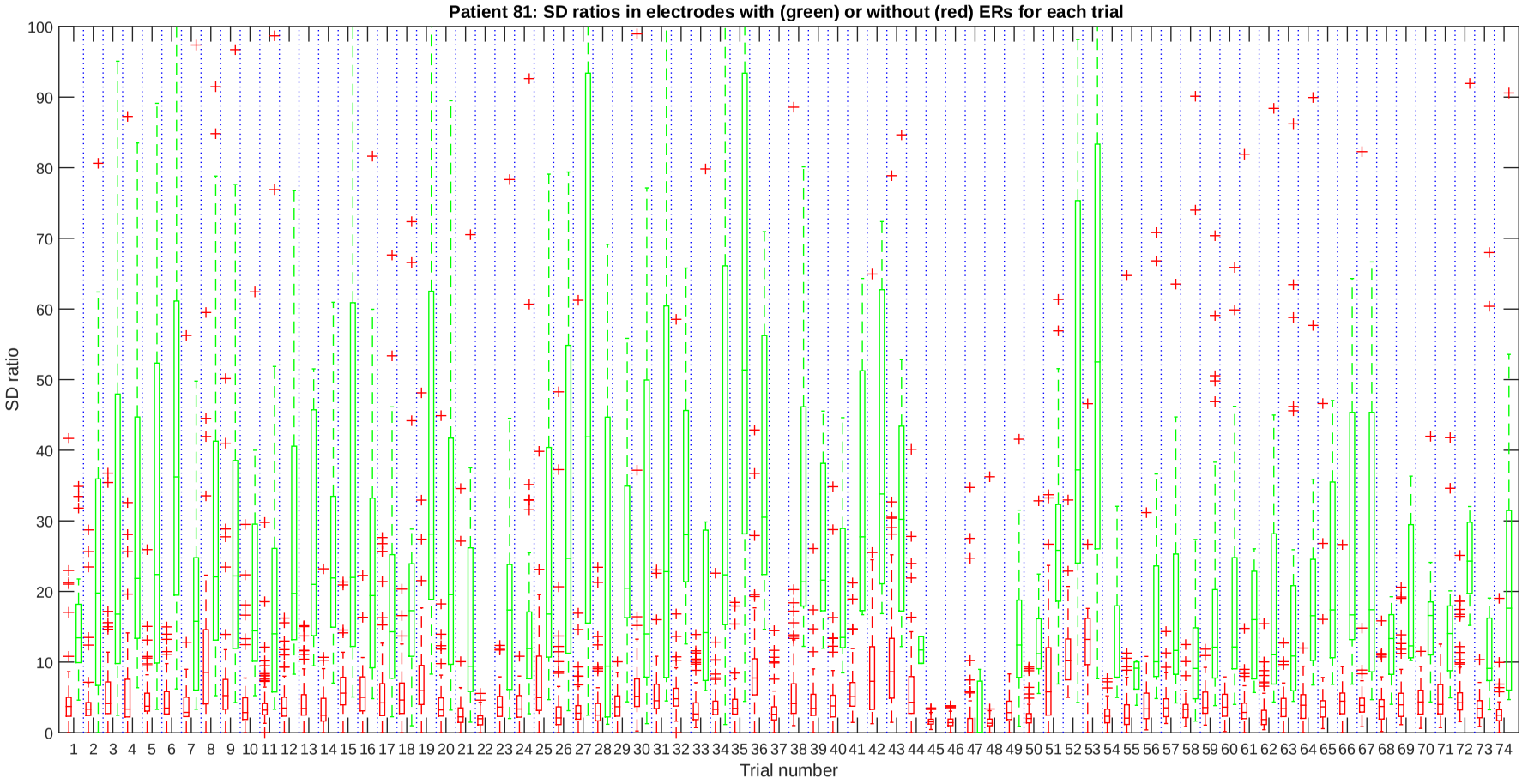
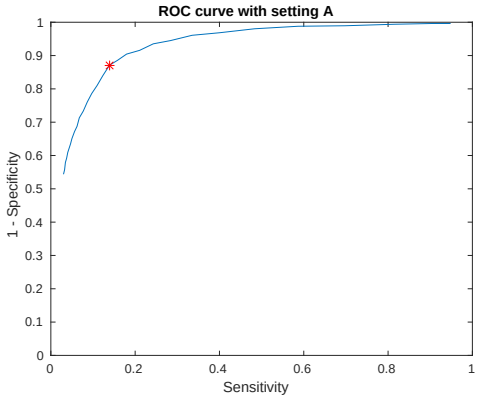
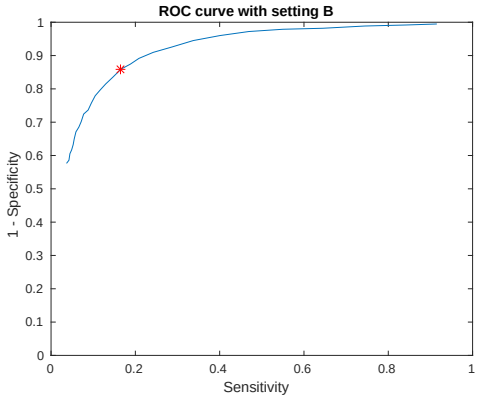


Figure 3.7: *For each trial (74 in total), two boxplots are displayed. In the red boxplot, the distribution of SD ratios for electrodes in the non ERs-group are displayed. In the green boxplot, the distribution of SD ratios for electrodes in the ERs-group are displayed.*

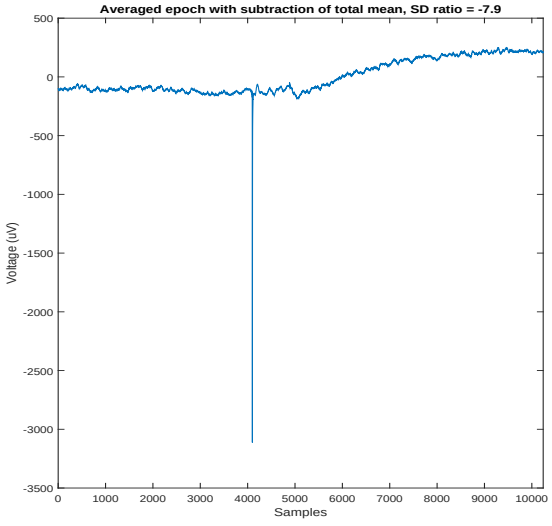
Figure 3.8



(a) *An ROC curve in patient 81. The optimal amplitude threshold is displayed with a red *.*



(b) *An ROC curve in patient 81. The optimal SD factor is displayed with a red *.*



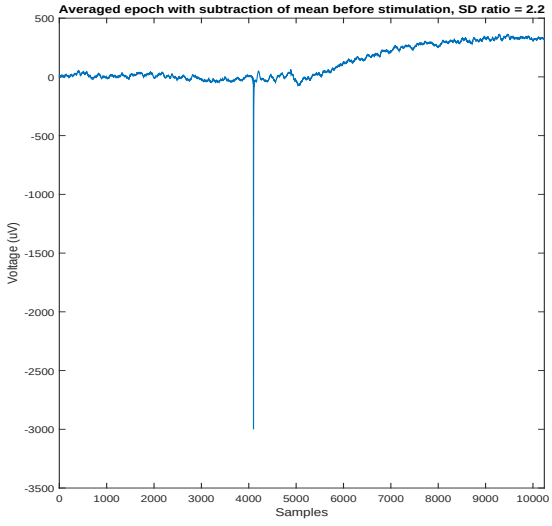


Figure 3.9: *The same signal is displayed in both figures. In the left figure, the mean value of the total epoch is subtracted. Due to the slope after the stimulation, this subtraction leads to a signal which is located far below 0 resulting in a peak with a high negative amplitude. This results in an SD ratio which is easily exceeding an SD factor. However, this is clearly not an ER. When the mean of only the part before the stimulation is subtracted from the signal. The signal deviates around 0 and as a result, the SD ratio is lower. This signal would not be detected as an ER.*

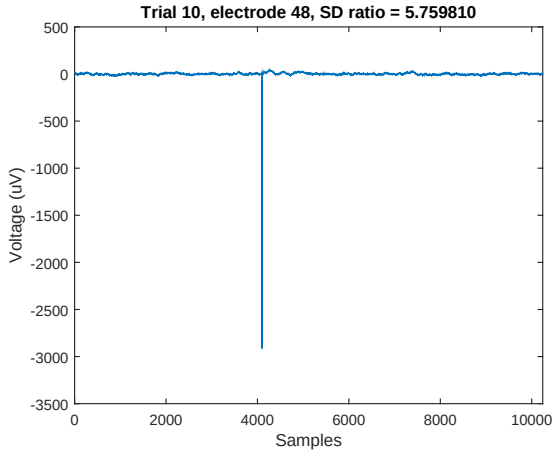


Figure 3.10: *In this epoch, no ER is visible. However, an ER was detected due to a very low SD in the signal previous to the stimulation artifact. Setting a minimal value for SD would prevent detection of those false positive responses.*

3.1.4 Discussion

In this section, we investigated whether ERs could be detected with a detector based on amplitude threshold (setting A) and SD factor (setting B). Our hypothesis was that epochs with an ER and epochs with no ER could be differentiated with both detectors. In Figure 3.4 and 3.7, it was shown that in each trial, epochs with an ER and epochs with no ER could be differentiated in both detectors. For the detector with setting A, in three trials, it was not possible to differentiate epochs with and without an ER. In trial 28 and 44, the distribution of amplitude values of epochs with an ER and the distribution of amplitude values of epochs without an ER overlapped. The median value of the distribution of amplitude values in the ERs-group was higher than the median value of the distribution of amplitude values in the non ERs-group. It is assumed that epochs with ERs and epochs without an ER could be differentiated in trial 28 and 44. In trial 47, a very low distribution of amplitude values of detected peaks in both the ERs-group and the non ERs-group was found. This was caused by annotation of epochs with a slow trend after the applied pulse instead of an ER peak. In the detector with setting B, it was not possible to differentiate the ERs-group from the non ERs-group either in trial 47, due to the slow trend after the pulse. In trials 8, 28, the distribution of SD ratios overlapped for the ERs-group and the non ERs-group. The median value of the distribution of the ERs-group was higher in both trial 8 and 28. This suggests that it is possible to differentiate the ERs-group and the non ERs-group. In all other trials, the distribution of the amplitudes of the detected peaks and the distribution of SD ratios in the ERs-group was higher than the distribution of the amplitudes of the detected peaks and the distribution of SD ratios in the non ERs-group. This suggests that it was possible to differentiate between ERs and non ERs-group in both the detector with setting A and the detector with setting B. However, the ranges of amplitude thresholds, to differentiate between the ERs-group and the non ERs-group, differed in several trials and a general threshold for all trials was not suitable. In Figure 3.7, it is observed that the 25th percentile of the distributions of the SD ratios in the ERs-group are similar in most trials. This suggests that a general SD factor might be easier determined than a general amplitude threshold.
However, several improvements can be added in both detectors. These improvements are described below. When trials in patient 81 were evaluated in more detail, we saw that a slow trend was present in trial 47 (Figure 3.5). In the Matlabfunction *peakfinder*, one parameter is "sel". It determines how much the amplitude of a peak should be higher than the surrounding data to be detected as a peak. Due to the settings in *peakfinder* (sel=10), even a small peak on this trend, which is 10 μV higher than the voltage values of its surrounding samples, generates a peak with a high amplitude. This small peak may be falsely considered as an ER. When sel > 10 μV, false positive detection of small peaks on slow

trends may be reduced. In addition, it was striking that some epochs showed a slow slope after the pulse (Figure 3.9). A slow slope after the pulse led to an increase in the median amplitude. When this value was subtracted, a small peak was detected as an ER. When the median value is calculated in only the signal before the pulse, this peak is correctly not detected as an ER. Finally, in some epochs (Figure 3.10), the SD is very low and this facilitates detection of peaks and false positive ERs with the detector with setting B. An improvement could be to implement a minimal SD to prevent detection of false positive ERs. Another suggestion for improvement is to implement a wave template to detect ERs. Due to the high variation in wave forms of ERs, this is not suitable.
We conclude that in both the detector with setting A and with setting B, it is possible to differentiate the ERs-group from the non ERs-group. However, Figure 3.7 assumes that obtaining a general threshold is more adequate in the detector with setting B. We mentioned some improvements such as implementing a minimal SD, subtracting the mean amplitude value in the signal before the pulse instead of subtracting the mean amplitude value in the total signal, and increasing sel, which is used in the matlabfunction *peakfinder*. In the next section, we test both detectors with varying parameters and determine which detector should be used in further research.

3.2 Evaluating the ER detector

3.2.1 Introduction

In the previous section, a detector with setting A and a detector with setting B were investigated. With both detectors, it was possible to differentiate between epochs with an ER and epochs with no ER. In the discussion, we described several parameters which may improve the performance of the detector. In this section, these parameters are varied. The combination of parameters with the best performance, may be validated in the next section. We hypothesize that the detector based on SD factor performs best, when a minimal SD is set and "sel" > 10 μV.

3.2.2 Method

Visual annotation In three patients, ERs are annotated by DvB using Micromed, SystemPlus Evolution with 5 s/page, no additional software filtering, and a variable scaling (usually 1200 μV/cm), depending on the amplitude of the signals and the number of electrodes shown in the display.

Detector The performance of the detectors with setting A and B, in which the described parameters are varied, is compared. In the detector with setting A, the threshold is based on amplitude values. In the detector with setting B, the threshold is based on the ratio between the SD and the amplitude of a detected peak. Both the detector with setting A and the detector with setting B calculated the mean amplitude of the averaged epoch of an electrode for each trial. This value is subtracted from the averaged epoch resulting in a signal fluctuating around zero. In this new signal, the Matlab function *peakfinder* (settings: sel = variable, thresh = []) is used to detect a positive and/or negative peak in a time window of 9 ms - 100 ms after the pulse. In the detector with setting B, the SD is calculated during 2 s before the pulse.

Varying parameters In the algorithm of the two detectors, several parameters are present which are varied to improve the performance of the detector. The amplitude threshold is varied in the detector with setting A and the SD factor is varied in the detector with setting B. The amplitude threshold is varied between 0 μV and 300 μV with steps of 10 μV. The SD factor is varied between 1 and 15 with steps of 0.5.
In the previous section, some improvements were suggested. These improvements are discussed below. The possible improvements are implemented in both detectors.
The sel is varied between 10 μV and 200 μV with steps of 10 μV.
Another parameter is the median value of amplitude of the signal which is subtracted from the signal to fluctuate around 0. The median value can be calculated in the total signal (2 s before the pulse - 3 s after the pulse) or in the signal before the pulse (2 s). In this section, it is investigated whether the detector performs better when the median value of the total signal is subtracted (setting A1 or B1) or when the median value of the signal before the pulse is subtracted (setting A2 or B2).
The last parameter is a minimal SD. A minimal SD is varied between 0 μV and 100 μV with steps of 10 μV. This parameter is only present in the detector with setting B.

These parameters are varied for both detector with setting A and the detector with setting B, resulting into four different settings:

- Setting A1: vary the amplitude threshold, sel and subtracting the median value of the total signal to obtain a signal deviating around 0.
- Setting A2: vary the amplitude threshold, sel and subtracting the median value of the signal before the stimulation artifact

- Setting B1: vary the SD factor, sel, a minimal SD and subtracting the median value of the total signal
- Setting B2: vary the SD factor, sel, a minimal SD and subtracting the median value of the signal before the stimulation artifact

For each possible combination of values for the parameters, peaks are detected in the time window for ERs. When the peak exceeds the amplitude threshold in setting A, it is considered as ER. When the ratio between the detected peak and the SD exceeds the SD factor in setting B, it is considered as ER. The detected ERs are compared to the visually annotated ERs and for each trial, the responses of each electrode to each trial are categorized as true positive, false positive, true negative or false negative. Sensitivity, specificity, PPV and NPV are calculated. The setting with the shortest distance to the upper left corner of the ROC curve is the setting with the best performance. This setting is validated in the next section.

3.2.3 Results

Patient specification The set contained three patients (Table 3.2): one male and two females, on average 11 years old (range: 8-12). The patients underwent grid implantation in December 2014 and January 2015. The grid contained on average 80 electrodes (range: 62-88). On average 70 stimuli (range: 40-74) were given. In total, 2779 ERs were annotated (median: 921, range: 522-1336).

Table 3.2: *Patient specification*

Patient	Gender	Age	Electrodes	Stimuli	Visual ERs (per trial: median, range)
81	m	11	88	74	1336 (15.5, 0-66)
83	f	12	62	40	522 (11.5, 2-35)
88	f	8	80	70	921 (12.5, 0-25)

Performance of detector in various settings In Table 3.3, the optimal values for the parameters resulting in the best performance are displayed for the four detectors. The sensitivity is 0.83 in detector A1 and A2, and 0.84 in detector B1 and B2. The specificity is 0.84 in detector A1, A2 and B2, and 0.83 in detector B1. The PPV is 0.55 in detector A1, A2 and B2, and 0.54 in detector B1. The NPV is 0.96 in detector A1, A2 and B1 and 0.95 in B2. The shortest distance to the upper left corner of the ROC curve is found in detector B1.

3.2.4 Discussion

Four detectors have been tested in three patients. All detectors had a comparable performance. However, the detector with settings B1 had the shortest distance to the upper left corner of the ROC curve when the parameter "sel" = 20 μv, minimal SD = 50 μV and the SD threshold = 2.5.
Burnos et al. [27] defined a threshold as the mean of an envelope around a signal + 3*SD. Staba et al. [30] uses a threshold of a mean energy + 5*SD. The same accounts for Crepon et al. [28]. Our threshold of 2.5*SD is not similar to other detectors. However, these detectors were set for detecting high frequency oscillations and not especially for ERs which are evoked after applying a pulse to two adjacent electrodes. These detectors are therefore not completely comparable, but there is not yet a detector available detecting ERs after stimulating tissue beneath electrodes. Lacruz et al [20] annotated CCEP responses when these exceeded twice the SD of the baseline. This is comparable to our threshold. We also included other parameters which could have resulted in a higher SD threshold.
A strong point of this research is that several parameters are varied and combined. This facilitates

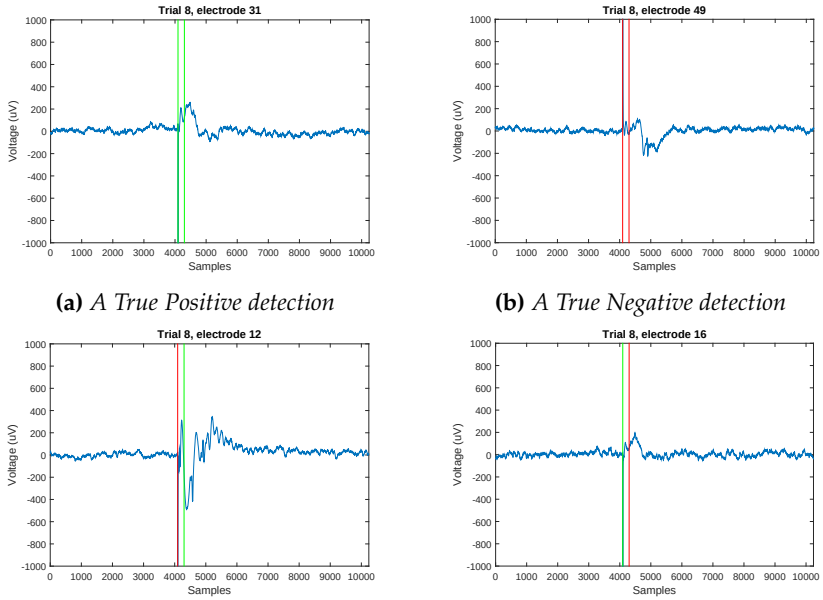
Table 3.3: *Performance of the detectors with varying settings.*

	Parameters			Performance				
Setting	Amplitude threshold (μV)	Sel (μV)	Minimal SD (μV)	Sensitivity	Specificity	PPV	NPV	Distance
A1	130	20	NA	0.83	0.84	0.55	0.96	0.2317
A2	60	180	NA	0.83	0.84	0.55	0.96	0.2327

	Parameters			Performance				
Setting	SD factor	Sel (μV)	Minimal SD (μV)	Sensitivity	Specificity	PPV	NPV	Distance
B1	2.5	20	50	0.84	0.83	0.54	0.96	0.2305
B2	2	180	30	0.83	0.84	0.55	0.95	0.2323

finding the optimal combination of parameters to obtain the best performance.
During evaluation of the false positive detections in Matlab, it was observed that many annotations were missed. In SystemPlus, all electrodes are packed on one screen and 5 s/page were displayed. Therefore, it is likely that some ERs were missed. On the contrary, other ERs were wrongly annotated as ER. In SystemPlus, it is not possible to differentiate a slow trend and an ER (Figure 3.5), since the signals are too packed on one screen. During visual annotation, an ER was not annotated because it looked like a slow trend and others were annotated although these signals showed a slow trend. During revision, the response in each electrode to some trials were magnified in Matlab and it was observed that it was difficult to distinguish ERs from slow trends during annotation in SystemPlus. For affirmation of the performance of the detector, a second observer should to test the discrepancies between the automatic detector and visual annotations.
The annotations in SystemPlus were made in ten individual epochs instead of the averaged epoch, which was used for detecting ERs in Matlab. This could explain the large number of false positive detections as well. However, an ER is assumed to occur during each pulse, so averaging would only filter out the false positive randomly occurring responses and improve the results. It is unlikely that when an ER is not present during ten separate epochs, it will show up in the averaged epoch. After a small investigation (Appendix 5), it is assumed that the averaged epoch is a reliable rendering from the ten separate epochs. Due to the limited time for this project, it was not possible to check the ten epochs visually for each electrode in each trial.
Remarkable is the low PPV. The number of false positive detections (median: 1897, range: 1840-1963) was high in each optimal setting. In Figure 3.11, an example of a true positive, true negative, false positive and false negative detection are displayed. The true positive detection is clearly an ER. The true negative detection shows a response after the specific time window for detecting ERs. Between the two vertical lines, no clear activity is visible. The false positive detection shows a large peak in the time window for ERs. This raises the question whether the visual annotation in this epoch was correct. Most false positive detections in the three patients may be reconsidered as ERs. Therefore, it is assumed that the actual number of false positive detections is lower than the current number.

In some epochs, an equivocal ER was visible, as displayed in Figure 3.12. The slope was slowly decreasing, resulting in a local minimum being detected as an ER. However, after 100 ms, a small wave is present. The question is whether the local minimum is an ER followed by a slow wave. In the second and third Figure, a plateau is visible. This is not a sharp deflection and according to Valentin et al. [15] it should not be considered as an ER. However, it is a change in signal which may be related to the pulse. The question is whether this plateau is an ER or not. Both are detected as ER in the detector with setting B1 and optimal parameters. According to Valentin et al. [15, 19, 16], the peak must start within 100 ms after the stimulation artifact. It is the question how strict this border is, since deterministic peaks



(a) *A True Positive detection*

(b) *A True Negative detection*

(c) *A False Positive detection according to the visual annotation. However, there is a clear response visible in the time window of an ER, so this may be reconsidered.*

(d) *A False Negative detection*

Figure 3.11: *The two lines show the time window in which a peak must be present to be detected as an ER. When the first line is green, an ER was visually annotated. When the first line is red, no ER was annotated. The second line is green when an ER is detected and red when no ER is detected.*

and slow waves were visible more than 100 ms after the stimulation artifact in the averaged epoch. So, it is doubtful that these peaks are delayed responses which occur only stochastic. To investigate whether these peaks are actual ERs is outside the scope of this study.

Further research should focus on investigating the origin of the peaks more than 100 ms after the stimulation artifact. This is important to improve constructing the networks using the early responses. We conclude that the performance of each detector was sufficient for usage in further research. However, in some cases the detector with amplitude threshold is not suitable. In Figure 3.13, two examples are shown. The upper Figures display an averaged epoch with no ER. However, when spontaneous activity is not taken into account, the small peak is detected as an ER. In the lower Figures, an ER was not detected in the detector with amplitude threshold, since the peak had a very low amplitude. However, there is a clear response visible which should be considered as an ER. This suggests that even though the performance of all detectors is comparable, the detector with settings B1 is the best to use in further research. Therefore, the performance of the ER detector with settings B1 will be tested in other patients in the next section.

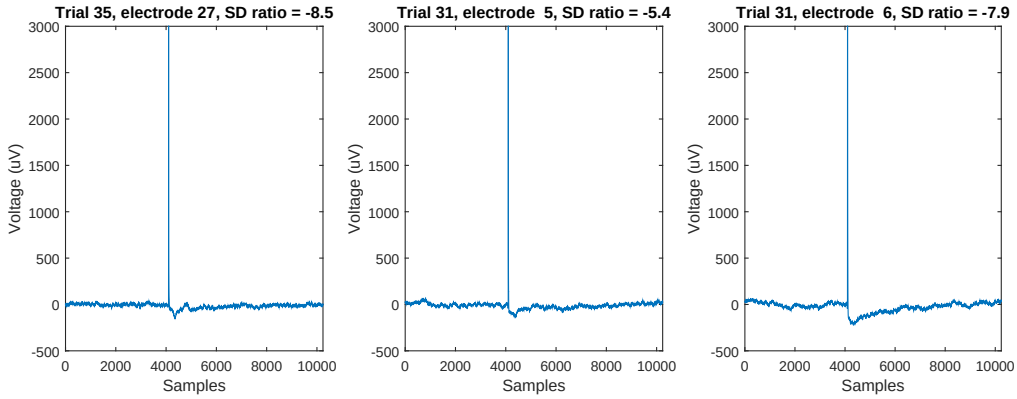
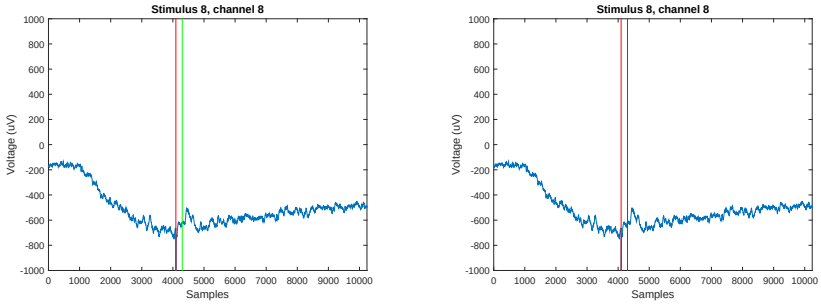
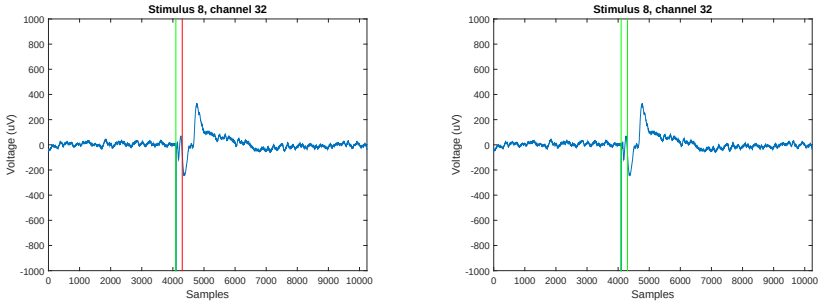


Figure 3.12: *These electrodes show a response after the pulse. However, the peak occurs after 100 ms (the first figure) or it is not a peak or slow wave, but a lower plateau level (second and third figure). It is doubtful whether these responses should be detected as early responses.*



(a) *A False Positive detection in the detector with setting A1.*

(b) *A True Negative detection in the detector with setting B1.*



(c) *A False Negative detection in the detector with setting A1.*

(d) *A True Positive detection in the detector with setting B1.*

Figure 3.13: *The two lines show the time window in which a peak must be present to be detected as an ER. When the first line is green, an ER was visually annotated. When the first line is red, no ER was annotated. The second line is green when an ER is detected and red when no ER is detected.*

3.3 Validating the ER detector

3.3.1 Introduction

In the previous section, four ER detectors were tested in three patients. The performances of these detectors were comparable, but we concluded that the detector with setting B1, SD factor = 2.5, sel = 20 μV, minimal SD = 50 μV was the best. In this section, this detector is validated. For this ER detector, both the specificity and sensitivity must be high to construct a functional network. Our final goal is to discard stimulus pairs which will not evoke any DRs by the physiological network based on ERs. Since we do not want to falsely discard any stimulus pairs, it is important to avoid false negative detections. Therefore, sensitivity must be as high as possible.

3.3.2 Method

Visual annotation In three patients, ERs are annotated visually by DvB using Micromed, SystemPlus Evolution with 5 s/page, no additional software filtering, and variable scaling (usually 1200 μV/cm), depending on the amplitude of the signals and the number of electrodes shown in the display. When amplitudes are too high to be able to differentiate ERs in neighboring electrodes, the scaling is decreased to 2000 μV/cm.

Detector In three patients, ERs are detected with the ER detector with B1 settings, SD factor = 2.5, sel = 20 μV, and minimal SD = 50 μV. The number of true positive, false positive, true negative and false negative ERs are determined based on comparison with visual annotations. Sensitivity, specificity, NPV and PPV (Formulas 3.1, 3.2, 3.3, 3.4 in chapter 3.1.2 are calculated in each patient to determine whether the detector is performing good enough for further usage.

3.3.3 Results

The validation set contained three patients (Table 3.4): 2 males and 1 female with an average age of 12 years old (mean: 776 per patient, range: 7-16). The ECoG data was recorded in February and March 2015. In total, 1564 ERs were annotated (mean: 819 per patient, range: 617-946). The detector detected 2458 ERs (range: 643-1112). The sensitivity, specificity, PPV and NPV are displayed in Table 3.5.

Table 3.4: *Patient characteristics of validation set*

Patient	Gender	Age	Electrodes	Trials	Visual ERs (per trial: median, range)	Detected ERs (per trial: median, range)
89	f	14	56	45	764 (17, 7-28)	643 (15, 4-24)
91	m	16	86	54	617 (13, 4-26)	703 (13.5, 1-30)
93	m	7	64	48	946 (20, 0-33)	1112 (22.5, 0-42)

Table 3.5: *The sensitivity, specificity, PPV and NPV for patients in the validation set*

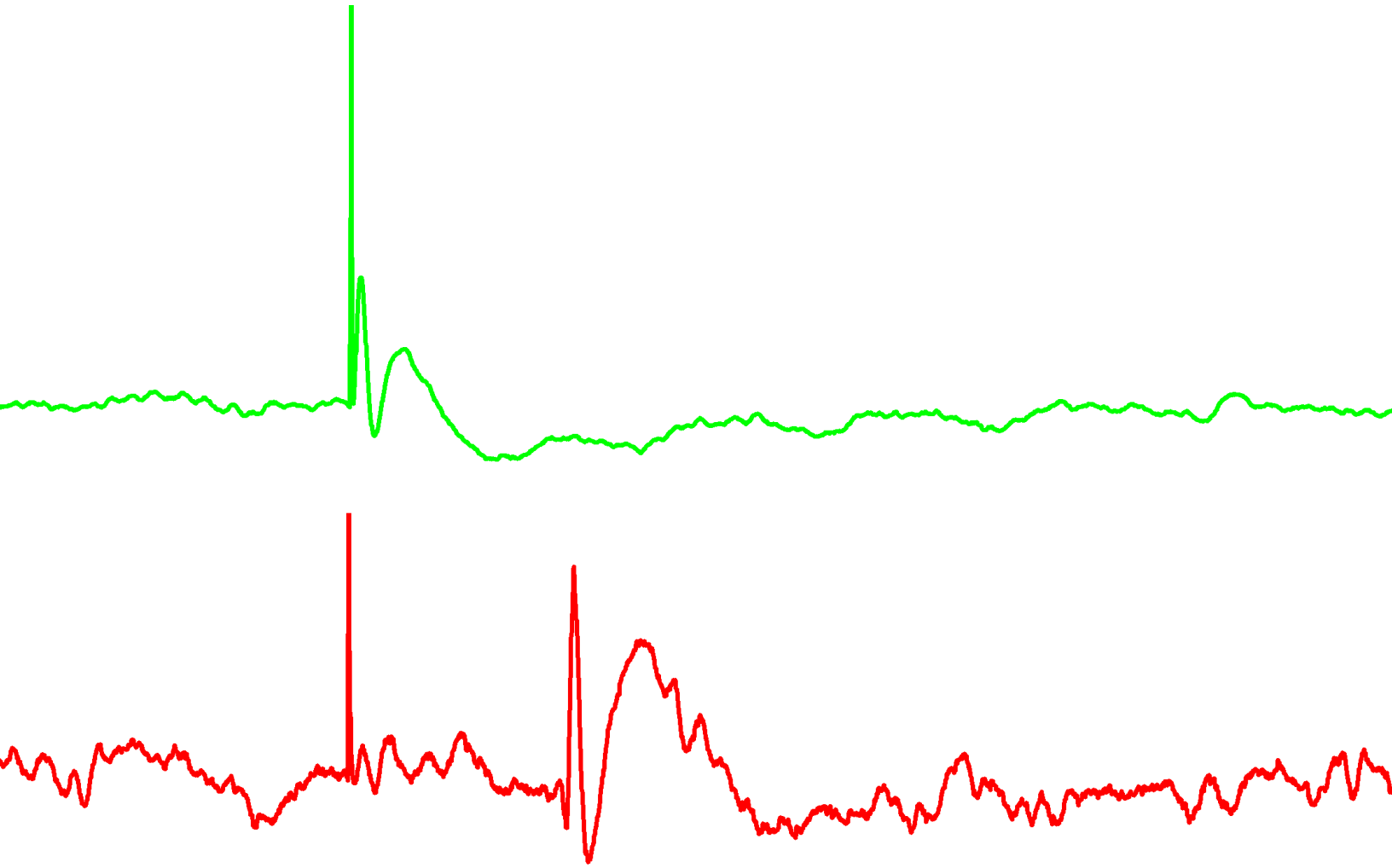
	detector B1			
Patient	Sensitivity	Specificity	PPV	NPV
89	0.71	0.94	0.84	0.88
91	0.76	0.94	0.67	0.96
93	0.86	0.86	0.73	0.93
Mean	0.78	0.91	0.75	0.92

3.3.4 Discussion

The mean sensitivity, specificity, PPV and NPV with the set variables in detector B1 were respectively 0.78, 0.91, 0.75, 0.92. Both the sensitivity and specificity are high. This means that most detected ERs are true ERs and not many ERs are missed. This is required for reconstructing a reliable physiological network. The sensitivity is not as high as the specificity. For now, we conclude that the ER detector performs sufficient to be used in further research. However, to reduce false negative detections, the ER detector must be improved before it may be used in a clinical setting.
A limitation of this study is that the visual annotations were only evaluated by DvB. No second observer had corroborated the annotations. Therefore, it is possible that ERs are wrongly annotated, missed or equivocal, so that the gold standard fails. Human scoring is by definition subjective. It is recommended that for further research, these annotations are determined by consensus between at least two observers.
Another limitation is that we try to reconstruct a physiological network out of ERs. As thousands of cells are located under each electrode of the grid, it is likely that only strong and fast connections are revealed and some connections remain undetected. Although the presence of ERs provides evidence of connections between the electrodes to which a pulse is applied and the areas where they are recorded, the absence of ERs does not imply lack of functional connection [20]. There might be significant spread of neuronal impulses, e.g. due to polysynaptic pathways, which may render responses undetectable by SPES [20].
The network is based on the presence of an ER. The strength of an ER [24] and its delay [31] could also be very important for the reconstruction of the physiological network. This may be a good focus for further research. The current ER detector is only the first step in evaluating the presence of ERs and its physiological network.
A lot of research is done on diffusion tensor imaging (DTI) in the last couple of years. With diffusion imaging methods and tractography algorithms, trajectories of white-matter fascicles (tracts) in the human brain can be estimated in vivo [32]. Such methods could help in getting more insight in the physiological networks below the electrode grid and across the rest of the brain . We conclude that this ER detector is a good first step in analyzing the network of ERs in individual patients. The performance of the ER detector with settings B1, SD factor = 2.5, sel = 20 μV, and minimal SD = 50 μV was sufficient and this ER detector is used in the retrospective analysis in chapter 5.

Chapter 4

Automatic detection of delayed responses



In this chapter, an algorithm to automatically detect DRs is constructed. In the first section, two detectors are compared. In the first detector, the amplitude threshold is varied. When the amplitude of a detected peak exceeds a set amplitude threshold, the peak is considered to be a potential DR. In the second detector, the SD factor is varied. When the ratio between the amplitude of a detected peak and the SD exceeds the set SD factor, the peak is considered to be a potential DR. When significant more often peaks are located after the stimulus artifact, the peaks are considered to be a DR. The one-tailed sign-test is used to determine whether the peaks are significantly related to the stimulus artifact [15]. Several parameters are varied to improve the detection algorithms in the second section. In the third section, the detector with the best performance in the section 2 is validated.

4.1 Constructing the DR detector

4.1.1 Introduction

Valentin et al. [15] describes delayed responses (DRs) as spikes or sharp waves occurring between 100 ms to 1 second after the stimulation artifact. They distinguished between spontaneous interictal spikes and truly evoked delayed responses by looking at the random occurrence of spikes during one second before each pulse. A one-tailed sign test was used to determine whether a peak occurred significantly more often after the pulse. The distribution of DRs is significantly associated with the regions where the seizure onset was located. Removal of areas with DRs correlated with a better seizure outcome [16].

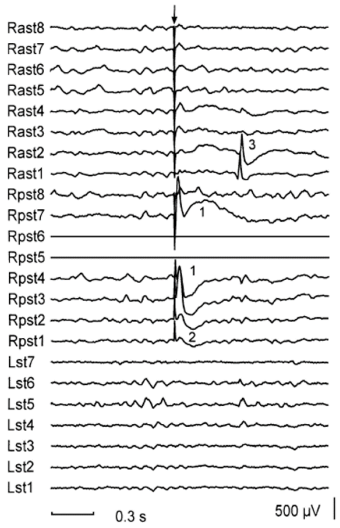


Figure 4.1: *The example of a DR according to Valentin et al. [15]. To the electrodes with the flat lines, a pulse is applied. 1: an ER located in an electrode within 3cm to the stimulated site. 2: an ER located in an electrode more than 3 cm away from the stimulated site. 3: A DR*

4.1.2 Method

Visual annotation In six patients, DRs were visually annotated in ten epochs for each electrode in each trial using Matlab. 33% of the trials was used for investigating possible improvements for the detector, 33% was used for training the detector, and 33% was used for validating the detector. Due to variety in number of DRs in different patients, it seemed more adequate to include part of the trials from all six patients in training and validating the detector, rather than using three patients for training

and the other three for validation.
Peaks during 1 s before the stimulation artifact and during 0.1-1 s after the stimulation artifact were annotated by DvB in Matlab (Figure 4.2), with a scaling of 1000 μV/cm. Matlab facilitated displaying ten epochs on one screen and magnification of these epochs. Due to these possibilities, annotation was less time consuming. As DRs often resembled spontaneous interictal discharges and were not seen after each stimulus, we assumed that a spike was a DR when its occurrence was statistically unexpected compared to the second prior to stimulation, with α <0.05 (one-tailed sign test) [15, 21].

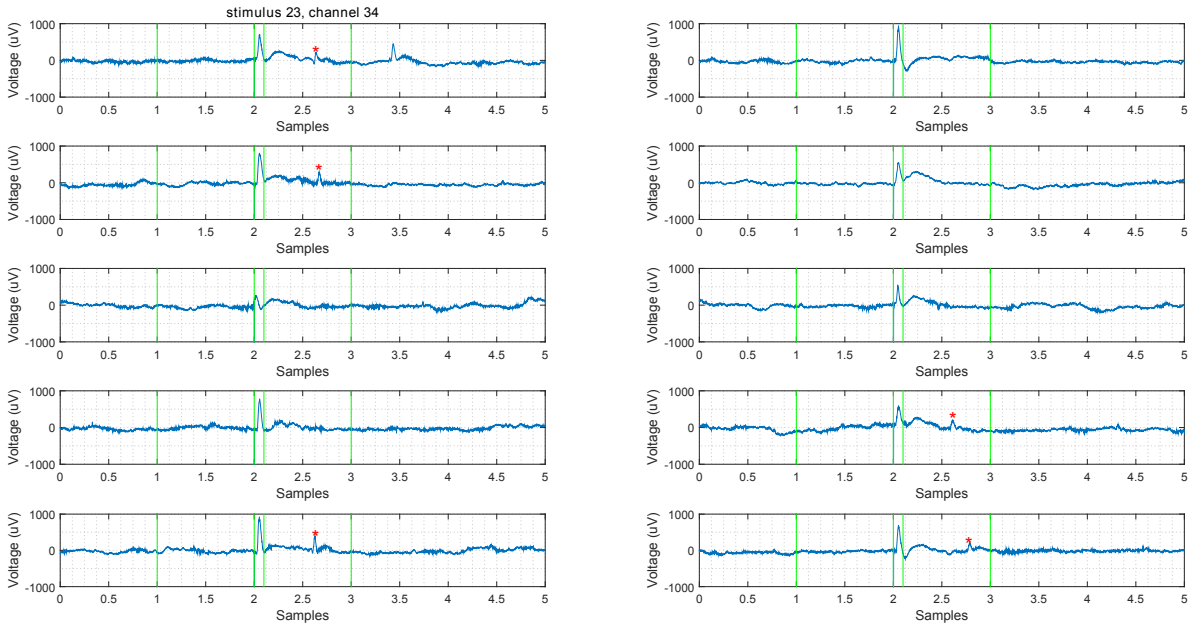


Figure 4.2: *This is an example of how the DRs were visually annotated. The small grid helps in deciding whether a wave is too slow or not. For each graph, peaks (indicated by a red *) were annotated before and after the stimulation artifact. This would be annotated as (order: left top to bottom, right top to bottom): before = [0 0 0 0 0 0 0 0 0 0], after = [1 1 0 0 1 0 0 0 1 1]. The one tailed sign test concludes that the number of peaks after the stimulation artifact is not significantly more than before the stimulation artifact. This would be annotated as non DR.*

Detectors For ten epochs of each electrode in each trial, the following procedure was performed (Figure 4.3). The SD was calculated in a time window of 1 second, located 2 seconds prior to the pulse. The mean amplitude was calculated in a time window of 2 s prior to the stimulus. This mean amplitude was subtracted from the epoch resulting in a signal fluctuating around zero. In the new signal, the Matlab function *peakfinder* (settings: sel = SD, thresh = not defined) was used to detect a peak in a time window of 1000 ms before and 100 - 1000 ms after the pulse. Using a one-tailed sign test, it was determined whether the detected peaks in ten epochs were significantly related to the pulse. If so, the electrode was considered as an electrode with a DR.

Varying thresholds As with the ER detector, we compared two different detectors. The first detector was based on an amplitude threshold (setting A). The second detector was based on an SD factor (setting B). Since DRs were not deterministic but more stochastic, ten epochs for each electrode in one trial were analyzed instead of the averaged epoch of ten epochs which was analyzed to determine the presence of an ER. In setting A, the amplitude threshold was varied from 20 and 400 μV with steps of 20 μV. A

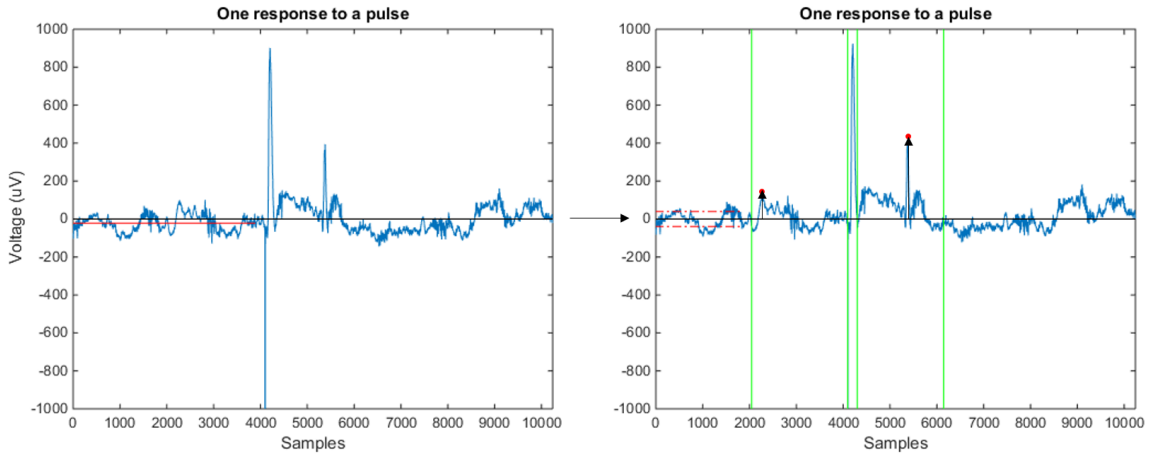


Figure 4.3: *The procedure in the DR detector. In two seconds baseline, the median amplitude is calculated per epoch for each electrode in each trial. The median value is subtracted from the epoch. In 1s baseline, which is located 2s prior to the pulse, the standard deviation (SD) is calculated. Peaks are detected, using peakfinder in Matlab, in 1s prior to the stimulus and 1s after the stimulus. In the detector based on an amplitude threshold, a peak is detected when it exceeds a set amplitude threshold. In the detector based on an SD factor, a peak is detected when the ratio between the amplitude of the peak and the calculated SD exceeds a set SD factor. When significantly more often a peak is detected after the stimulus, a DR is considered in this electrode.*

peak was detected in 1000 ms prior to the pulse and 1000 ms after the pulse when the peak exceeded the amplitude threshold. In setting B, the SD threshold was varied between 1 and 10 with steps of 1. A peak was detected in 1000 ms prior to the pulse and 100-1000 ms after the pulse, when the ratio between the amplitude of the detected peak and the SD exceeded the specific SD factor.
For each setting and varying thresholds, the detected DRs were compared to the visually annotated DRs and categorized as true positive, false positive, true negative and false negative. The sensitivity, specificity, PPV and NPV were calculated. The false positive and false negative detections were analyzed to see where the detector needed improvement. These improvements may be investigated further in the next section.

4.1.3 Results

Patient characteristics Six patients underwent grid implantation between February 2009 and November 2010 (Table 4.1). The patients (4 males, 2 females) had a median age of 12 years (range:8-31). In Matlab, 100 DRs were visually annotated (median: 18.5, range: 5-22) in 33% of the trials (median: 17, range: 16-19).

Table 4.1: *Patient characteristics for testing automatic detection of delayed responses*

Patient	Gender	Age	Electrodes	Annotated trials	Visual DRs (per trial: median, range)
16	m	13	64	16	5 (0, 0-1)
19	f	31	64	16	22 (0, 0-10)
21	m	8	64	19	16 (0, 0-7)
22	f	13	64	16	19 (0, 0-19)
27	m	9	64	18	20 (0, 0-7)
29	m	11	64	18	18 (0, 0-6)

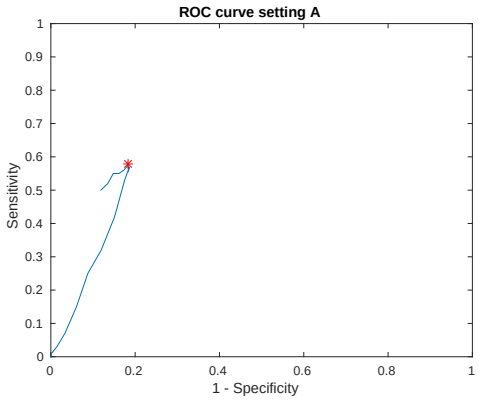
Performance In Table 4.2, the performance of the detector with setting A and B is displayed. The performance of setting B was better than the performance of setting A. The sensitivity and PPV were low in both settings. This suggests that the number of false positive and false negative detections compared to the true positive detections was high. An ROC-curve is displayed in Figure 4.4.

Table 4.2: *Performance of the detector in 33% of the trials in six patients.*

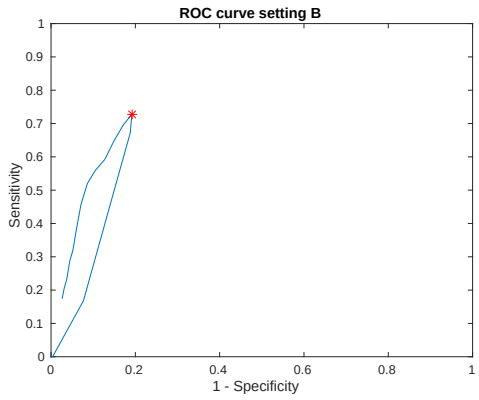
Setting	Amplitude threshold μV	Sensitivity	Specificity	PPV	NPV	Distance
A	280	0.58	0.82	0.05	0.99	0.4580

Setting	SD factor	Sensitivity	Specificity	PPV	NPV	Distance
B	4	0.64	0.79	0.05	0.99	0.4145

Figure 4.4



(a) *An ROC curve in 33% of the trials in six patients. The optimal amplitude threshold is displayed with a red *.*



(b) *An ROC curve in 30% of the trials in six patients. The optimal SD factor is displayed with a red *.*

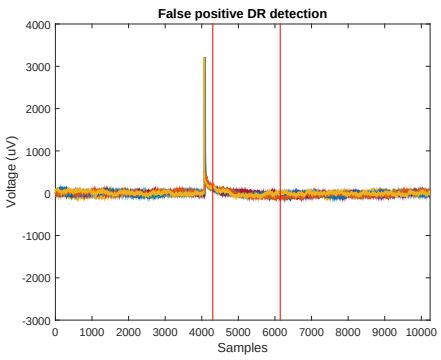
4.1.4 Discussion

In this set, the best performance was found in the detector with setting B. The sensitivity and specificity were respectively 0.64 and 0.79. However, the PPV was low (0.05) due to the high amount of false positive detections. In the next section, a high pass filter and minimal SD are implemented to improve the performance of the DR detector. The ROC curve in Figure 4.4 had a form that was different from usual. In a normal ROC curve, a low threshold value results in a low specificity and a high sensitivity, and a high threshold value results in a high specificity and a low sensitivity. Positive detections are still positive when a lower threshold is used [33]. The current ROC curve started and ended with a low sensitivity and a high specificity. Peaks were detected both before and after the pulse. A low value for the SD factor or amplitude threshold resulted in more peaks detected before and after the pulse. Since a sign-test was used to determine whether peaks after the pulse were considered as a DR, a low value for the SD factor resulted in a low number of detected DRs and therefore a low sensitivity and a high specificity. A high value for the SD factor resulted in less detections before and after the pulse and a low number of detected DRs. This resulted in a low sensitivity and a high specificity. Due to the statistic test used in the DR detector, the detector was not a clear binary classifier [33]. Therefore, constructing an ROC curve was not the correct method to determine the performance of the detector. In this chapter, the ROC curve is used due to the limited time of the study. In further research, it is preferable to annotate

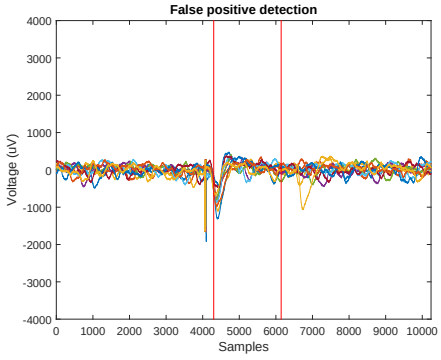
peaks without using a sign-test. These peaks should be detected by the detector and the sign-test determines whether these peaks are significantly related to the pulse. When the sign-test is excluded, the ROC curve would have a normal curve in which more positive detections are present when the threshold is lowered.
Another weaker point of this study is that the visual annotation was performed only by one observer. This was her first time annotating DRs. Therefore, it is possible that peaks were wrongly considered as DRs or wrongly missed. An important next step would be to include a second observer to corroborate the annotations.
In Table 4.3, the number of detected DRs with the optimal amplitude threshold and SD factor are displayed. In four patients, the maximal number of electrodes with a detected DR per trial was more than 15. It is not realistic that more than 15 electrodes are part of the SOZ.
In patient 21 and 27, with the optimal amplitude threshold and SD factor, still 11 false negative DRs were detected. These DRs were detected when the amplitude threshold or SD factor was exceeded too many times in the time window before the pulse, resulting in an insignificant sign test. Visual annotation of DRs is subjective, which sometimes resulted in annotation of a DR when one peak before the stimulation artifact was missed. Therefore, it is disputable whether the false negative DR detections are true DRs.
The high amount of false positive detections in patient 19 and patient 27 were caused by a slow trend after the stimulation artifact, as shown in Figure 4.5a. The same problem was observed in the ER detector. In the ER detector, sel was increased from 10 μV to 20 μV. This solved the problem of detecting small peaks on the trends after the stimulation artifact. In the DR detector, sel = SD. In most of the false positive detections, the SD was lower than 30 μV resulting in a detection when a the amplitude of a peak was larger than 140 μV. Setting a minimal SD may decrease the number of false positive detections.
Another cause of the high number of false positive detections were the slow waves occurring in the time window of DRs. These slow waves were not excluded from DR detection when the top of these peaks was located in the time window of DRs. Valentin et al. [15] described slow waves occurring after a peak in the time window of ERs as part of the ER. Matsumoto et al. [34] stimulated N1 and N2 peaks with cortico-cortical evoked potentials (CCEP). They found two negative peaks. The first one had a mean latency of 27.9 ms. The second peak had a mean latency of 144.6 ms. The N2 was blunter than the N1. Boido et al. [25] described an early (2-60 ms) and a late response (60-500 ms) as phase 1 and 2 in stereo-electroencephalogram (SEEG) data. Phase 1 consists of a sharp component, phase 2 is a slow wave. This is similar in our data. Valentin et al. [15] described the slow wave following the ERs. We assume that these slow wave and N2 and late responses are similar. This suggests that the slow wave is indeed in the time window of DRs, but part of the ER. In the current detector, no differentiation is made between slow waves and sharp waves or peaks resulting in many false positive detections. Differentiation between slow waves and sharp waves or peaks could be an improvement in detecting DRs. A high pass filter with a cut off frequency of maximally 5 Hz may differentiate between slow and sharp waves or peaks. Slow waves in the time window of DRs can be filtered and the peaks are still visible in the signal. In the next section, we try to prevent detection of these 'late' ERs by using a high pass filter which suppresses the slow waves.

Since slow waves present itself before a DR, another idea is to increase the start of the time range for DRs. This was investigated in Appendix D, but did not lead to new improvements.
Another suggestion for improvement was to implement a wave template to detect DRs. Template matching was used to detect spikes in previous studies [12], but the ideal spike detector does not exist yet. A DR is always a negative peak or sharp wave, but does not have a consistent form. Therefore, a wave template to detect DRs is not added to improve the DR detector.
The detector may be improved by setting a minimal SD and a cut off frequency. These improvements are implemented and tested in the next section.

Figure 4.5: *False positive DR detections*



(a) *The slow trend after the stimulation artifact resulted in detection. Setting a minimal SD or a larger value for sel would prevent detection of this DR.*



(b) *The slow wave in the time window of DRs is wrongly detected as DR. Implementing a high pass filter would prevent detection of this DR.*

Table 4.3: *With optimal amplitude threshold (setting A) and SD factor (setting B), the number of detected DRs in 33% of the trials. Between brackets, the median and range per trial are displayed.*

	Setting A					Setting B				
Patient	Detected DRs (per trial: median, range)	TP	TN	FP	FN	Detected DRs (per trial: median, range)	TP	TN	FP	FN
16	40 (2, 0-10)	3	770	37	2	107 (2.5, 0-42)	3	703	104	2
19	299 (14, 0-44)	19	548	280	3	354 (18.5, 2-48)	18	492	336	4
21	32 (2, 0-4)	5	1062	27	11	34 (2, 0-5)	7	1062	27	9
22	49 (1, 0-18)	11	738	32	8	46 (0.5, 0-21)	14	738	32	5
27	474 (30, 1-46)	9	597	461	11	458 (29.5, 1-47)	11	611	447	9
29	185 (8, 0-20)	11	821	170	7	204 (9, 0-24)	11	798	193	7

4.2 Evaluating the DR detector

4.2.1 Introduction

In the previous section, we concluded that the detectors with setting A and B can be improved to reduce the number of false positive and false negative detections by implementing a high pass filter and a minimal SD. Peaks have a duration of maximally 80 ms. Assuming that a peak is a half sinus, the total period is 160 ms, which has a frequency of 6.25 Hz. A sharp wave has a duration of 80-120 ms (4.2-6.25 Hz), slow wave has a duration of > 120 ms (< 4.2 Hz). Therefore, a cut off frequency of 4 Hz may prevent detection of slow waves in the time window of DRs. We hypothesize that the detector with setting B is performing best, when a minimal SD and cut off frequency are set. In Figure 4.6, the effect of a high pass filter on a signal with a slow wave and a signal with a slow wave and DR are displayed. With increasing fc, the slow wave decreases and it is not detected as DR when fc is larger than 3 Hz. The DR is still detected as DR when the slow wave is filtered.

4.2.2 Method

Patients The same patients were included as in the previous section. In a training set of 33% of the trials, DRs were detected.

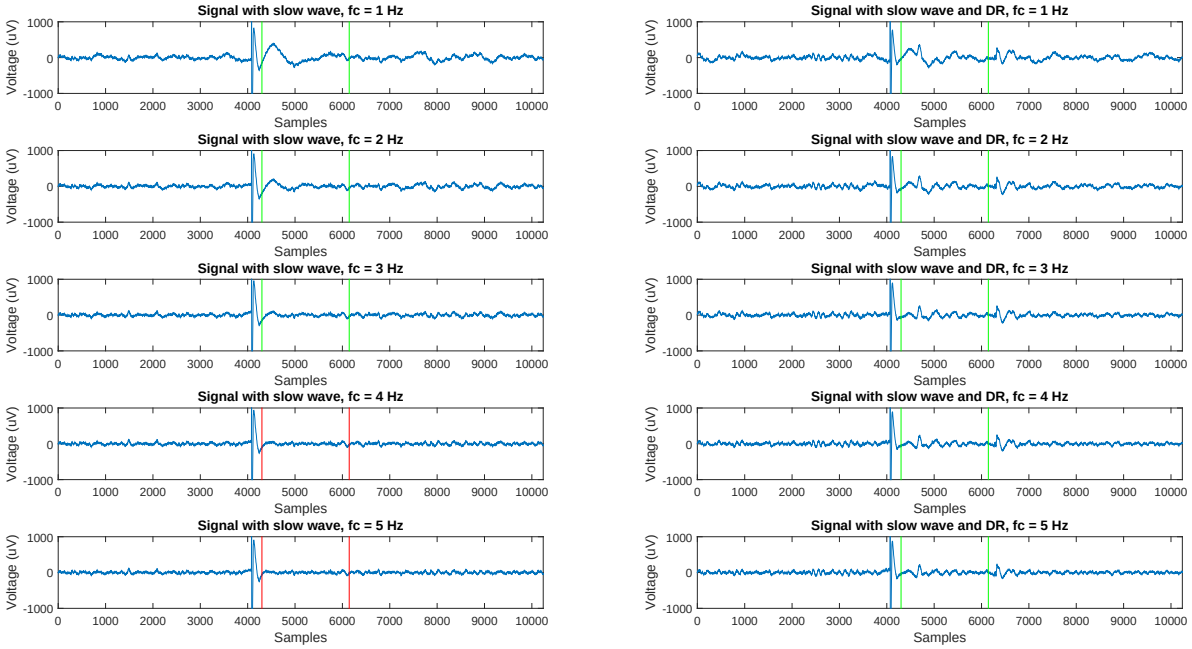


Figure 4.6: *The effect of the high pass filter on a signal with a slow wave and a signal with a slow wave and DR. The cut off frequency is increased from 1 Hz to 5 Hz. In the figure with green lines, the peak is detected as a DR. In the figure with red lines, the peak is not detected as DR. The lines indicate the time window in which DRs are detected.*

Detector As during testing the ER detector, the performance of the detector with setting A and the detector with setting B were compared. The algorithm of the detector is explained in section 4.1.2.

Varying parameters Several parameters were varied to find the combination which resulted in the best performance for the detector. The amplitude threshold was varied in the detector with setting A. The SD factor was varied in the detector with setting B. The amplitude threshold was varied between 20 μV and 400 μV with steps of 20 μV. The SD factor was varied between 1 and 10, with steps of 1. The minimal SD was varied between 0 and 100 μV with steps of 20 μV. The cut off frequency of the high pass FIR-filter (3rd order) was varied from 1 to 5 Hz with steps of 1 Hz.

Varying the parameters in the detector with setting A or B resulted into two different settings:

- Setting A1: vary the amplitude threshold, sel, the cut off frequency
- Setting B1: vary the SD factor, minimal SD, the cut off frequency

Testing For each setting, detections were compared with visual annotation and categorized as true positive, true negative, false positive and false negative detections. Sensitivity, specificity, PPV and NPV were calculated. The distance from the ROC-curve to the upper left corner was calculated using the theorem of Pythagoras (Formula 3.5 in Section 3.1.2). The setting with the shortest distance may be validated in the next section.

4.2.3 Results

Six patients underwent grid implantation between February 2009 and November 2010 (Table 4.4). The patients (4 males, 2 females) had a median age of 12 years (range:8-31). In Matlab, 66 DRs were annotated (median: 10, range: 4-20) in 33% of the trials (median: 17.5, range: 17-20).

Table 4.4: *Patient characteristics for testing automatic detection of delayed responses*

Patient	Gender	Age	Electrodes	Annotated trials	Visual DRs (per trial: median, range)
16	m	13	64	17	4 (0, 0-2)
19	f	31	64	17	20 (0, 0-8)
21	m	8	64	20	10 (0, 0-7)
22	f	13	64	17	6 (0, 0-2)
27	m	9	64	19	16 (0, 0-6)
29	m	11	64	18	10 (0, 0-3)

In Table 4.5, the optimal values for varying parameters in each setting with the accompanying sensitivity, specificity, PPV and NPV are displayed. The shortest distance was obtained in the detector with setting B1. The sensitivity, specificity, PPV and NPV were respectively 0.67, 0.92, 0.09 and 1.00. In Table 4.6, the number of true positive, true negative, false positive and false negative detections are displayed. Still many false positive detections are observed. This results in a very low PPV.

Table 4.5: *The performance of the detector with setting A1 or B1 in 33% of the trials in six patients.*

Setting	Amplitude (μV)	sel	fc (Hz)	Sensitivity	Specificity	PPV	NPV	Distance
A1	240	20	1	0.59	0.93	0.09	1.00	0.4145

Setting	SD factor	Minimal SD (μV)	fc (Hz)	Sensitivity	Specificity	PPV	NPV	Distance
B1	4	40	1	0.67	0.92	0.09	1.00	0.3430

4.2.4 Discussion

The performance of the detector with settings B1, SD factor = 4, minimal SD = 40 μV, fc = 1 Hz was best with sensitivity, specificity, PPV and NPV were respectively 0.67, 0.92, 0.09 and 1.00. However, the PPV is very low in this setting due to the high number of false positive detections (463). The number of false positive detections is lower in the detector with setting A1, but the number of false negative detections is higher. We want to discard stimuli which will not evoke any DRs. Therefore, it is important that no DRs are missed, because these responses correlate with the location of the SOZ. The number of false negative detections must be as low as possible. Therefore, we conclude that the detector with settings B1, SD factor = 4, minimal SD = 40 μV and fc = 1 Hz has the best performance and is validated in the next section.

Table 4.6: *True positive, true negative, false positive, false negative detections*

Setting	Amplitude (μV)	sel	fc (Hz)	TP	TN	FP	FN
A1	240	20	1	39	5336	380	27

Setting	SD factor	Minimal SD (μV)	fc (Hz)	TP	TN	FP	FN
B1	4	40	1	44	5267	463	22

Most false positive detections contained slow waves in the time window of DRs. A higher cut off frequency may prevent detection of these slow waves as DRs. However, this may result in more false negative detections as well. Some false negative detections are disputable whether these are true DRs. Visual annotation is subjective. Sometimes, it depends on annotation of one peak to consider the responses to ten pulses as significant DRs. A strong point of this study is that several parameters are included and combined to optimize the detector. However, still a lot of false positive detections are observed. Partly, this is the result of fc = 1 Hz. Waves of maximally 500 ms are still visible in the signal. These waves are detected as significant DRs and lead to a higher amount of false positive detections.
More improvements for this DR detector are possible to reduce the number of false positive detections. However, due to the limited time of this study, these improvements are not implemented. In the next section, the DR detector with settings B1, SD factor = 4, minimal SD = 40 μV and fc = 1 Hz is validated in 33% of the trials of six patients.

4.3 Validating the DR detector

4.3.1 Introduction

In the previous section, we concluded that the best performance was obtained in the DR detector with settings B1, SD factor = 4, minimal SD = 40 μV, fc = 1 Hz. In this section, this detector is validated. We hypothesize that the PPV is not very high, because many FP detections were already present in the test set.

4.3.2 Method

In 33% of the trials of six patients, DRs were annotated in Matlab by DvB. DRs were detected in 33% of the trials of six patients with the DR detector with settings B1, SD factor = 4, minimal SD = 40 μV, fc = 1 Hz. Detected DRs were compared with the visual annotation and the signals were divided into true positive, true negative, false positive and false negative detections. Sensitivity, specificity, PPV and NPV were calculated for each individual patient.

4.3.3 Results

Patient Characteristics In Matlab, 102 DRs were annotated (median: 16, range: 1-41) in 33% of the trials (median: 17.5, range: 16-19) (Table: 4.7). 588 DRs were detected (median: 100.5, range: 19-184) in 33% of the trials.

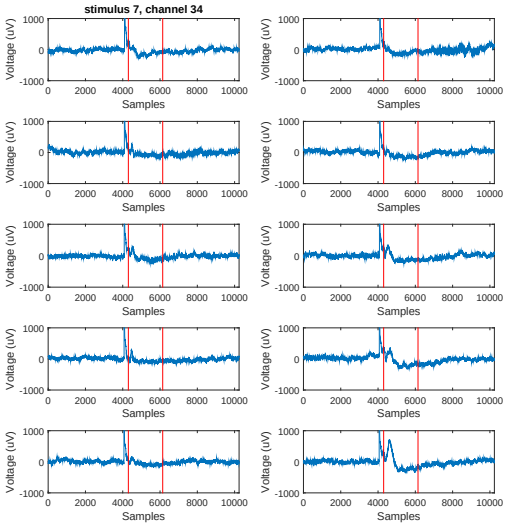
Table 4.7: *Patient characteristics for testing automatic detection of delayed responses. Between brackets, the median value and range of DRs are displayed per trial.*

Patient	Gender	Age	Electrodes	Annotated trials	Visual DRs (per trial: median, range)	Detected DRs (per trial: median, range)
16	m	13	64	16	1 (0, 0-1)	19 (0, 0-6)
19	f	31	64	17	41 (0, 0-11)	138 (9, 0-19)
21	m	8	64	19	21 (0, 0-6)	91 (1, 0-27)
22	f	13	64	16	7 (0, 0-4)	46 (1, 0-19)
27	m	9	64	19	18 (0, 0-3)	110 (5, 0-20)
29	m	11	64	18	14 (0.5, 0-3)	184 (8.5, 0-22)

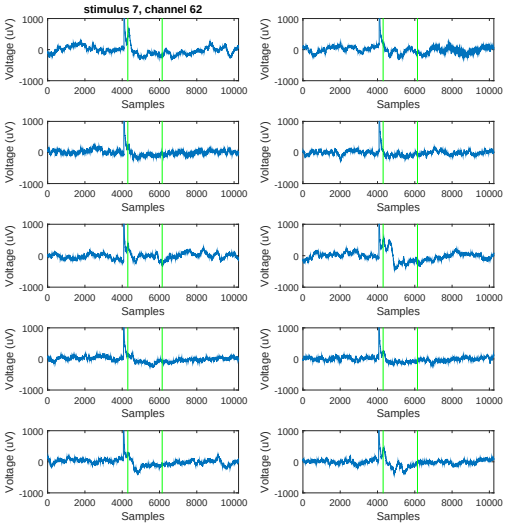
Validation In Table 4.8, the performance of the detector in each patient is displayed. The median sensitivity is 0.68 (0.54-1.00), the median specificity is 0.92 (0.82-0.98), the median PPV is 0.10 (0.05-0.21), the median NPV is 1.00 (0.97-1.00).

Table 4.8: *Performance of the detector in validation set which contains 33% of the trials in six patients.*

Patient	Sensitivity	Specificity	PPV	NPV
16	1.00	0.98	0.05	1.00
19	0.54	0.86	0.16	0.97
21	0.90	0.93	0.21	1.00
22	0.71	0.95	0.11	1.00
27	0.56	0.91	0.09	0.99
29	0.64	0.82	0.05	0.99
Median	0.68	0.92	0.10	1.00



(a) *This DR is not detected but was annotated as a DR. However, it is disputable whether the annotated waves are sharp waves or peaks or whether some are slow waves. This could explain why this DR is not detected.*



(b) *This DR is detected but was not annotated as a DR. However, it is disputable whether the small peaks present in the time window of DRs should not have been annotated as a DR.*

Figure 4.7: *Examples of false negative and false positive DR in Patient 29.*

The sensitivity and PPV are lower than the specificity and NPV are high. This is mainly the result of the high number of true negative detections and only a low number of true positive detections. In total, 522 false positive and 36 false negative detections were observed (Table 4.9). In most of the false negative detections, it can be argued whether these were true DRs. Those annotated DRs were subjective and during a revision, it is possible that no significant DR would be found in this electrode. In most of the false positive detections, no DR was present after revision of these responses. Especially in patient 29, the number of false positive detections is very high. An example of a false positive DR and a false negative DR are shown in Figures 4.7a and 4.7b.

Table 4.9: *The number of true positive, true negative, false positive and false negative detections and the median number of detections per trial in each patient.*

Patient	Detected DRs (per trial: median, range)	TP	TN	FP	FN
16	19 (0, 0-6)	1	772	18	0
19	138 (9, 0-19)	22	719	116	19
21	91 (1, 0-27)	19	1072	72	2
22	46 (1, 0-19)	5	740	41	2
27	110 (5, 0-20)	10	967	100	8
29	184 (8.5, 0-22)	9	802	175	5

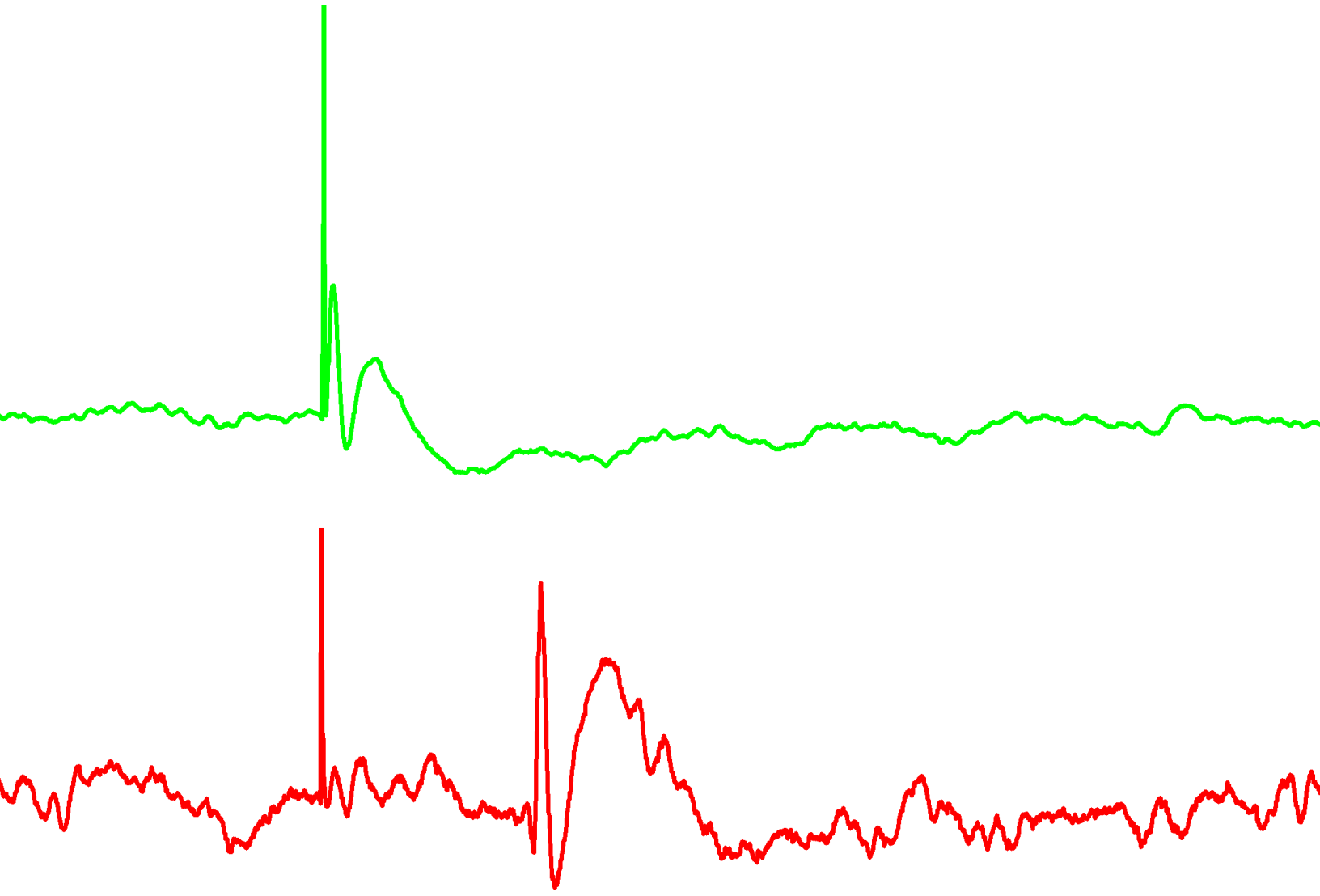
4.3.4 Discussion

The specificity and NPV were very high and the sensitivity and PPV were very low. The detector was not good enough for use in further study. The sensitivity and PPV were too low (respectively, 0.68

and 0.10). The number of annotated DRs per patient was low with maximally 11 DRs in a trial and a median number of 0 DRs per trial. The number of false positive detections was very high. Therefore, we conclude that the detected DRs in the rest of the study need to be visually checked to reduce the false positive detections.
The visual annotation was performed in Matlab. The DRs were annotated by DvB. She did not have any experience in annotating DRs. No consensus between two observers was obtained. Therefore, it is possible that DRs were wrongly annotated or missed.
The detector does not differentiate between RRs [19], SRs [21] and DRs [15]. RRs occur in the time window of both ERs and DRs, but correlate with pathogenic tissue. SRs occur in the time window of DRs, but are supposed to reflect physiological networks. Therefore, it is very important to distinguish these different responses.
Further research should focus on improving the DR detector. A solution may be to build a DR detector with two parts. In the first part, peaks are detected in the time window of ERs. When a peak is detected in the time window for ERs, peaks are detected in the time window of DRs. When three consecutive waves are observed, this response may be considered as an RR. Since these responses can be slow waves as well, a cut off frequency should not be implemented. In a second step, peaks should be detected in the time window of DRs. To differentiate between SRs and DRs, it may be possible to average ten epochs and subtract the averaged signal from an individual epoch. When no peak is detected any more, the response in this electrode could be considered as an SR. When significantly more often peaks are detected after the pulse, it could be considered as a DR. However, when in nine out of ten epochs, a DR occurs, these could be visible in the averaged signal as well and results in wrongly consideration of this DR as an SR. More research should be done to investigate whether DRs can be visible in an averaged epoch. Investigating such a detector is not done in this study due to the limited time. At last, it is important that consensus between two observers is obtained for the DR detections to prevent optimizing on wrong DRs.
The detector is not optimal yet. Further improvements should be implemented to distinguish SRs, RRs and DRs. Due to the limited time of this study, these improvements are not investigated. In this study, the detector is only used as a first detection. All detected DRs will be visually checked.

Chapter 5

Persistence of ERs



5.1 Introduction

Valentin et al. [15] assumed that ERs are deterministic and present after each pulse when the response is seen once, since it is a physiological connection. This suggests that a pulse does not have to be applied ten times but only a few times to establish a reliable ER. In this chapter, we investigate how many pulses should be applied to establish a reliable ER. We hypothesize that a reliable ER can be established with three pulses per trial instead of ten pulses per trial.

5.2 Method

ERs were annotated in SystemPlus in six patients. For each trial, the ten single epochs of the visual ERs were evaluated in Matlab. ERs were detected using the Matlab function *peakfinder*, SD factor = 2.5, minimal SD = 20, sel=50. We counted how many ERs were detected in the epochs where ERs were visually annotated. For each patient, we made a histogram in which it is displayed how often an ER was detected after ten pulses.
For each patient, the number of ERs in ten epochs were divided into two variable groups: group1 varied from 0 detected ERs out of 10 pulses (0/10) to less than 10 ER out of 10 pulses (<10/10). Group2 varied from >1/10 to 10/10. The Mann Whitney U test was used to find when a significant difference (p=0.05) was found between both groups. When the difference was significant, more ERs were detected in group2 than in group1 and the amount of stimuli, after which an ER could be reliably established, would be found.

5.3 Results

In Table 5.1, the specification of six patients are displayed. The median age was 11.5 years old (range: 7-16 years). Three patients were male. The median number of electrodes was 72 (range: 62-88), the median number of stimuli was 51 (range: 40-74). In total, 5106 ERs were annotated, a median of 842.5 ERs per patient (range: 617-1336).

Table 5.1: *Patient specification*

Patient	Gender	Age	Electrodes	Trials	Visual ERs (median, range per trial)
81	m	11	88	74	1336 (15.5, 0-66)
83	f	12	62	40	522 (11.5, 2-35)
88	f	8	80	70	921 (12.5, 0-25)
89	f	14	56	45	764 (17, 7-28)
91	m	16	86	54	617 (13, 4-26)
93	m	7	64	48	946 (20, 0-33)

In Figure 5.1, three examples are displayed in which less than 5 ERs were detected. In each situation, I would not reconsider the ER annotation.

In Figure 5.2, six histograms are displayed, one for each patient. Most ERs were present in ten out of ten epochs. In all patients together, in median 80% (range: 74-93%), an ER was present in at least five out of ten epochs.

In Figure 5.3, the frequency of ERs of the individual patients are collected together. A significant difference was found between group1 and group2 when group2 contained the number of electtrodes with ERs occurring in 8-10 stimuli and group1 contained the number electrodes with ERs occurring in 0-7 stimuli. In median 61% (53-80%), at least eight out of ten epochs showed an ER.

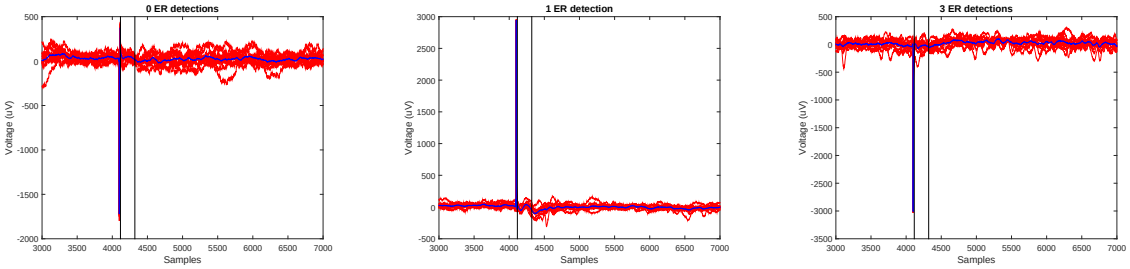


Figure 5.1: *Examples of epochs with 0, 1 or 3 detected ERs. The red lines show the separate ten epochs. The blue line shows the averaged epoch. The black lines indicate the time window in which the ER is detected.*

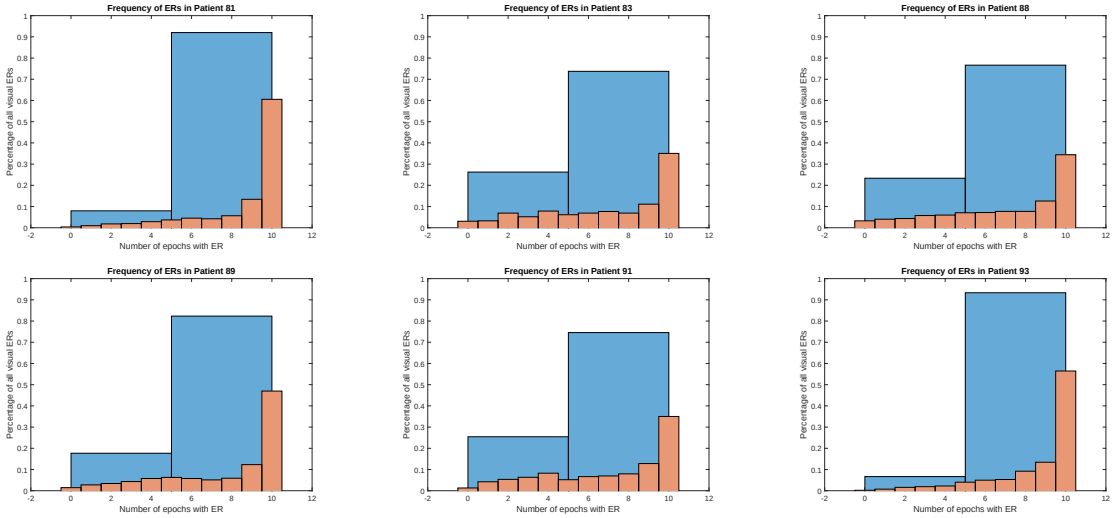


Figure 5.2: *Histograms of number of ERs in ten epochs (the red histograms) in Patient 81, 83, 88, 89, 91, 93. The blue histograms show the number of ERs detected in less than five stimuli or in more than five stimuli.*

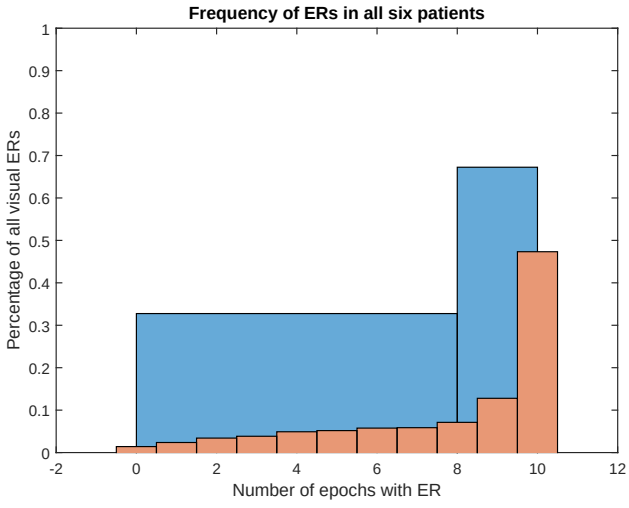


Figure 5.3: *The frequency of ERs in the six patients are collected into one histogram.*

5.4 Discussion

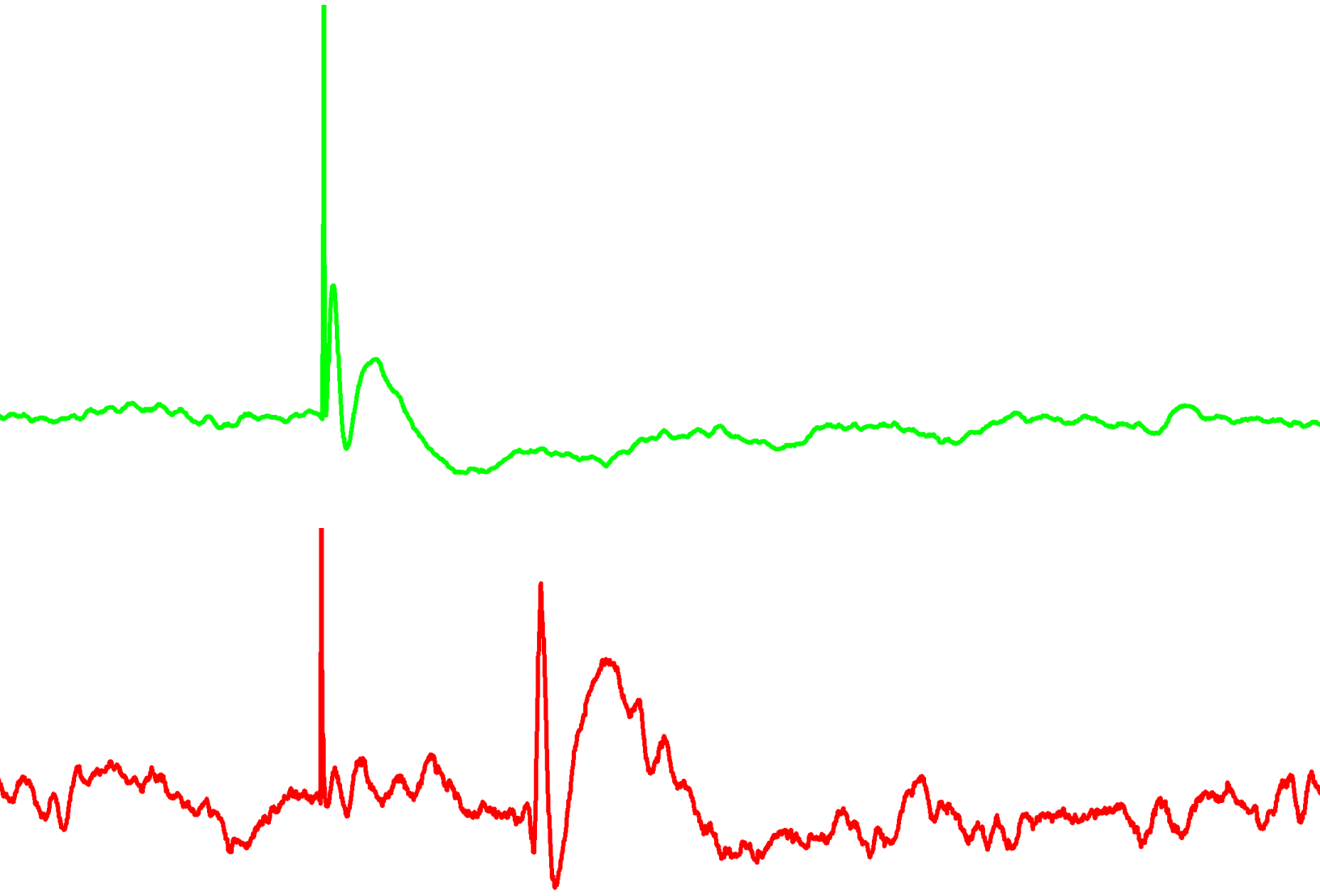
We conclude that the majority of the ERs (80%) were present in at least five out of ten out of ten epochs. Since we averaged the ten epochs to decrease the effect of noise, it was important to determine how many pulses should be applied to obtain a reliable rendering of an averaged epoch of an electrode in which an ER was displayed when it was visible in the separate epochs. Since a significant difference was found between group1 and group2 when group1 contained the electrodes with ERs which were detected in 0-7/10 epochs and group2 contained the electrodes with ERs which were detected in >8/10 epochs, we conclude that significantly more ERs are present in at least 8 out of 10 epochs. It may be possible that in the first two epochs, an ER is not present. When three more pulses are applied, the ER may be present in 3/5 epochs. This suggests that an ER can be reliable rendered, since it is present in more than half of the epochs. We conclude that instead of 10 epochs, a reliable rendering is possible with only five epochs. This decreases the duration of the SPES protocol with 50%.

We hypothesized that only three epochs would be enough to reliably establish an ER. Boido et al. [25] calculated a variability index on each average response to evaluate the reliability of SPES-evoked responses. This is an accurate method for determination whether responses are reliable. Due to the limited time of this study, this was not used in our study. In further research, we could evaluate the reliability of SPES-evoked responses with such method and compare it with the method used in this chapter to confirm the conclusions in this chapter.

Due to the significant results, showing that ERs were significantly more often visible in at least 8 responses to pulses than in less than 8 responses to pulses, we conclude that five stimuli are sufficient to reliably establish ERs.

Chapter 6

Stimulation in retrospective data



6.1 Introduction

DRs occur in epileptic tissue [15], but are more stochastic: ten stimuli are needed to reliably establish a response. ERs occur in healthy and diseased tissue [15] and a reliable ER can be established with only five stimuli (Section 5). This would already result in a SPES protocol which takes half of the time to reconstruct a reliable physiological network of ERs. If the network of ERs can predict which stimulus pairs can be discarded because no DRs will be evoked, the protocol of stimulation could be even more time efficient. The relation between ERs and DRs is investigated in this section.

Networks Network analysis of the brain and connectivity measures are increasingly used to investigate the integrity of brain networks in epilepsy [35]. These measures offer a framework to characterize the organization of brain networks [35, 36]. Network analysis has given more insight in seizure onset, propagation and termination [35]. Early studies used the small-world and scale-free model to describe structural and functional networks [37]. A deviation from a topology with both small-world and scale-free characteristics is linked to cognitive and clinical symptoms in neuropsychiatric diseases [37, 38]. In later years, modularity, hierarchy, and mixing properties as degree correlations were regions of interest to characterize the complex brain networks [37]. The conventional graph metrics as the clustering coefficient, shortest path length and degree distribution have increased understanding of the complexity of the brain's architecture [37]. However, methodological issues have limited comparability between experimental conditions, cohorts and studies [37].
During network analysis, the brain is reduced to nodes and edges [35]. Nodes represent functional or structural elements and edges represent the structural or functional relations between those nodes [35]. Edges are connections between nodes and represent (functional) connectivity values [36]. Together, nodes and edges form the basic elements of a network, and from these elements various global and local network measures can be inferred [36].
Functional networks are based on the strength or consistency of functional interactions between the network nodes. In a weighted network, the strength of this interaction is taken into account, whereas in an unweighted (binary) network only the existence or absence of an interaction is taken into account [36]. Such a binary network is obtained by setting a threshold for the functional connectivity above which a functional connection is considered to be present [36]. A motivation to use a binary network could be to discard spurious connections that are potentially influenced by, for example noise [36].

SPES and networks During SPES, electrodes are stimulated and responses are detected in other electrodes. The nodes reflect the individual electrodes. The edges between the nodes are reflected by the ERs evoked by applying a pulse to a stimulus pair. This results in a directed network in which information is found of the directionality of communication [36]. We construct a binary, unweighted network in which the existence or absence of an interaction is taken into account instead of the strength of an interaction [36].
Parameters like the average path length, average clustering coefficient, degree, betweenness centrality, and eigenvector centrality can be used to describe networks. In this study, we want to have a practical view on how ERs are related to DRs. Therefore, we will not use these parameters in this study.

6.2 Method

Patient characteristics Patients were included when they underwent grid recordings between September 2012 and June 2015. The new stimulator was used and the stimuli were marked with a trigger. This facilitated pre-processing the signals as mentioned in Appendix A. No other inclusion or exclusion criteria were used. The SOZ was assessed visually by two clinical neurophysiologists. The SOZ was considered here as the site of the beginning of the epileptic seizures.

SPES protocol In all neighboring electrode pairs in one direction, ten monophasic pulses (8 mA (lowered to 4 mA in the motorcortex), 1 ms, interval = 5s) were applied. Responses in the other electrodes were detected with the automatic detectors constructed in Chapters 3 and 4.

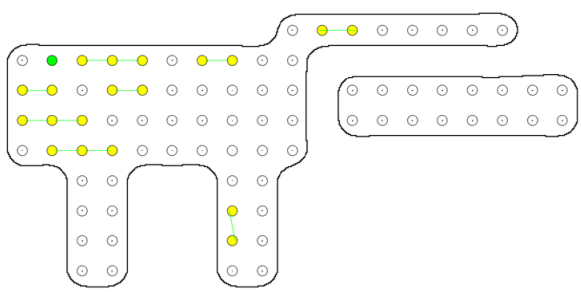
Detection of ERs The raw data was processed towards averaged signals of ten epochs in one trial with 2 seconds before and 3 seconds after the stimulation artifact. This process was explained in Appendix A. In the averaged signal, ERs were found with the ER detector with SD factor = 2.5, sel = 20 μV and minimal SD = 50 μV.

Detection of DRs In ten epochs of an electrode in each trial, DRs were found with a detector with SD factor = 4, minimal SD = 40 μV, fc = 1 Hz. Due to the over-detection of DRs, the detected DRs were visually checked in Matlab with a scale of 1000 μV/cm (Figure 4.2).

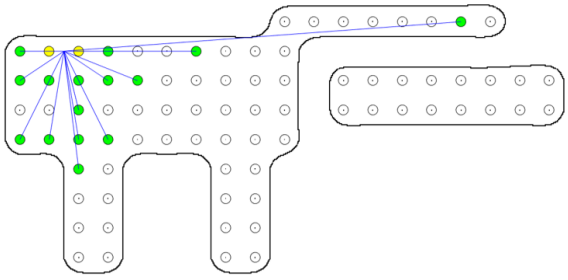
6.2.1 Are ERs evoked in the same population of stimulus pairs or electrodes as DRs?

Per electrode, the number of stimulus pairs that evoked ERs in this electrode were counted (sER) (Figure 6.1a). Per stimulus pair, the number of ERs that were evoked (eER) were counted (Figure 6.1b). This was similar for DRs (sDR and eDR).

Figure 6.1



(a) *An ER is evoked in the green electrode, when applying a pulse to the electrodes which are connected with a green line.*



(b) *An ER is evoked in the green electrodes when a pulse is applied to the yelow pair of electrodes.*

Per electrode, the number of stimulus pairs that evoked both ERs and DRs (sERDR) were counted, and per stimulus pair, the number of electrodes in which both ERs and DRs were evoked (eERDR) were counted. Electrodes and stimulus pairs with only ERs or DRs were called eoER, eoDR, soER, and soDR respectively. We tested whether the ratios of eERDR/eDR and sERDR/sDR differed significantly from 0.5 (Figure 6.2). When ERs occurred equally in either epochs with or without DRs, the ratio eERDR/eER would be 0.5. When ERs were evoked equally by either stimulus pairs evoking DRs and stimulus pairs, which did not evoke DRs, the ratio sERDR/sER would be 0.5. To test whether the ratios eERDR/eER, and sERDR/sER were significantly other than 0.5, a two sided t-test was used (α = 0.05). Same analysis was used for the ratios eERDR/eDR, and sERDR/sDR.

6.2.2 When DRs are evoked in an electrode or by a stimulus pair, are more ERs evoked?

Since not many DRs are found in general, we investigated whether stimulus pairs which evoked DRs in other electrodes also evoked significantly more or less ERs (Figure 6.1a). The other way around, we

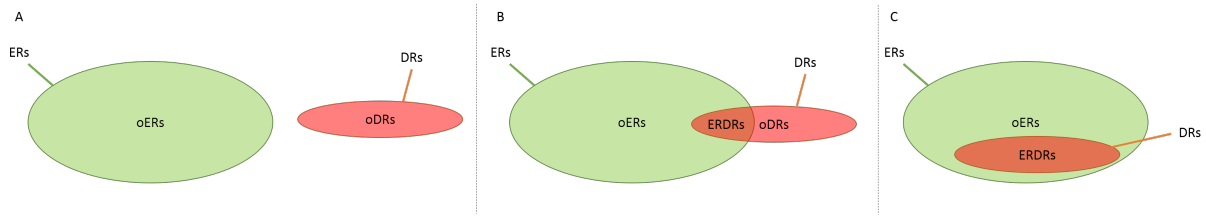


Figure 6.2: *Are DRs evoked in the same population of electrodes as ERs? In A: both group of electrodes do not overlap (eERDR/eER and eERDR/eDR = 0). In B: both groups overlap partly. In C: DRs are always evoked in electrodes in which ERs are evoked. (eERDR/eDR = 1)*

investigated whether electrodes in which DRs were evoked, significantly more or less ERs were evoked as well (Figure 6.1b). A Mann Whitney U test was used to investigate whether in electrodes with DRs significantly more ERs were evoked as well ($\alpha = 0.05$). Similar analysis was used in stimulus pairs which evoked DRs.

6.3 Results

Patient specification 20 patients (6 females, 14 males) (Table 6.1), with a median age of 15 years (range: 2-49 years), underwent grid implantation between September 2012 and June 2015. 9 patients are currently not seizure free, 1 patient complains about specific emotional fluctuations, but these are not confirmed as epileptic seizures, 10 patients are seizure free (Engel class Ia or Ib).

Table 6.1: *Patient characteristics. MCA = middle cerebral artery, MTS = mesiotemporal sclerosis, FCD = focal cortical dysplasia, TSC = tuberous sclerosis complex, F = frontal, C = central cortex, T = temporal, P = parietal, O = occipital, L = left, R = right, ● = no gamma onset, ⋆ = diffuse onset, ◇ = not operated*

Patient	Gender	Age	Pathology	Location of grid on lobe	Side	SOZ electrodes	Seizure free? (Engel class)
37	f	35	-	F C	L	8 ●	no
38	m	15	MTS	F C	L	NA ⋆	no ◇
39	m	7	congenital MCA infarct	F C	L	NA ⋆ ●	no ◇
40	m	15	FCD	F C	L	8 ●	yes (Ib)
48	m	5	FCD	F	L	7	yes (Ia)
49	m	14	TSC	F C T	R	16	no
50	m	45	tumor	C T	L	2	yes (Ia)
51	m	2	Encephalopathy	C T	R	NA	no ◇
52	f	6	TSC	T O	R	1	yes (Ib)
53	f	10	FCD	F C	L	● ⋆	pseudo seizures?
54	m	15	-	T O	R	8	yes (Ia)
55	f	42	FCD	T P	L	4	yes (Ia)
77	m	4	TSC	C	L R	3 ●	yes (Ia)
78	f	15	-	F C	L	12	yes (Ia)
79	m	19	tumor	T O	L	10	no
80	f	25	post-encephalopathical gliosis	T P	L	NA ⋆	no
90	m	17	Rasmussen encephalitis	F C	R	NA ⋆	no ◇
95	m	49	FCD	T O	L	7	yes (Ia)
96	m	11	FCD	F	R	5 ●	no
97	m	13	FCD	C	L	4 ●	yes (Ia)

SPES specifications The grids contained a median number of 74 electrodes (range: 63-116). In each patient, a pulse was applied in a median number of 59 trials (range: 46-96). ERs and DRs were detected with the detector described in chapters 3 and 4. 22369 ERs (median: 1118, range: 391-2524 per patient) and 141 DRs (median: 7, range: 0-37 per patient) were detected. In 6 patients, no DRs were detected. The characteristics are also displayed in Table 6.2.

Table 6.2: *Grid specifications. Between brackets, the median number and range of responses per stimulus pair are displayed.*

Patient	Electrodes	Stimuli	ERs (per trial: median, range)	DRs (per trial: median, range)
37	88	71	1051 (14, 1-34)	37 (0, 0-9)
38	104	86	800 (15, 4-31)	0
39	92	68	762 (8, 0-69)	3 (0, 0-1)
40	96	84	2524 (31.5, 1-74)	20 (0, 0-7)
48	70	53	859 (15, 1-41)	0
49	112	96	1375 (15, 1-41)	5 (0, 0-2)
50	64	55	851 (15, 3-57)	16 (0, 0-3)
51	64	54	874 (16, 2-30)	0
52	56	46	581 (13, 0-32)	1 (0, 0-1)
53	76	55	918 (15, 2-36)	5 (0, 0-2)
54	64	45	922 (18, 4-55)	0
55	88	64	1582 (23.5, 1-70)	16 (0, 0-5)
77	64	51	584 (12, 2-24)	0
78	64	55	624 (10, 0-33)	5 (0,0-1)
79	80	68	2042 (30, 10-61)	4 (0, 0-1)
80	74	62	1004 (16.5, 0-69)	5 (0, 0-4)
90	112	89	2023 (17, 1-102)	3 (0, 0-1)
95	72	62	391 (5, 0-67)	0
96	64	54	1671 (29.5, 5-55)	8 (0, 0-4)
97	62	53	931 (17, 1-34)	13 (0, 0-3)

In Figure 6.3, an example is displayed of the grids in patient 97. The number of evoked ERs and DRs in each electrode for all trials, and the stimulation pairs which evoked ERs and DRs are displayed. A darker blue electrode or line connecting two electrodes indicates that more ERs or DRs were evoked in this electrode (Figure 6.3a and 6.3b) or by this stimulus pair (Figures 6.3c and 6.3d).

6.3.1 Are ERs evoked in the same population of stimulus pairs or electrodes as DRs?

In Table 6.3, the electrodes with evoked ERs and DRs for all patients are collected. The ratios of eERDR/eER and sERDR/sER were significantly lower than 0.5. The ratios of eERDR/eDR and sERDR/sDR were significantly higher than 0.5. In Figure 6.4, the overlap between ERs and DRs is displayed.
In Table 6.4, the results are displayed for twenty individual patients. In all patients in which we found DRs, we found that the ratios eERDR/eER and sERDR/sER were significantly lower than 0.5. In only three patients, the ratios eERDR/eDR and sERDR/sDR were significantly higher than 0.5. In 13 patients, we saw that the ratios eERDR/eDR and sERDR/sDR was higher than 0.5.

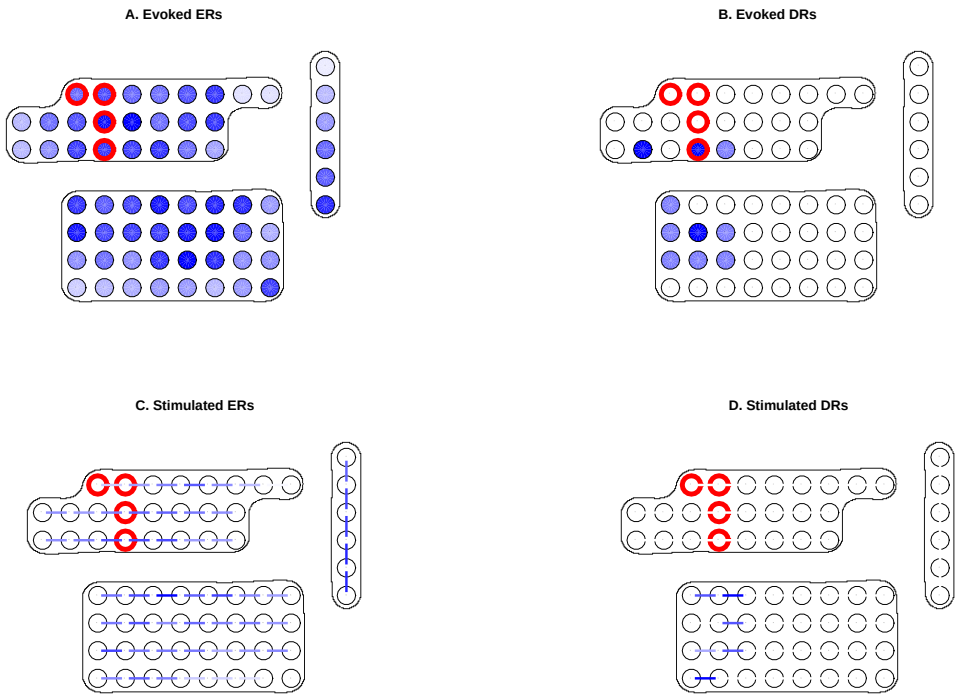


Figure 6.3: *An example of evoked ERs and DRs per electrode and per stimulus pair are displayed for Patient 97. When the electrode or line is dark blue, many ERs or DRs were evoked. In A, the number of evoked ERs are displayed in each electrode for all trials. In B, the number of evoked DRs are displayed in each electrode for all trials. In C, the number of evoked ERs by each stimulus pair is displayed. Each stimulus pair is indicated by the small line connecting two electrodes. In D, the stimulus pairs evoked DRs are displayed. Each stimulus pair is indicated by a blue line connecting two electrodes. The red electrodes reflect the SOZ.*

Table 6.3: *Ratios between electrodes (e) or stimulations (s) with both ERs and DRs and electrodes or stimulations with only ERs or DRs. Between brackets, the range is displayed.*

eERDR/eER	eERDR/eDR	sERDR/sER	sERDR/sDR
0.0038 (0-0.26)	**0.78** (0-1)	**0.0053** (0-0.43)	**0.76** (0-1)

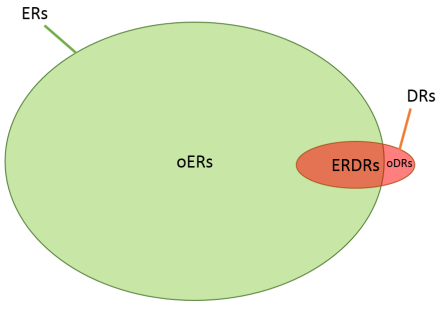


Figure 6.4: *Are DRs evoked in the same population of electrodes as ERs? The ratios of eERDR/eER and sERDR/sER is very low (<0.01), the ratios of eERDR/eDR and sERDR/sDR is on average 0.77.*

Table 6.4: *Ratios between electrodes (e) or stimulus pairs (s) with both ERs and DRs and electrodes or stimulus pairs with only ERs or only DRs. Between brackets, the range of ratios is displayed. NA = not applicable, due to no detected DRs.*

Patient	eERDR/eER	eERDR/eDR	sERDR/sER	sERDR/sDR
37	**0.02** (0-0.26)	**0.85** (0-1)	**0.04** (0-0.43)	**0.78** (0-1)
38	NA	NA	NA	NA
39	**0.002** (0-0.08)	0.67 (0-1)	**0.003** (0-0.13)	0.67 (0-1)
40	**0.004** (0-0.11)	**0.89** (0.5-1)	**0.006**(0-0.10)	**0.88** (0.5-1)
48	NA	NA	NA	NA
49	**0.002** (0-0.09)	0.5 (0-1)	**0.002** (0-0.2)	0.4 (0-1)
50	**0.01** (0-0.22)	0.63 (0-1)	**0.02** (0-0.40)	0.65 (0-1)
51	NA	NA	NA	NA
52	0	0	0	0
53	**0.003** (0-0.07)	0.63 (0-1)	**0.003** (0-0.08)	0.75 (0-1)
54	NA	NA	NA	NA
55	**0.005** (0-0.07)	**0.81** (0-1)	**0.007** (0-0.28)	0.68 (0-1)
77	NA	NA	NA	NA
78	**0.006** (0-0.1)	0.8 (0-1)	**0.005** (0-0.2)	0.67 (0-1)
79	**0.001** (0-0.04)	0.75 (0-1)	**0.001** (0-0.04)	0.75 (0-1)
80	**0.006** (0-0.17)	0.88 (0.75-1)	**0.004** (0-0.13)	0.80 (0-1)
90	**0.0004** (0-0.01)	1 (1-1)	**0.002** (0-0.07)	1 (1-1)
95	NA	NA	NA	NA
96	**0.005** (0-0.14)	1 (1-1)	**0.005** (0-0.08)	1 (1-1)
97	**0.01** (0-0.19)	0.75 (0-1)	**0.01** (0-0.11)	**0.85** (0-1)

Table 6.5: *Number of stimulus pairs which evoke ERs when DRs are observed. Number of stimulus pairs: total ERs (median per stimulus pair, range)*

Stimulus pairs		
Number of ERs with 0 DRs	Number of ERs with > 0 DRs	p
1462: 20657 (13, 0-47)	92: 1722 (17.5, 3-42)	**<0.0001**

Table 6.6: *Number of evoked ERs in electrodes when DRs are observed. Number of electrodes: total ERs (median per electrodes, range)*

Electrodes		
Number of ERs with 0 DRs	Number of ERs with > 0 DRs	p
1135: 199444 (15, 0-72)	81: 2435 (25, 2-102)	**<0.0001**

6.3.2 Are DRs evoked by stimulus pairs which evoked more ERs as well?

In Table 6.5, the number of stimulus pairs of all patients, which evoked at least 1 DR and the number of stimulus pairs evoking no DRs are displayed. When DRs were evoked by a stimulus pair, significantly more ERs were evoked by these stimulus pairs as well.
In Table 6.7, the analysis is repeated per individual patient. In six patients, we found that significant more ERs were evoked by stimulus pairs which evoked DRs. In six patients, more ERs were evoked by stimulus pairs which evoked DRs. In these patients, this was not significant. In one patient, less ERs were evoked in stimulus pairs which evoked DRs. The results in this patient were not significant.

6.3.3 Are DRs evoked in electrodes in which more ERs are evoked as well?

In Table 6.6, the number of ERs in electrodes in all patients, in which at least 1 DR was evoked and the number of ERs in electrodes in which no DRs are evoked, are displayed. When DRs were evoked in an electrode, significantly more ERs were evoked in these electrodes as well.
In Table 6.8, the analysis is repeated per individual patient. In ten patients, we found that in an electrode in which at least one DR was evoked, more ERs were evoked as well. In one patient, this difference was significant. In one patient, the median number of ERs in electrodes with an evoked DR was the same as the median number of ERs in electrodes with no evoked DRs. In two patients, the median number of ERs in electrodes with no evoked DRs was higher than the median number of ERs in electrodes with evoked DRs. These results were not significant.

6.4 Discussion

In this chapter, we investigated the spatial relation between ERs and DRs. First, we investigated whether ERs and DRs were evoked by the same population of stimulus pairs and in the same population of electrodes. We found that DRs were evoked by the same population of stimulus pairs as ERs, but that ERs occurred often without DR. This is as expected, since much more ERs were found than DRs. We also found that DRs were evoked in the same population of electrodes as ERs, but that ERs were evoked often without DR. This is also as expected. The analyses in all patients collected together were very convincing. The results were not significant in all individual patients, due to the low number of detected DRs. In all patients in whom DRs were detected, the ratio of sERDR/sDR was higher than 0.5, except patient 49. In patient 49, the ratio of sERDR/sDR was 0.4. This suggests that DRs were not evoked by the same population of stimulus pairs as ERs. This patient was diagnosed with tuberous sclerosis and had multiple tubers. It is possible that physiological networks are affected by these tubers and that the

Table 6.7: *Number of stimulus pairs with ERs when DRs are observed. Number of stimulus pairs: total ERs (median per stimulus pair, range)*

Stimulus pairs			
Patient	Number of ERs with 0 DRs	Number of ERs with > 0 DRs	p
37	53: 641 (9, 1-31)	18: 410 (24, 10-34)	**0.00002**
38	NA	NA	NA
39	65: 730 (8, 0-69)	3: 32 (13, 2-17)	0.76
40	71: 2066 (29, 1-61)	9: 458 (50, 35-74)	**0.0004**
48	NA	NA	NA
49	84: 1302 (15, 1-41)	4: 73 (16, 11-30)	0.51
50	46: 628 (14, 3-24)	9: 223 (19, 9-57)	**0.02**
51	NA	NA	NA
52	45: 569 (13, 0-32)	1: 12	0.79
53	51: 808 (15, 2-33)	4: 110 (30, 14-36)	**0.03**
54	NA	NA	NA
55	55: 1243 (22, 1-61)	9: 339 (29, 24-70)	**0.007**
77	NA	NA	NA
78	50: 558 (9.5, 0-33)	5: 66 (13, 10-18)	0.15
79	60: 1906 (30, 10-61)	4: 136 (34.5, 12-55)	0.78
80	58: 979 (16.5, 0-69)	2: 25 (12.5, 6-19)	0.66
90	86: 1744 (16, 1-72)	3: 279 (96, 81-102)	**0.004**
95	NA	NA	NA
96	50: 1524 (29.5, 5-55)	4: 147 (34, 27-52)	0.40
97	43: 806 (17, 1-34)	6: 125 (21, 16-25)	0.10

Table 6.8: *Number of electrodes with ERs when DRs are observed. Number of electrodes: total ERs (median per electrode, range)*

Electrodes			
Patient	Number of ERs with 0 DRs	Number of ERs with > 0 DRs	p
37	68: 794 (10.5, 0-28)	20: 257 (11.5, 3-30)	0.43
38	NA	NA	NA
39	77: 736 (8, 0-28)	3: 26 (8, 7-11)	0.98
40	85: 2186 (27, 0-47)	11: 338 (32, 15-42)	0.08
48	NA	NA	NA
49	107: 1304 (12, 0-26)	5: 71 (16, 5-19)	0.22
50	58: 779 (12.5, 3-25)	6: 72 (11.5, 5-22)	0.56
51	NA	NA	NA
52	55: 576 (11, 0-19)	1: 5	0.16
53	72: 860 (11, 0-28)	4: 58 (14, 12-18)	0.28
54	NA	NA	NA
55	77: 1322 (16, 0-40)	11: 260 (23, 17-32)	**0.017**
77	NA	NA	NA
78	61: 584 (9, 0-32)	3: 40 (14, 10-16)	0.13
79	76: 1919 (25.5, 7-40)	4: 123 (30, 24-39)	0.17
80	69: 921 (14, 0-31)	5: 83 (17, 8-23)	0.17
90	109: 1967 (19, 0-44)	3: 56 (16, 15-25)	0.99
95	NA	NA	NA
96	58: 1504 (27, 10-39)	6: 167 (28.5, 20-33)	0.44
97	52: 765 (15, 2-25)	10: 166 (18.5, 11-23)	0.36

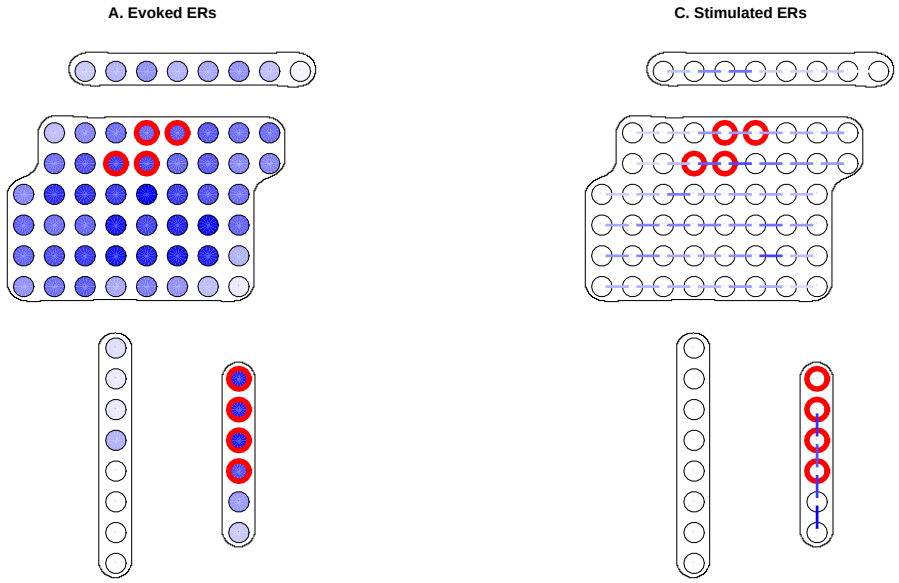


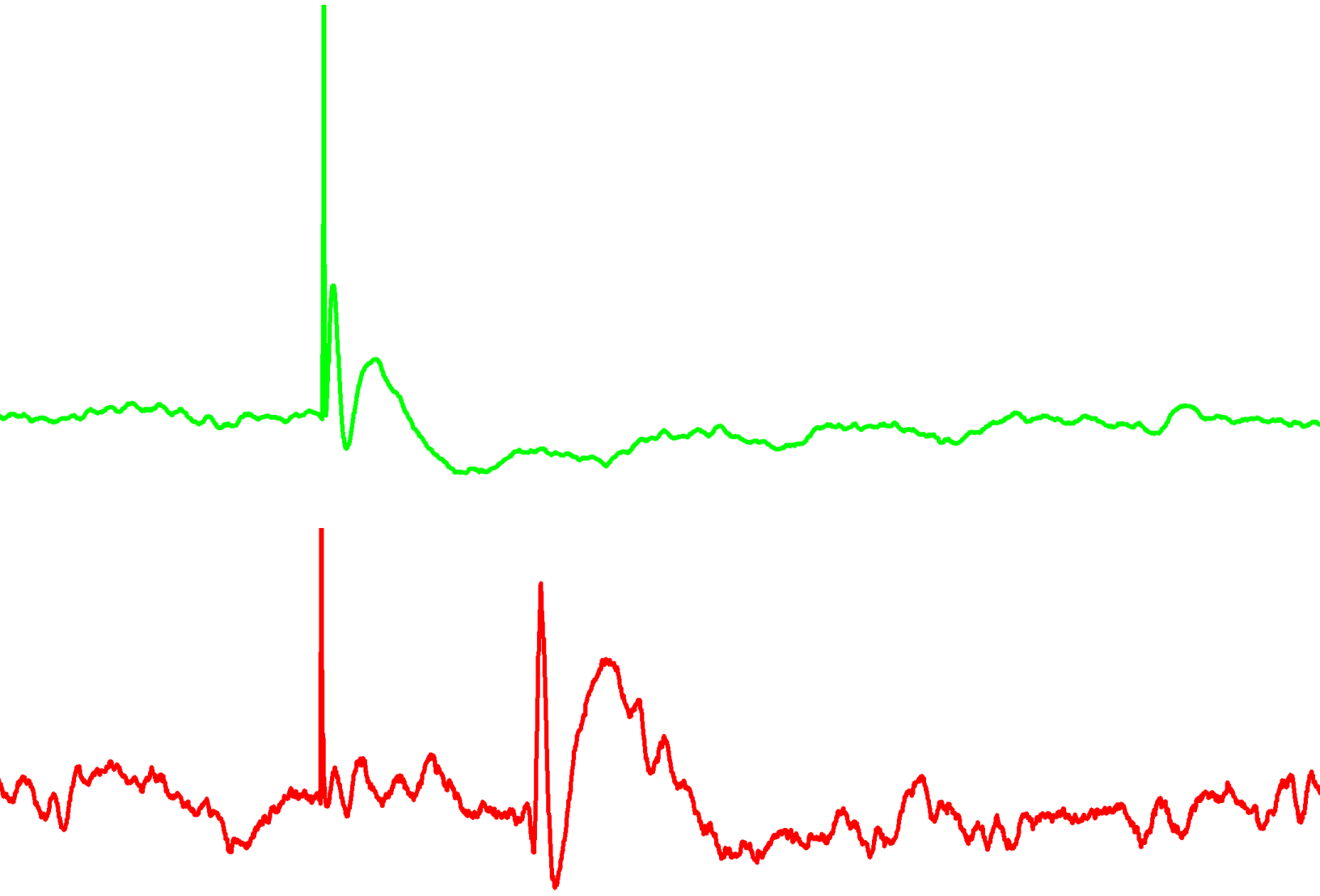
Figure 6.5: *Patient 48 is seizure free (Engel class Ia). The SOZ was characterized by gamma onset.*

ratio of sERDR/sDR is affected as well.
Secondly, we investigated whether electrodes in which DRs were evoked, more ERs were evoked as well. We found that when DRs were evoked in electrodes, more ERs were evoked as well. The analyses in all patients collected together, were very convincing. We did not find significant results in each patient, due to the low number of detected DRs. We only found that significantly more ERs were evoked in electrodes in which evoked DRs were found in patient 50. In patient 39, the median number of ERs evoked in electrodes without evoked DRs was similar to the median number of ERs in electrodes with DRs. In this patient, only 3 DRs were detected. In patient 50 and 90, the median number of evoked ERs in electrodes without DRs was higher than the median number of ERs in electrodes with evoked DRs. Patient 50 underwent surgery due to a tumor. Perhaps, the previous resection influences the physiological network. Patient 90 was diagnosed with Rasmussen encephalitis. This could have influenced the results, since an inflammation may cause changes in the physiological network.
We also investigated whether stimulus pairs which evoked DRs, evoked more ERs as well. We found that when DRs were evoked by stimulus pairs, more ERs were evoked as well. In six patients, this result was significant. In all other patients in which DRs were detected, except patient 80, the median number of ERs was higher in stimulus pairs which evoked DRs as well. Patient 80 was diagnosed with post-encephalopathical gliosis. Perhaps, physiological networks are changed due to the encephalopathy and the gliosis.
Our results are in agreement with the results in the study of Biodo et al. [25]. Boido et al. defined activators and receivers. Activators induces evoked responses in other contacts (receivers). They also defined bidirectional contacts that received from the same activated contacts. They found a trend for a higher probability of bidirectional connections in the epileptic zone. We did not investigate bidirectional connections, but we did find that in electrodes with evoked DRs, more ERs were evoked as well. Similarly, we found that stimulus pairs that evoked DRs, evoked more ERs as well. This suggests that the electrodes can be reached from many other electrodes directly. This suggests that these electrodes are both activators and receivers. In further research it should be investigated whether the connections in these electrodes are bidirectional. Further research could also investigate other network measures like

the clustering coefficient and minimal spanning tree. Since a lot of research is done on these network measures [39], such measures could help in obtaining more insight in the network based on ERs and categorizing it. A strong point of this research is that the detection of ERs is reliable, resulting in reliable reconstruction of the physiological network. The detection of DRs has a lot of over-detection. This was visually checked. It is possible that some DRs are missed since only the detected DRs and not all responses to stimuli were visually checked. This was too time-consuming. Due to the visual inspection, no SRs are falsely included as DR.
A weaker point in this study is that we did not investigate the presence of RRs. RRs are pathogenic as well [15], although these responses occur partly in the time window of ERs. Further research should focus on enabling detection of RRs as well.
In further research, more information can be achieved from the relation between ERs and DRs. We did not look at indirect, secondary connections. When looking at the grid configurations in Appendix E and for example Figure 6.5, it was striking that electrode pairs in the SOZ evoked a lot of ERs in other electrodes. This is an interesting observation and it should be investigated whether this was significant in patient 48 and in other patients. Another possibility for improvement is to correct the number of evoked ERs in electrodes and by stimulus pairs to the location of the electrodes on the grid. It is plausible that in an electrode on the edge of the grid less ERs were evoked than in an electrode in the middle of the grid, since the electrode in the middle is surrounded by more electrodes which may be connected to this electrode. An electrode on the edge may have many connections with brain tissue where no grid is located. These connections are not detected. This could result in the wrong assumption that the electrode on the edge will not evoke any DRs when stimulated.
The number of ERs varied from patient to patient, depending on the size of the grid. Therefore, it was not possible to determine a specific number of ERs after which the presence of DRs is more presumable. A next step could be to normalize the data to the size of the grid. Perhaps, this leads to an ER threshold. When a stimulus pair evokes less ERs than the ER threshold, the stimulus pair can be safely discarded because it is not assumed that this stimulus pair is going to evoke any DRs.
This research is a good start in gaining more insight in the physiological network and the pathogenic DRs in which it is found that DRs are evoked in electrodes and by stimulus pairs in which more ERs are evoked. With this study, we attempted to find ways to speed up the SPES protocol. Currently, we did not find specific values which could help in distinguishing stimulus pairs which are assumed to evoke DRs and stimulus pairs which are not going to evoke any DRs. More research, in which the number of ERs is normalized and not depending on grid size, and in which the number of ERs is corrected for the location of the electrode on the grid, must be done before this general number of evoked ERs can be determined.

Chapter 7

Stimulation settings evaluated



7.1 Introduction

In the UMCU, the standard SPES protocol is performed with monophasic pulses, with positive polarity, 8 mA, 0.2 Hz, for 50 s, and a pulse duration of 1 ms. However, it is unknown whether the SPES protocol could be optimized by using for example a biphasic pulse, or by using pulses with another current intensity. It is unknown what current amplitude should be used to elicit responses that best resemble physiological ones and further studies are required in that direction [29].
Previous studies assumed that pulses with opposite polarity might stimulate slightly different regions [20]. In the same study, it is assumed that the neural stimulation is the greatest at the cathode [20]. According to Matsumoto et al. [34], it is assumed that anodal and cathodal stimulation generates respectively direct and indirect orthodromic discharges in the corticospinal pathway. On the other hand, David et al. [29] mentions that monophasic pulses are thought to be more efficient to initiate action potentials, and are commonly used for short trains of stimulation in patients implanted with depth electrodes [15, 21, 40]. He [29] assumes that both orthodromic and antidromic action potentials are propagated, since the primary targets of stimulation are large myelinated axons.
The differences in assumptions presumes that it is important to investigate the stimulus settings.

In this study, several questions are investigated:

- Are the ERs reproducible when the standard protocol is repeated?
- Is neural stimulation the greatest at the cathode, the anode, or do both contribute equal?
- Are the same ERs evoked when a lower current intensity is used?
- Are the same ERs evoked when a biphasic stimulus is used instead of a monophasic positive pulse?

7.2 Method

Two SPES sessions were acquired in patients who underwent grid implantation between December 2014 and June 2015. In the first session, the standard SPES protocol was used. In the second session, an alternative SPES protocol was used. In the first session, monophasic, positive, pulses of 1 ms, 8 mA (4 mA in the motor cortex), 0.2 Hz were stimulated on neighboring electrodes. In the second session, we applied three pulses with the standard protocol, and seven pulses with an alternative protocol. The several protocols are displayed in Table 7.1.

7.2.1 Are the ERs reproducible when the standard protocol is repeated?

For each electrode, the first three epochs ($epochs1_3$) of the first SPES session were averaged for each trial. For each electrode, the first three epochs of the second SPES session were averaged for each trial ($epochs2_3$). The ER detector, which was optimized in chapter 3, was used for detection of ERs in the averaged epochs for each electrode for each trial in both SPES sessions. For the stimulus pairs to which a pulse was applied in both sessions, the number of epochs with ERs detected in both sessions, only in the first session, only in the second session, and the pulses without ERs in both sessions are determined (Table 7.2). The overall agreement, the positive agreement and the negative agreement are calculated with the following formulas:

$$agreement_{overall} = \frac{w + z}{w + x + y + z} \tag{7.1}$$

$$agreement_{positive} = \frac{2 * w}{2 * w + x + y} \tag{7.2}$$

Table 7.1: *The standard and alternative protocols for SPES sessions 1 and 2*

Protocol	Pattern	Electrodes	Pulses	Mono/ biphasic	Polarity	Current intensity (mA)	Frequency (Hz)	Pulse duration (ms)
Standard	NA	all	10	mono	positive	8	0.2	1
a	chess	50%	3	mono	positive	8	0.2	1
			7	mono	negative	8	0.2	1
		50%	3	mono	positive	8	0.2	1
			7	mono	positive	4	0.2	1
b	alternating rows	50%	3	mono	positive	8	0.2	1
			7	mono	negative	8	0.2	1
		50%	3	mono	positive	8	0.2	1
			3	mono	positive	4	0.2	1
			4	mono	positive	6	0.2	1
c	alternating rows	50%	3	mono	positive	6	0.2	1
			7	mono	negative	6	0.2	1
		50%	5	mono	positive	4	0.2	1
			5	mono	positive	6	0.2	1
d	NA	all	3	mono	positive	8	0.2	1
			7	bi	both	8	0.2	1
e	on the same gyrus	all	10	mono	positive	8	0.2	1

$$agreement_{negative} = \frac{2 * z}{x + y + 2 * z} \tag{7.3}$$

When the overall, positive and negative agreement were higher than 0.5, the agreement is not random and both SPES sessions are comparable.

Table 7.2: *Determine the agreement between two SPES sessions. w = electrodes with ERs in both the first and second session, x,y = electrodes with ERs in only one of the two sessions, z = electrodes without ERs in both sessions.*

		Session I	
		Electrodes with ER	Electrodes without ER
session II	Electrodes with ER	w	x
	Electrodes without ER	y	z

7.2.2 Is neural stimulation the greatest at the cathode, the anode, or do both contribute equal?

The first three epochs of each trial for each electrode were averaged ($epochs2_3$). The fourth-sixth epochs of each trial for each electrode were averaged ($epochs2_6$). The ER detector detected ERs in both averaged epochs for each electrode, in each trial.

Three different situations were possible:

1. Both the anode and cathode contributed equal in evoking ERs.
2. At the cathode, the ERs were evoked.

3. At the anode, the ERs were evoked.

Each of these situations was investigated. For investigating the first situation, the number of electrodes with ERs detected in both $epochs2_3$ and $epochs2_6$, only in $epochs2_3$, only in $epochs2_6$, and the electrodes without ERs in both $epochs2_3$ and $epochs2_6$ were determined (Table 7.2) for each trial to which both a negative and positive monophasic pulse wass applied. The total, positive and negative agreement were calculated. When the agreement was very high, both the anode and cathode contributed equal in evoking ERs, since it did not matter whether a negative or positive pulse was supplied between the two electrodes.
For investigating the second situations, $epochs2_3$ contained the first three responses of each electrode to positive pulses of for example stimulus pair 1-2 and $epochs2_6$ contained the fourth-sixth responses of each electrode after applying a negative pulse to stimulus pair 2-3. This made electrode 2 the anode. Again, the electrodes were divided according to Table 7.2. Total, positive and negative agreement were calculated.
For investigating the third situation, the same procedure was repeated in neighboring electrodes as in situation 2, but now for example stimulus pair 1-2 contained the responses to applying a negative pulse and 2-3 contained the responses to applying a positive pulse. This made electrode 2 the cathode.
The agreements between the three different situations were compared with the Mann Whitney U test (α=0.05). When the median agreement of one of the situations was higher than the median value of agreement in the other situations, the specific situation was the most reliable.

7.2.3 Are the same ERs evoked when a lower current intensity is used?

The first three epochs $epochs2_3$ in SPES session 2 were averaged for each electrode in each trial. In these electrodes, the standard SPES protocol was executed. The fourth-sixth epochs in SPES session 2 ($epochs2_6$) were averaged for each electrode in each trial. In these epochs, pulses with a lower current intensity were supplied according to the alternative SPES protocol. The ER detector detected ERs in both $epochs2_3$ and $epochs2_6$ for each electrode in each trial. The electrodes were divided into four groups, according to Table 7.2. The overall, positive and negative agreement were determined. When the agreement was high, the same ERs were evoked with 8 mA and 4 mA. This would suggest that the same ERs were evoked with a pulse with a current intensity of 8 mA and with a pulse with a current intensity of 4 mA.

7.2.4 Are the same ERs evoked when a biphasic stimulus is applied instead of a monophasic positive pulse?

The first three epochs in SPES session 2 were averaged ($epochs2_3$)for each electrode in each trial. Pulses were applied according to the standard SPES protocol. The fourth-sixth epochs in SPES session 2 were averaged as well ($epochs2_6$). In these epochs, a biphasic pulse was supplied. The ER detector detected ERs in both $epochs2_3$ and $epochs2_6$ for each electrode in each trial. The electrodes were divided into four groups according to Table 7.2. The overall, positive, and negative agreement was determined. When the agreement was higher than 0.5, some ERs were evoked after both applying a biphasic pulse and a monophasic pulse. This would suggest that the same ERs woould be evoked with a biphasic and a monophasic pulse.

7.3 Results

In 8 patients (3 female, 5 male), we performed a second SPES session (Table 7.3). These patients underwent chronic grid implantation between December 2014 and June 2015. The median age was 11.5 years (range: 7-16 years). In 6 patients, we varied the polarity of the pulse. In 4 patients, we varied the

current intensity. In 2 patients, we stimulated all electrodes on the same gyrus. In Appendix G, the patient specific SPES sessions are displayed.

Table 7.3: *Patient specification and the protocol used in the second session.*

Patient	Gender	Age	Protocol
81	m	11	a
83	f	12	a
88	f	8	b
89	f	14	c
91	m	16	d
93	m	7	d
96	m	11	e
97	m	13	e

7.3.1 Are the ERs reproducible when the standard protocol is repeated?

In patient 81, 83, 88, 89, 91, 93, three pulses with the standard SPES protocol were supplied. So, these patients were included to answer this question (Table 7.4). The median total, positive and negative agreement were respectively 0.77, 0.60, 0.84. In patients 81, 88, 93, the positive agreement was very low compared to the positive agreement in patients 83, 89, 91. The mean values for total, positive and negative agreement in patients 81, 88, 93 were respectively 0.67, 0.52, 0.75. The other three patients had a total, positive and negative agreement of respectively 0.85, 0.67, 0.90 on average.

Table 7.4: *The six patients with the number of detected ERs in sessions I and II and the interagreement.*

			Agreement		
Patient	ERs (median, range) I	ERs (median, range) II	Total	Positive	Negative
81	1612 (29, 0-83)	1346 (22, 4-83)	0.71	0.58	0.78
83	626 (13.5, 3-42)	597 (13.5, 3-36)	0.86	0.72	0.91
88	1602 (26, 3-49)	1099 (19, 3-36)	0.70	0.49	0.79
89	721 (17, 6-27)	555 (12, 2-32)	0.83	0.67	0.88
91	890 (17, 4-46)	704 (14, 0-36)	0.86	0.61	0.91
93	1084 (28, 2-44)	809 (22, 4-38)	0.61	0.49	0.69
Median			0.77	0.60	0.84

7.3.2 Is neural stimulation the greatest at the cathode, the anode, or do both contribute equal?

In patient 81, 83, 88, 89, three positive pulses according to the standard SPES protocol and negative pulses were supplied. These patients were included to investigate this question. In Figure 7.1, three figures are displayed with each three boxplots. A trend towards significance was observed when the total and negative agreement of the situation in which both electrodes contribute equally were compared with both the situation in which the contribution of the cathode and the situation in which the contribution of the anode was investigated.

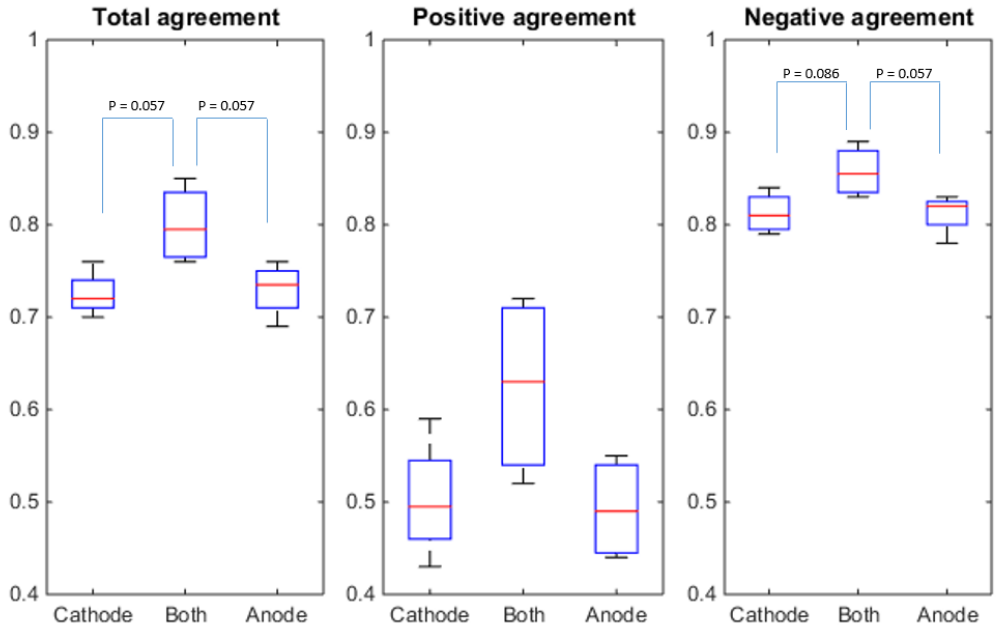


Figure 7.1: *In this figure, the agreement of detected ERs between the first three responses to a positive monophasic pulse and the second three responses according to a negative monophasic pulse are compared. The highest agreement is achieved when the averaged response to a positive pulse is compared with the averaged response to a negative pulse in the same stimulus pair. This suggests that both the anode and cathode contribute equally to the neural stimulation. A trend towards significant results is obtained when the total agreement and negative agreement of the situation in which both electrodes contribute equally is compared with the situation in which the anode or cathode contributes more.*

7.3.3 Are the same ERs evoked when a lower current intensity is used?

In patient 81, 83, 88, three pulses with the standard SPES protocol and pulses with a lower current intensity were supplied. In patient 89, pulses with an current intensity of 6 and 4 mA were applied, because the pulses were applied in the motor cortex. So, these patients were included to answer this question (Table 7.5). In patient 88, also pulses with a current intensity of 6 mA were supplied. In this patient, the overall, positive, and negative agreement between 6 mA and 8 mA is also determined. The mean total, positive, and negative agreement were respectively 0.82, 0.52 and 0.89. The positive agreement is not much higher than 0.5, suggesting that some ERs are evoked when applying a pulse of both 4 and 8 mA, but more ERs were evoked only when applying a pulse of 4 or 8 mA. The low values for positive agreement are also visible when responses to pulses of 8 mA are compared with responses to pulses of 6 mA and responses to pulses of 6 mA and responses to pulses of 4 mA.

7.3.4 Are the same ERs evoked when a biphasic stimulus is used instead of a monophasic positive pulse?

In patient 91 and 93, three pulses according to the standard SPES protocol and seven biphasic positive pulses were supplied. So, these patients were included to answer this question (Table 7.6). The positive agreement was lower than 0.7 in both patient 91 and 93, suggesting that many ERs are evoked when applying both a biphasic or monophasic pulse, but also some ERs are only evoked when a biphasic or monophasic pulse is applied.

Table 7.5: *Four patients with the number of detected ERs when applying pulses with 8, 6 or 4 mA and the total, positive and negative agreement.*

8 mA vs 4 mA					
Patient	ERs (median, range) I	ERs (median, range) II	Total	Positive	Negative
81	621 (20, 4-64)	259 (9, 0-34)	0.80	0.49	0.87
83	337 (10.5, 1-36)	203 (7.5, 0-24)	0.85	0.55	0.91
88	526 (18.5, 4-35)	330 (10.5, 3-27)	0.81	0.51	0.89
Mean			0.82	0.52	0.89

8 mA vs 6 mA					
Patient	ERs (median, range) I	ERs (median, range) II	Total	Positive	Negative
88	526 (18.5, 4-35)	438 (13.5, 5-34)	0.81	0.55	0.88

6 mA vs 4 mA					
Patient	ERs (median, range) I	ERs (median, range) II	Total	Positive	Negative
88	438 (13.5, 5-34)	330 (10.5, 3-27)	0.85	0.56	0.91
89	408 (15, 6-23)	278 (9.5, 2-32)	0.86	0.70	0.91

Table 7.6: *Two patients with the number of detected ERs after applying monophasic (I) or biphasic (II) pulses and the total, positive and negative agreement.*

			Agreement		
Patient	ERs (median, range) I	ERs (median, range) II	Total	Positive	Negative
91	750 (14, 0-36)	573 (10, 0-29)	0.89	0.59	0.93
93	897 (22, 4-38)	794 (17, 4-32)	0.80	0.67	0.85

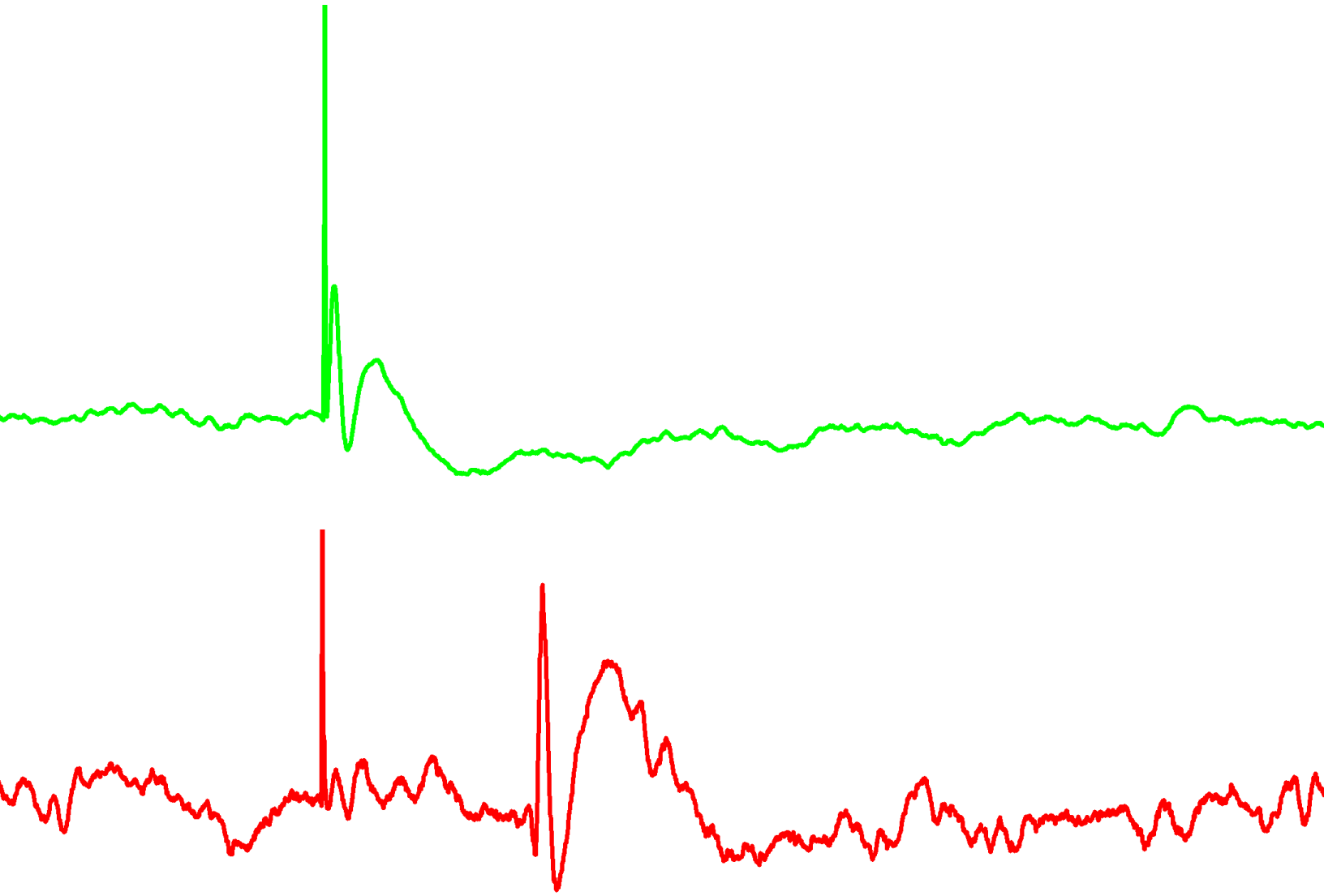
7.4 Discussion

In this chapter, several research questions were investigated. First, we investigated whether ERs are reproducible when the standard protocol is repeated. In patients 83, 89, 91, the total agreement is on average 0.85. In patients 81, 88, 93, the total agreement was on average 0.67. In patients 83, 89, 91, the first SPES session was executed at least one day after the surgery. In patients 81, 88, 93, the first SPES session was executed on the same day as the implantation surgery. These patients were still sleepy. Perhaps, anesthetics were still present in the brain, effecting physiological connections. Propofol is known for its anti-epileptic effects. Zijlmans et al. [41] found that propofol reduced epileptic high frequency oscillations (HFOs). The results in this small population suggests that SPES should not be executed on the same day as surgery, since connections influence the physiological connections.
Secondly, we investigated whether the cathode, anode, or both contribute most during neural stimulation. The total agreement of the situation in which both the cathode and anode contribute equally was almost significantly higher (p=0.057) compared with both the situation in which the contribution of the cathode was investigated and the situation in which the contribution of the anode was investigated. This suggests that both the cathode and anode contribute equally in the neural stimulation. This is not in agreement with the assumption in the study by Lacruz et al. [20], who assumed that the cathode contributes slightly more and the study by Matsumoto et al. [34], who assumed that both anodal and cathodal stimulations generate different mechanisms. However, it is in agreement with the study by David et al. [29] who assumes that in a monophasic pulse action potentials in both orthodromic and antidromic direction.
Thirdly, we investigated whether different physiological connections were exposed when stimulating with a different current intensity. We found that the positive agreement was on average 0.52 when

stimulating with 8 mA was compared with stimulating with 4 mA. This suggests that different fibers are exposed when another current intensity was used, since the positive agreement is only 0.52. This is in agreement with other studies [29]. However, we only tested several current intensities in four patients. More patients must be included to get more insight in the mechanism behind applying pulses with different current intensity.
At last, we investigated whether stimulating with a biphasic pulse results in the same ERs as stimulating with a monophasic pulse. The positive agreement was on average 0.63. This suggests that ER detections differed when monophasic or biphasic pulses were applied. According to David et al. [29], monophasic pulses are assumed to be more efficient in evoking responses. When we look at the number of evoked ERs when applying a biphasic or monophasic pulse, more ERs were evoked when applying a monophasic pulse. However, these results are only observed in two patients. More patients must be included to obtain more insight in the mechanism behind applying monophasic or biphasic pulses.
A strong point in this study is that we were able to execute two SPES session. We were able to investigate whether a second SPES session resulted in the same detected ERs as the first SPES session. We were also able to investigate the difference between the current SPES protocol and an alternative SPES protocol, by applying three pulses according to the current SPES protocol and seven pulses according to an alternative SPES protocol. Since stimulating one stimulus pair took less than one minute, we can assume that the network did not change significantly and both protocols could be compared per stimulus pair.
A weaker point in this study is that we averaged three epochs. In the averaged epoch, ERs were detected. It is possible that we missed ERs since it occurred only once of three stimuli and was minimized during the averaging. Since we applied three pulses according to the current protocol, we averaged also three epochs of the alternative protocol.
More research can be done to investigate the stimulation settings. This was only the first step in obtaining insight in the effect of stimulation settings on evoked ERs. More patients should be included to investigate the effect of propofol on physiological connections in the brain, the difference in current intensity and biphasic or monophasic pulse forms. More patients should be included to investigate whether the hypothesis that both anode and cathode contribute equally in neural stimulation is significant. Perhaps DTI could help in investigating whether different fibers are stimulated when different current intensities are applied. Due to the limited time of this study, we were not able to investigate whether applying pulses to grids on the same gyrus gives more insight in the connections in the network, although in two patients, we executed a second SPES protocol in which we only applied pulses in electrodes which were localized on the same gyrus. An interesting hypothesis could be that ERs are more often evoked in the same gyrus than abroad.
We conclude that this study is a small step in obtaining more insight in the effects of the stimulation settings on the brain tissue. More patients should be included to get more conclusive results.

Chapter 8

General conclusion and discussion



In this research, we tried to find ways to make the SPES protocol and analysis more time efficient. In chapter 3, we automated the detection of ERs by constructing an automatic ER detector. The sensitivity, specificity, PPV and NPV of this detector were respectively 0.78, 0.91, 0.75 and 0.92. This means that detection of ERs is reliable. Detected ERs are true ERs and not many ERs are missed. With this perfomance, the ER detector may be used in automatic detection of ERs.
In chapter 4, we automated the detection of DRs. The sensitivity, specificity, PPV and NPV of this DR detector were respectively 0.68, 0.92, 0.10 and 1.00. Due to the low number of detected DRs, any false positive or false negative detection had a big influence on the sensitivity and PPV. Since the performance of this detector was not very good, this detector alone was not applicable in further research. We decided to detect DRs with this detector, but to check the DRs visually to reduce the number of DRs due to over-detection.
In chapter 6, we investigated the relation between ERs and DRs. In 20 patients, ERs and DRs were detected with the automatic ER and DR detectors. We found that DRs and ERs are evoked in the same population of electrodes and by the same population of stimulus pairs. ERs were evoked much more, so most ERs were observed without a DR. We found that when DRs are evoked, more ERs were evoked in these electrodes and by these stimulus pairs. This suggests that these electrodes are connected more widely than electrodes in which no DRs were observed. This is in agreement with the study of Boido et al. [25], who found that the epileptic area was highly connected to other areas. They also found that epileptic area contained especially many bidirectional connections. We did not investigate this in the current study. This could be a good focus in further research.
In chapter 7, we evaluated the stimulation settings. We found that anesthetics may have a big influence on the detection of ERs. We also found a trend towards significance that both the anode and cathode contribute equally when applying a monophasic pulse. We found that different ERs were evoked when the current intensity was varied between 4 and 8 mA. At last, we found that biphasic pulses evoke different ERs than monophasic pulses. Besides, less ERs were evoked during biphasic pulses. This was in agreement with the assumption of David et al. [29] that a monophasic pulse is more efficient in evoking resonses.
A strength of this study is that first an automatic detection algorithm for detecting ERs and DRs was reconstructed. Automatic detection is less subjective than visual annotation. Another strength is that 20 patients were included. A larger population facilitates obtaining significant results.
The DR detector is not performing sufficiently. More effort should be put in improving this detector. Since the detections were visually checked, the poor performance of the DR detector did not have a big influence on the analysis of the relation between ERs and DRs.
DvB annotated both the ERs and DRs. She was not experienced in annotating ERs and DRs and the annotations were not validated by an expert. The ER and DR detector are tested and validated on these annotations. It is possible that the detectors fundamentally detect signals without ERs and DRs since the annotation was not good enough. It is important that the annotations are corroborated by a second observer.
This study gives a lot of new starting points for further investigations. First, both the ER and DR detector must be improved. RRs and SRs are not detected with the current detectors. Since RRs reflect pathogenic tissue and SRs reflect physiological connections, it is important that these are detected sufficiently in both the ER and DR detectors.
Secondly, the study of the retrospective data is only a the beginning of investigating the spatial relation between ERs and DRs. We did not investigate bidirectionality in the network based on ERs. When observing the grid configurations in appendix E, it was striking that electrodes in the SOZ evoked many ERs in other electrodes. It is interesting to investigate whether this is significant.
The retrospective study shows that ERs expose the underlying physiological network. However, some information about pathogenicity is probably hidden in the information about ERs. Bernhardt et al. [38] concludes that networks are altered in epileptic areas. In further research, it is recommended to investigate other network measures like the eigenvector centrality, average path length, average

clustering coefficient, degree and betweenness centrality. These networks measures could help in getting more insight in the networks based on ERs.
This research is a first step in obtaining a more time efficient SPES protocol and facilitates analysis of ERs and DRs. The ultimate goal was to make the protocol more time efficient. We conclude that the acquisition of the SPES protocol can be halved, since reliable ERs can be established in only five pulses. With these ERs, we can reconstruct the underlying physiological network. The electrode pairs with more ERs are more suggestible for evoking DRs in other electrodes.
We have approval from the METC to execute a third SPES session in a patient when informed consent is signed. In the next patients, we could reconstruct the network based on ERs. We could determine which stimulus pairs evoked probably not sufficient ERs. In the third session, we could investigate whether we are able to find the same DRs in the alternative protocol in which stimulus pairs are discarded and whether we are able to determine what area should be resected. This could be the first step forward in obtaining a time-efficient SPES protocol.
In the future, more insight in the relation between ERs and DRs will hopefully lead to a shorter SPES protocol in which the epileptic area is delineated without obviating the need for days of chronic grid monitoring.

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Appendix A

Technical aspects of automated SPES analysis

A.1 Procedure

The Electrocorticography (ECoG) was recorded using the Micromed SD128 headbox or the LTM128 express EEG headbox with integrated programmable stimulators and SystemPlus Evolution acquisition software. The data was recorded at a sample frequency of 2048 Hz. The ground electrodes were usually placed on the scalp, outside the grid. In the ECoG, signals from electrodes on the grid were recorded. Grids consisted of 8*1, 8*2 or 8*4 electrodes. A typical grid placement covered the suspected seizure onset zone and preferably the hippocampus, because of epilepsy possibly originating in the hippocampus. An average grid lay-out contained 72 electrodes.

During classic SPES, ten single monophasic pulses of 1 ms duration, 8 mA amplitude, 0.2 Hz repetition frequency were given in all neighboring electrodes. After stimulating all stimulus pairs (average 60 trials), the file was saved in a proprietary format that could be imported in Matlab.

In the University Medical Center Utrecht (UMCU), over the last ten years of SPES stimulation had been performed using two different stimulators. In the old stimulator, there was no trigger channel recorded. In the new stimulator, there was a trigger channel in which the moments of stimulation were indicated with a Dirac's delta function. This trigger channel facilitated processing and therefore resulted in a more efficient way of preprocessing. Both methods were explained below.

A.2 Determination of stimulation instants

A.2.1 New stimulator

The ECoG data was loaded in Matlab. In the trigger channel, the location of the delta functions indicating a stimulation, were found using an algorithm in Matlab called *peakfinder*. When ten consecutive peaks with a time difference of 5 seconds were found, these peaks belonged to the same stimulus pair. A time window of two seconds before the first pulse and three seconds after the tenth pulse was used to delimit the stimulations of one electrode pair. In line with Valentin et al. [15], this period will be called a trial. The response to one single stimulation will be referred to as 'an epoch'. The separate epochs in each electrode for each trial were saved with a time window of 2 seconds before, and 3 seconds after the pulse. Ten epochs were also averaged for determination of the stimulation electrodes. This procedure is visualized in Figure A.1.

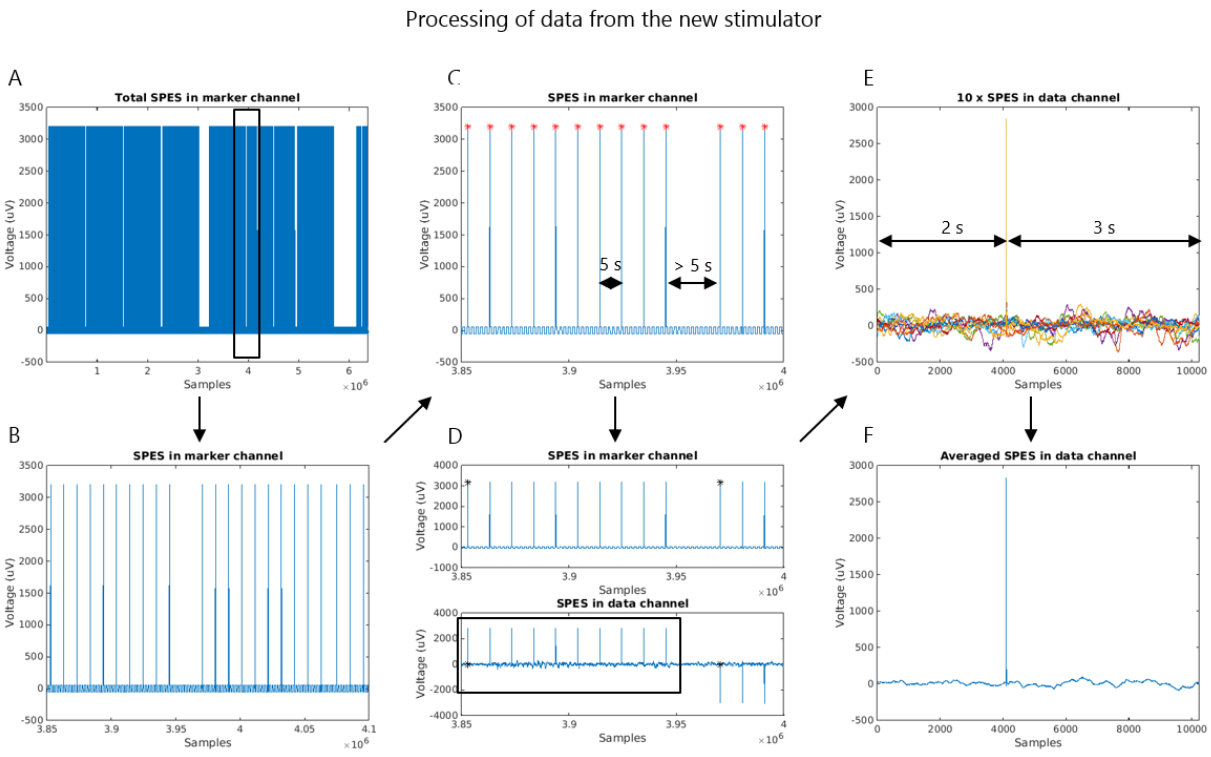


Figure A.1: *Processing of data from the new stimulator. In A, the total SPES signal of the marker channels is displayed. The signal in the black square is magnified in B. In C, all peaks were detected. Peaks with 5s interval belonged to one stimulation pair. When the interval was longer than 5s, the next peak belonged to the ten pulses of the next stimulation pair. The first peak was displayed with a black * in D. The location of the first peak in the marker channel was used to delimit the ten pulses belonging to one stimulation pair in data channels. The epoch around each pulse was 2s before and 3s after stimulation. The epochs of the ten pulses in the black square in D are displayed in E. The averaged epoch of the ten epochs is displayed in F.*

A.2.2 Old stimulator

The ECoG data was loaded in Matlab. Since there was no trigger electrode, the stimulation instants were found in the signals from grid electrodes. When only one arbitrary electrode was used to detect peaks, there was a chance that some trials were not detected or a peak was detected later than it was truly occurring as was the case when this electrode was stimulated itself. Therefore, peaks were detected in all electrodes using *peakfinder*. When ten consecutive peaks with a time difference of 5 seconds were found, these peaks belonged to the same stimulation pair: a trial. For each electrode, the first peak of each trial was saved. All these first peaks of all electrodes were collected, sorted in consecutive order and grouped into groups with first peaks occurring within 1 s. To distinguish between groups with first peaks from a true stimulus and groups with false first peaks, a group of first peaks was regarded as belonging to a stimulus when at least 20% of the electrodes detected a first peak in a group. The groups, in which less than 20% of the electrodes detected a first peak, were removed. In the remaining groups, the median value of the instant of the first peaks was calculated and this was assumed to be the moment of stimulation. The median value was calculated instead of the mean value, because the stimulation instant was seen at the same sample in most electrodes except the stimulation electrodes. In the stimulation electrodes, the stimulation instant was shifted. A median value takes into account which instant sample was found in most electrodes. A time window of two seconds before and $(5 * 10 =) 50$

seconds after the first pulse was used to delimit the stimulations of one electrode pair: a trial. The separate epochs in each electrode for each trial were saved with a time window of 2 seconds before, and 3 seconds after the stimulation. These epochs were also averaged for determination of the stimulation electrodes.

A.3 Determination of the stimulation electrodes

For each stimulus, the stimulation pair was found. This procedure was the same for both the old and new stimulator. Two properties of the signals during stimulation were apparent:

1. The stimulation electrodes were saturated for a long time, resulting in a high area under the curve.
2. One stimulation electrode was saturated with a positive voltage (+3000 μV). The other stimulation electrodes was saturated with a negative voltage (-3000 μV).
3. The electrodes were neighbors on the grid.

These properties were used to determine the stimulation electrodes in each trial. The median value of an averaged epoch for each electrode and each trial was subtracted from the averaged epoch for each electrode and each trial. The area under the curve was calculated. The five electrodes with the highest (positive) and the five electrodes with the lowest (negative) value for the area under the curve are found. When a neighbor of an electrode with a high area under the curve was found in the group with a low area under the curve, electrodes with reverse polarity were found and these were regarded as the stimulation electrodes. This procedure was also visualized in Figure A.2.

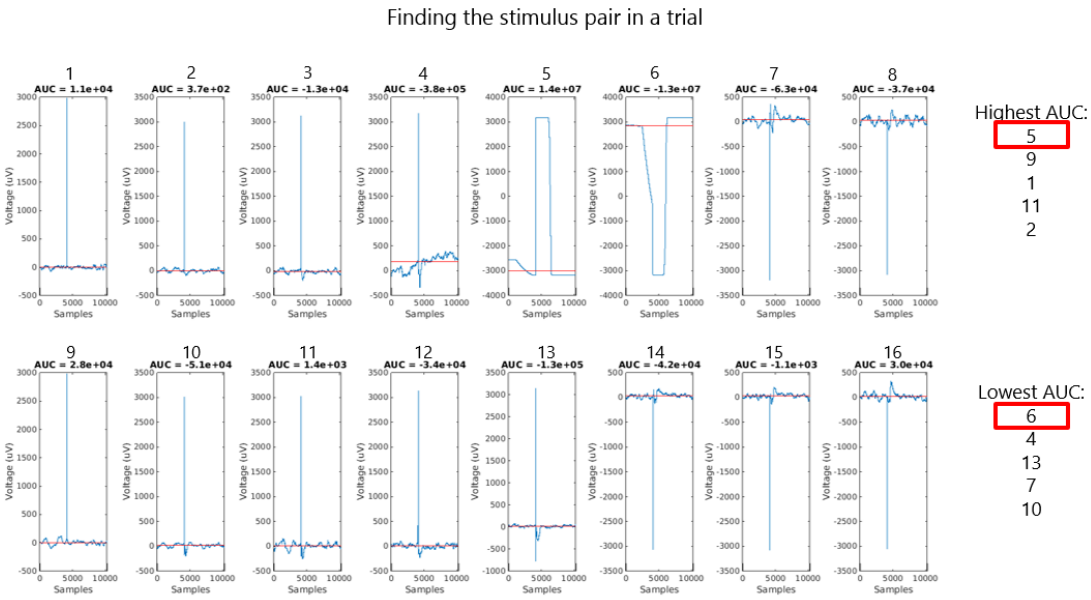


Figure A.2: *Finding the stimulus pairs in each trial. The averaged epoch of 16 electrodes during stimulation in one stimulation pair are displayed. Electrodes 5 and 6 were stimulated. The median amplitude of each electrode is displayed with a red line. This value was subtracted from the averaged epoch. The area under the averaged epoch (AUC) was calculated. The stimulation electrodes had the highest positive and negative value for the AUC. Since stimulation occurred always between neighboring electrodes, one electrode with a high positive AUC should have a neighboring electrode with a high negative AUC to be considered as a stimulation electrode.*

Appendix B

Averaging ten epochs into one epoch

B.1 Introduction

According to Valentin et al. [15], an ER is present in ten epochs of one electrode in one trial when it is seen in one epoch, because it is a physiological response. This would suggest that in the average of ten epochs, an ER is still visible. This was never investigated. Therefore, in this appendix, we investigate whether ten epochs can be averaged without reducing the present ER.

B.2 Method

In one patient, we annotated ERs visually using Micromed, SystemPlus Evolution with 5s/page, no extra filter settings and variable scaling (usually 1200 μV/cm), depending on the amplitude of the signals and the number of electrodes shown in the display.
In Matlab R2014b, we picked six visually annotated ERs at random. For each visual ER, we plotted ten epochs and averaged the ten epochs in one figure. We evaluated whether in the averaged epoch an ER was visible as well.

B.3 Results

In patient 81 (male, 11 years), 1336 ERs were annotated in 74 trials (per trial: median = 15.5, range = 0-66). In Figure B.1, it is displayed that an ER is reliably rendered in the averaged epoch.

B.4 Discussion

In this appendix, we investigated whether it was possible to average ten epochs without reducing a visible ER. We randomly picked six visual ERs and observed whether in the averaged epoch, an ER was still visible. Each ER was visible in the averaged epoch, so we conclude that in an averaged epoch in which ten epochs are averaged, an ER is still visible. We also observed that the background activity is reduced, facilitating detection of ERs instead of background activity.
Boido et al. [25] evaluated the reliability of SPES-evoked responses by calculating a variability index on each average response, computed as the ratio between the summation of the standard deviations and the summation of the averaged points of each response, normalized for the number of points. They excluded averaged responses with variability index values >0.1. This is a more accurate method for determination whether responses are reliable. Due to the limited time of this study, this was not used in our study. It could be a good improvement in further research. We conclude that, since we observed

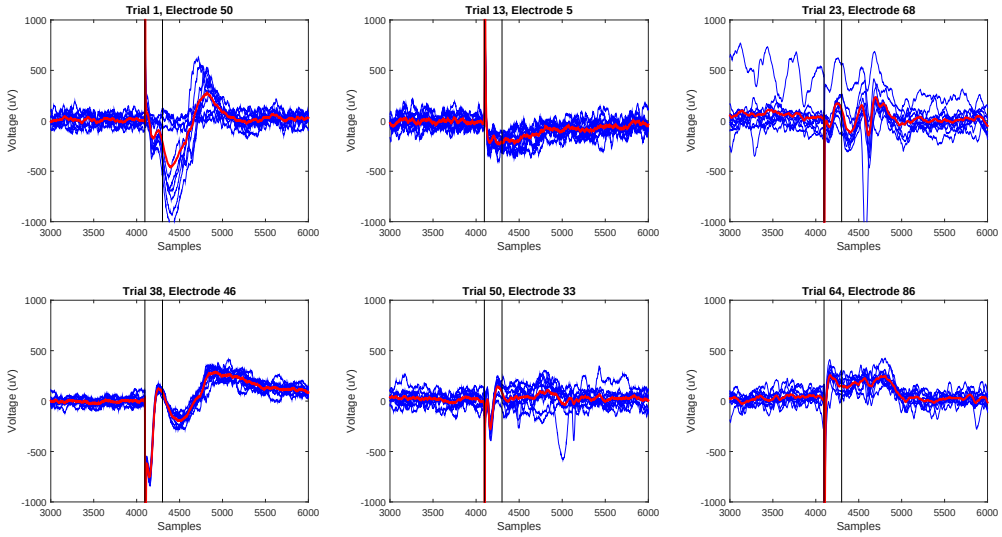


Figure B.1: *In this Figure, for six electrodes, in six different trials, ten epochs (blue lines) and the averaged epoch (red line) are displayed. The vertical black lines indicate in which time window ERs are annotated. In all figures except the upper left figure, it is shown that an ER was visible in each epoch. This ER was reliably rendered in the averaged epoch. In the upper left figure, the ER was only visible in 6 out of ten epochs. Still, the ER was reliably rendered in the averaged epoch. We also see that the amount of background activity is reduced, facilitating the detection of an ER instead of peaks from background activity.*

that an ER was clearly visible in the averaged epoch, we will construct a detector in which ERs are found in averaged epochs.

Appendix C

Stimulator and acquisition characteristics in MicroMed: an independent test

C.1 Introduction

During visual observation of responses to stimulations in Micromed, SystemPlus Evolution, ERs were seen with an amplitude varying between 50-500 μV. This raised questions whether one early response is more predictive than the other. Furthermore, the influence of the stimulation artifact was unknown. The company Micromed told us that prior to stimulation, the potential difference is measured between an electrode on the grid and the reference electrode. During stimulation, the potential difference is measured between the reference electrode and the ground electrode. After stimulation, the potential difference is measured between an electrode on the grid and the reference electrode. This is reflected in the stimulation artifacts. However, it is unknown when the switch from ground electrode to grid electrode occurs. Therefore, it is unknown when activity of the brain is measured again and reliable ERs can be detected. We decided to investigate the stimulation artifact during application of pulses to grid electrodes lying on cotton pads. The purpose was to get more insight in the stimulation artifact and when ERs could be detected. This could help in determining the stimulation settings in the rest of this study. Enatsu et al. [24] found the first response in CCEP data with a latency of 9 ms.

C.2 Method

In a small tray, two layers of cotton pads were laid. These cotton pads were moistened with salty water (NaCL, unknown concentration). Two grids (8*2) were laid on the moistened cotton pads. Two separate electrodes were used as ground and reference. These electrodes were laid on the same layer of cotton pads, but they did not touch the electrode grids. The measurement set up is displayed in Figure C.1. On top of the grids, an extra layer of moistened cotton pads were laid to obtain signals with less noise. We stimulated ten times during 50 seconds in electrode 22-23 with a positive and biphasic pulse of 1 ms, and current intensity of 8 mA. We measured the response in the other grid electrodes. We chose an electrode with visible 50 Hz noise and assumed that this is similar to brain activity. During the switch between grid electrode to ground electrode, this noise would not be visible. After the switch, this noise is visible again. The number of samples after stimulation and before visibility of 50 Hz noise gives us insight when ERs can be detected after stimulation.

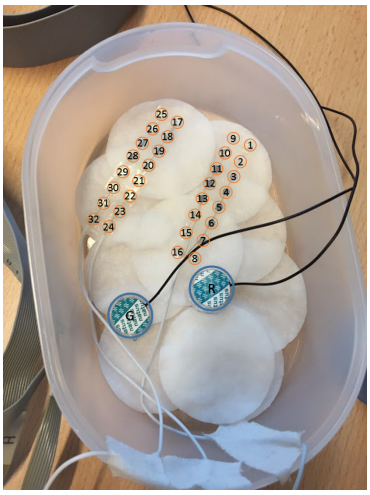
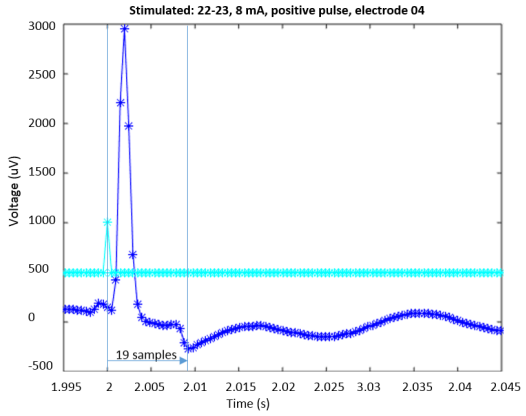
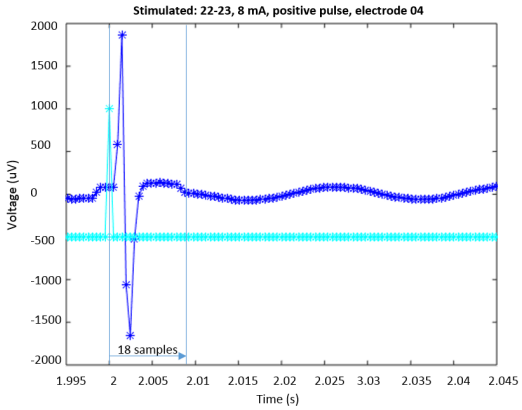


Figure C.1: *Measurement set up of cotton pads and grids.*



(a) *The response in electrode 4 after stimulating electrode 22-23 with 8 mA and a positive pulse.*



(b) *The response in electrode 4 after stimulating electrode 22-23 with 8 mA and a biphasic pulse.*

Figure C.2: *The amount of samples between the stimulus in electrode 22-23 and the appearance of 50 Hz noise in electrode 4.*

C.3 Results

Electrode 4 had visible 50 Hz noise in the signal. During and a short period after the given stimulus, this noise was absent. It is assumed that physiological signal in the brain is comparable to 50 Hz noise: it disappears during and a short period after the given stimulus. During this period, it is not possible to detect physiological signals from the brain. Any peaks in this interval would be evoked artificially by the stimulus. In Figure C.2, the delay in electrode 4 is displayed and the amount of samples between the given stimulus and the appearance of the 50 Hz noise. The amount between the stimulus and the appearance of 50 Hz noise was 18-19 samples. This suggests that the earliest physiological response could be detected after (19/2048 =) 9 ms.

C.4 Discussion

In this experiment with SPES in cotton pads, we investigated the stimulation artifact after stimulating with varying SPES settings. We compared the response to two pulse forms (positive, biphasic) in an electrode with 50 Hz noise. We found that during the switch this noise was absent. 9 ms after the stimulation, this noise was visible again. We conclude that brain activity is not measured during 9 ms after the stimulation. Therefore, detection of ERs in Section 3 will be performed between 9-100 ms after stimulation.

In literature, responses are detected in depth electrodes [25, 24, 42]. Enatsu et al. [24] found negative peaks with a latency of 9 ms after stimulation. The median latency was 60 ms. Conner et al. [42] explicitly says that data within the first 8 ms after stimulation were excluded to eliminate stimulation artifacts. Even though the stimulation settings were different than ours, their latencies were similar to ours.

This experiment was a small project and improvements are possible. The reference and ground are both close to the electrode grid. Therefore stimulation artifacts could have been noticed by the reference and ground and could have led to more fluctuations in measured potential difference. The concentration of NaCl to moisten the cotton pads was unknown and therefore possibly different from the concentration in the brain. The stimulation artifact could look different in this experiment due to different conductivity. However, we assume that the latency before brain activity is visible is similar in this experiment as in the brain.

When this experiment would be repeated, it is recommended to use a salty solution with a concentration similar to cerebral fluid to simulate a more reliable situation.

Our conclusion is that data within 9 ms after the stimulation must be excluded from ER detection because potential difference between the reference and ground is measured. Physiological brain activity is present after 9 ms and therefore, ERs can be detected between 9-100 ms after stimulation.

Appendix D

Time window for detection of delayed responses

D.1 Introduction

Valentin et al. describes ERs as sharp deflections following the stimulus artifact or occasionally merging with it. This deflection is followed by one or two slow waves of alternating polarity. In our observations, these slow waves occur in the time window of DRs: 100 ms-1 s after the stimulus artifact. In this section, we investigate whether the time window of 100 ms-1s after the stimulus artifact is right for detection of DRs or that this window should be narrowed due to the slow waves after the initial ER.

D.2 Method

In six patients, DvB annotated DRs. In these patients, DRs are detected in Matlab with the function *peakfinder*, SD factor = 4, sel = SD, minimal SD = 0. The start of the time window is varied from 100 ms to 200 ms with steps of 10 ms. In a graph, the number of detected DRs are displayed for each start of the time window. When an outstanding drop in detection of DRs is present, the detected DRs are compared with the visual DRs. The sensitivity, specificity, PPV and NPV are calculated. When the performance of the detector is better with narrower time window for DR detection, this time window will be used in further research.

D.3 Results

In six patients (4 males, mean age: 12, range: 8-31), DRs were annotated. In total 268 DRs were annotated (median 44.5 per patient, range: 10-83) In Table D.1, the patient specification are displayed.

In Figure D.1, the number of detected DRs are displayed for varying start of time window. The number of detected DRs decreased when the start of time window increased from median 437.5 (139-1328) detected DRs to median 160 detected DRs (23-518). The decrease was linear. No outstanding drop in detected DRs is observed.

D.4 Discussion

In this section, we investigated whether the time window in which DRs are detected should be adapted due to the slow waves of ERs present in the time window of DR detection. We saw that the number

Table D.1: *Patient characteristics for varying time window for detecting DRs. Between brackets, the median number of annotated DRs per trial, the minimal number and the maximal number are displayed.*

Patient	Gender	Age	Electrodes	Trials	Visual DRs (median, range)
16	m	13	64	49	10 (0, 0-2)
19	f	31	64	50	83 (0, 0-11)
21	m	8	64	58	47 (0, 0-7)
22	f	13	64	49	32 (0, 0-19)
27	m	9	64	56	54 (0, 0-7)
29	m	11	64	54	42 (0, 0-6)

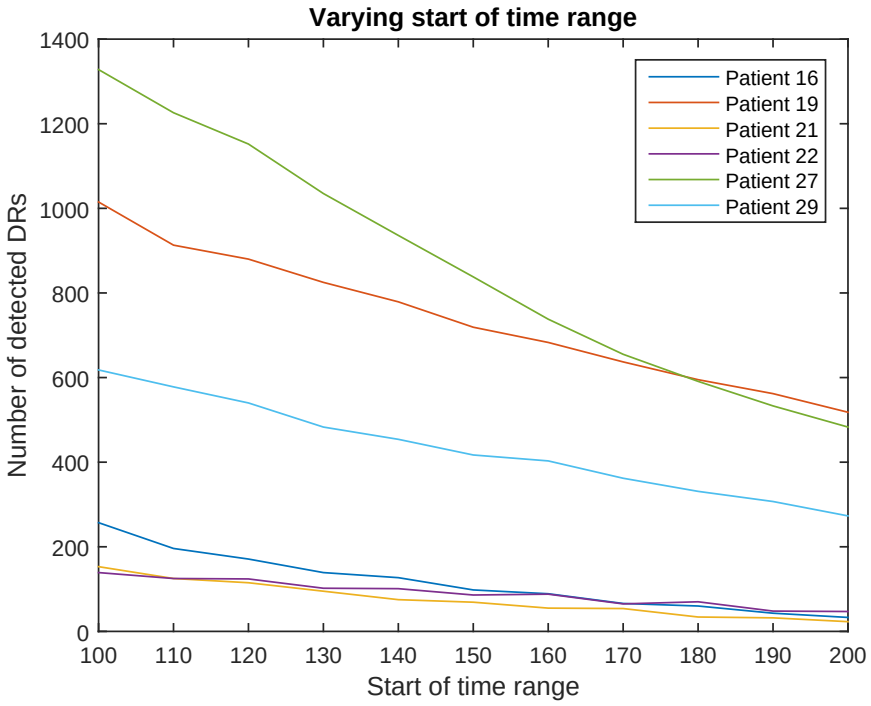


Figure D.1: *The time window is varied and DRs are detected.*

of detected DRs dropped with time. The lowest amount of detected DRs were observed when 200 ms-1s after the stimulation artifact. However, we did not see a outstanding drop in DR detections. This suggests that peaks of DRs and slow waves overlap and it is not possible to differentiate between the two by only adapting the time window of DR detection.

At the UMCU, researchers have a lot of experience in annotating DRs in time frequency plots [18]. Their experience is that most DRs are observed within 200-400 ms after the stimulation artifact. Therefore, changing the time window of DR detection to 200 ms-1s after the stimulation artifact would likely increase the number of false negative detections.

In this small section, we only considered the number of automatically detected DRs. We did not validate these detections with visual annotation. Further research should compare the detection with visual annotation to see whether the performance of the detector increases when the time window is varied. Besides, filtering the slow waves could help in facilitating the detection of DRs.

We conclude that the time window for DR detection is sufficient when the window is 100 ms-1 s after the stimulation artifact.

Appendix E

Networks in retrospective data of twenty patients

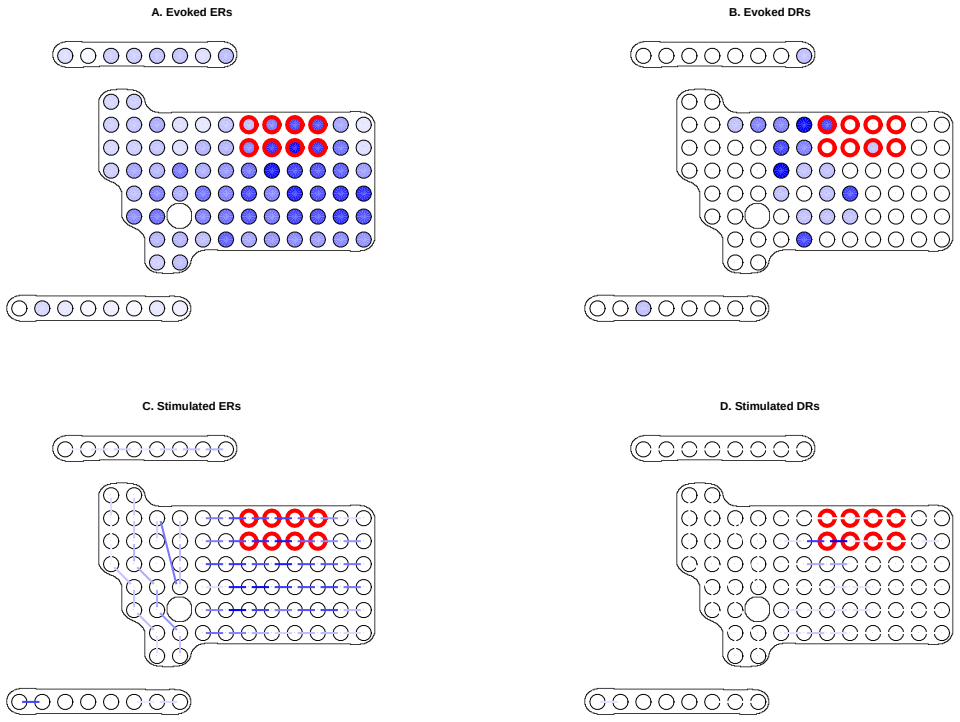


Figure E.1: *Patient 37 is not seizure free. The red electrodes reflect the SOZ. The SOZ was not characterized by gamma onset.*

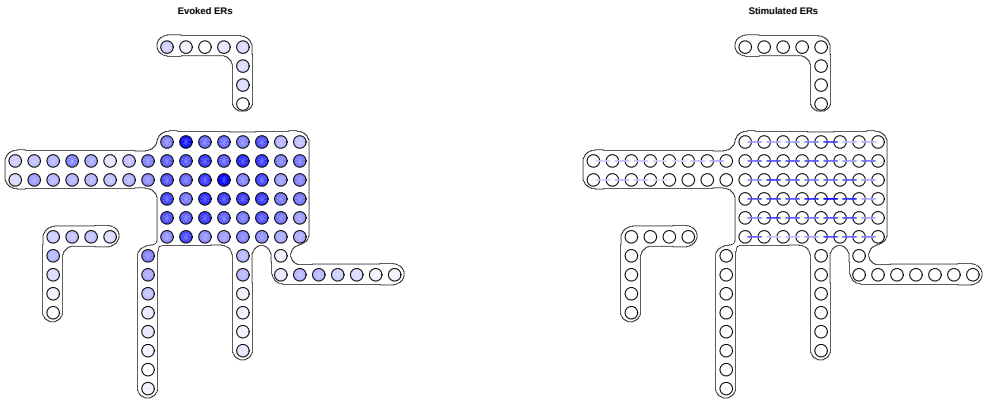


Figure E.2: *Patient 38 is not seizure free, since no tissue was resected. The SOZ was not determined since the seizures had a diffuse onset.*

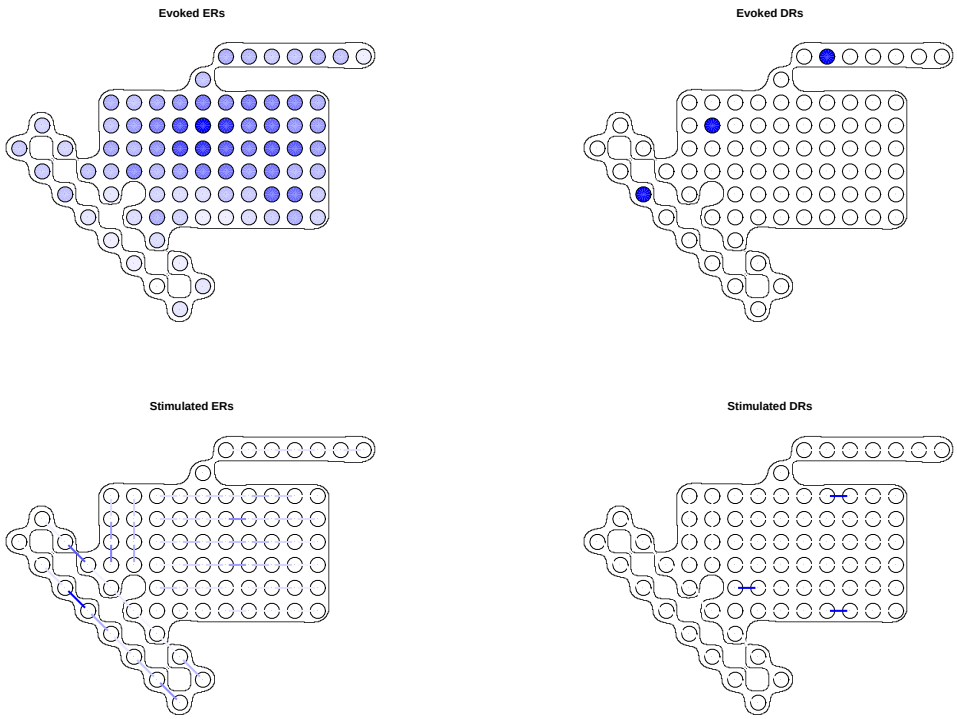


Figure E.3: *Patient 39 is not seizure free, since no tissue was resected. The SOZ was not determined since it was a diffuse onset.*

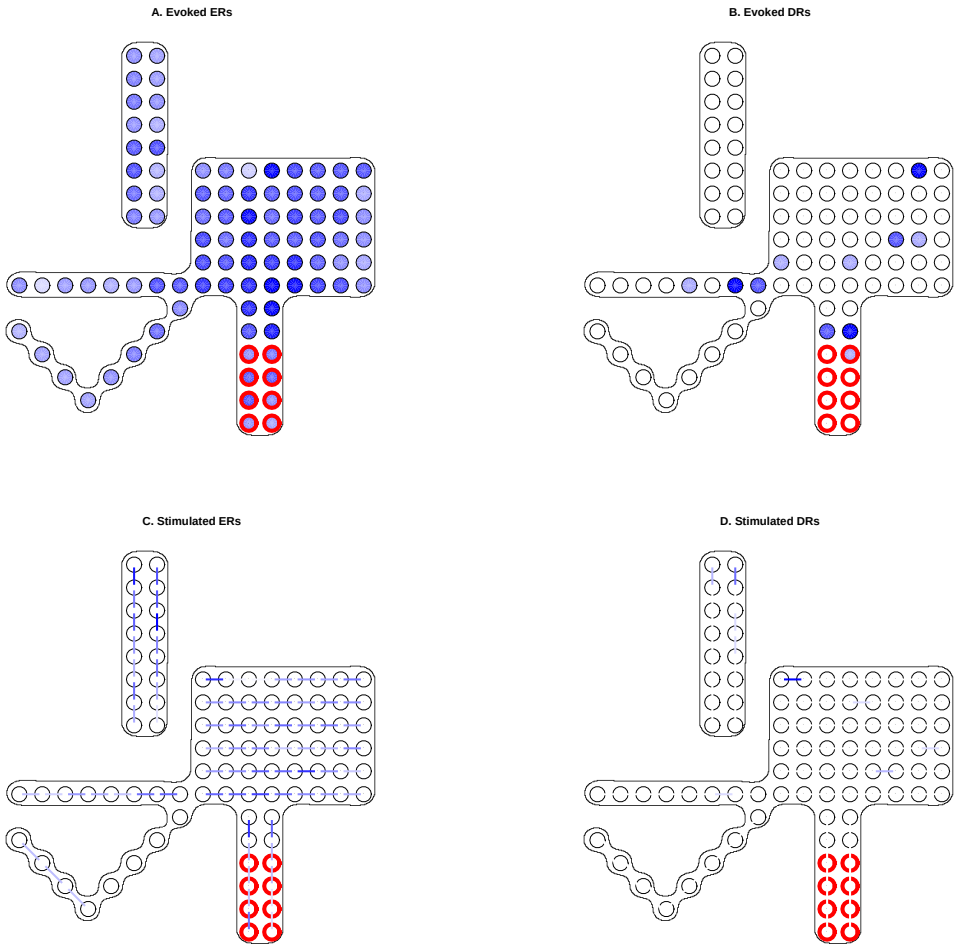


Figure E.4: *Patient 40 is seizure free (Engel class Ib). The red electrodes reflect the SOZ. The SOZ was not characterized by gamma onset.*

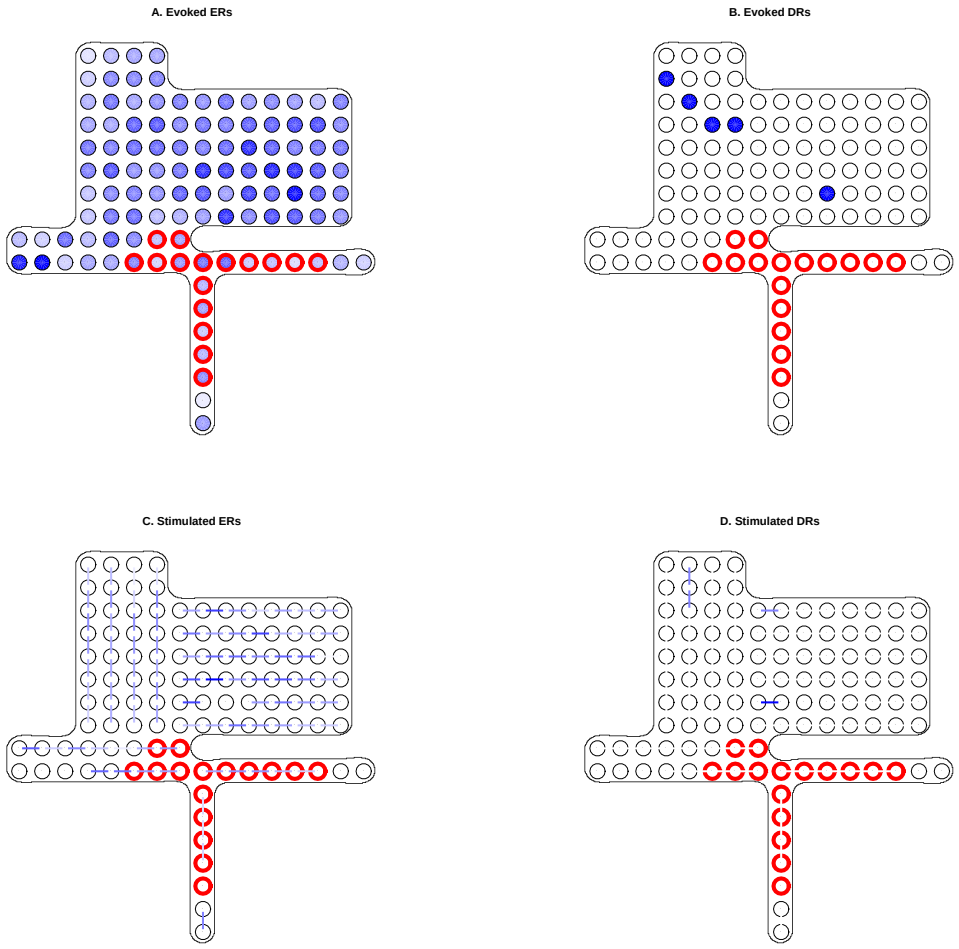


Figure E.5: *Patient 49 is not seizure free. This was not the purpose of the implantation. The severity of the seizures became less severe after surgery. The red electrodes reflect the SOZ. The SOZ was characterized by gamma onset.*

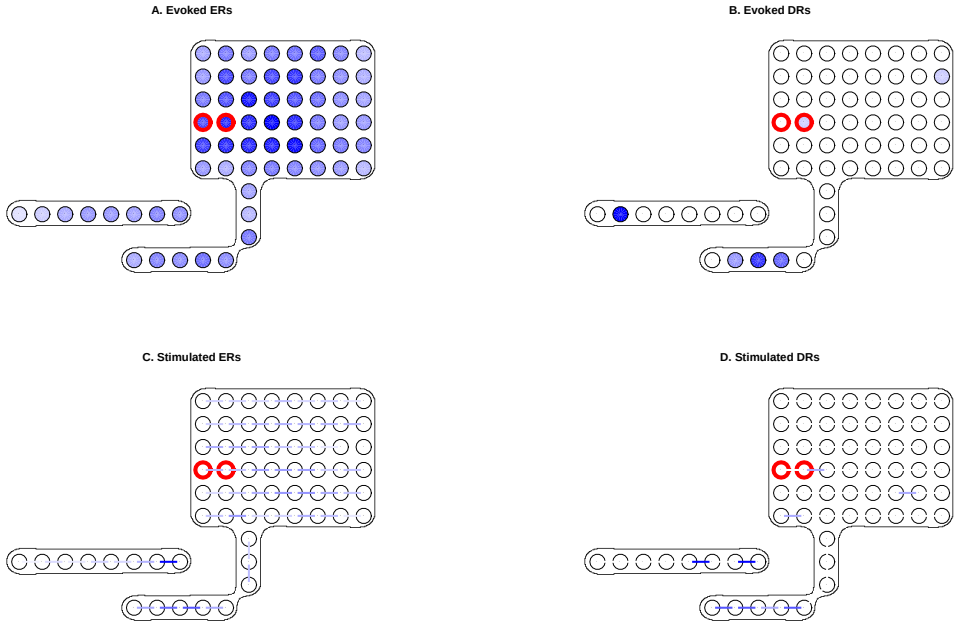


Figure E.6: *Patient 50 is seizure free (Engel class Ia). The red electrodes reflect the SOZ. The SOZ was characterized by gamma onset.*

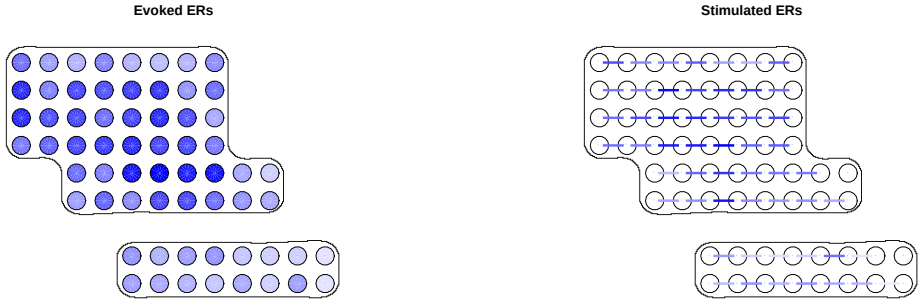


Figure E.7: *Patient 51 is not seizure free, because he was not operated. This patient underwent a frontal disconnection. No SOZ was determined, due to the short duration of the grid implantation. The grid implantation was only performed to localize functional areas.*

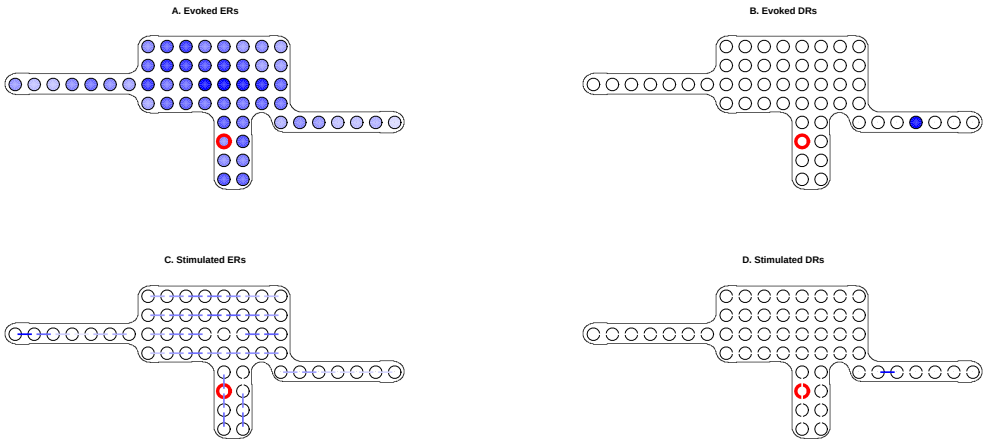


Figure E.8: *Patient 52 is seizure free (Engel class Ib). The red electrodes reflect the SOZ. The SOZ was characterized by gamma onset.*

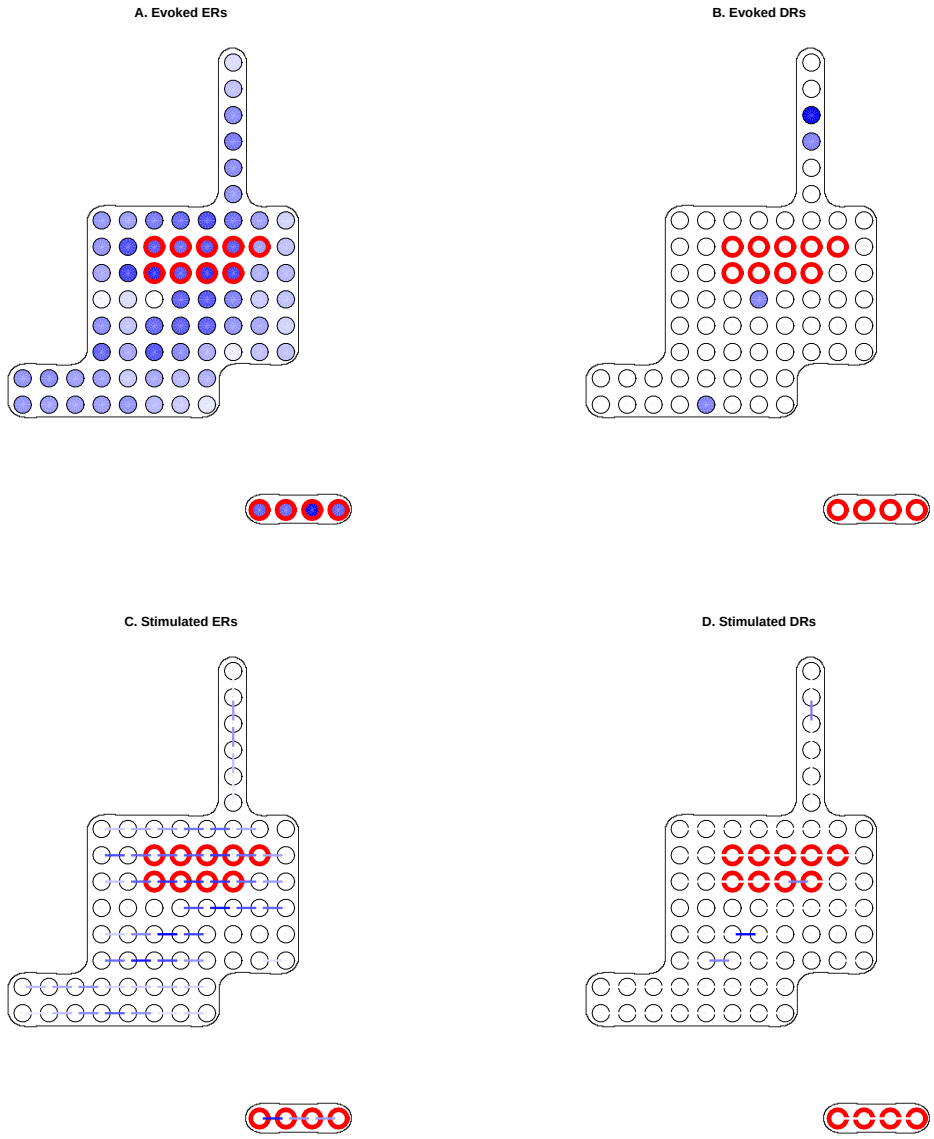


Figure E.9: *Patient 53 was operated but complains about emotional feelings. It is not confirmed that these feelings are epileptic seizures. The red electrodes reflect the resection area. The SOZ was not determined since the onset was diffuse. The resection area was determined based on interictal spikes from the dysplasia.*

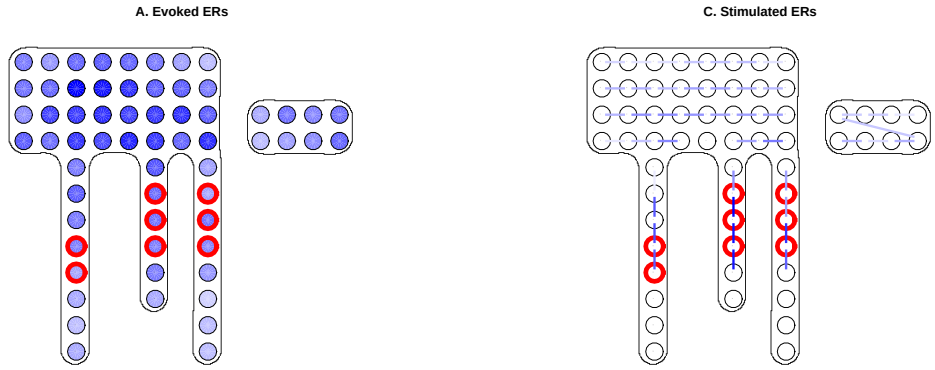


Figure E.10: *Patient 54 is seizure free (Engel class Ia). The red electrodes reflect the SOZ. The SOZ was characterized by gamma onset.*

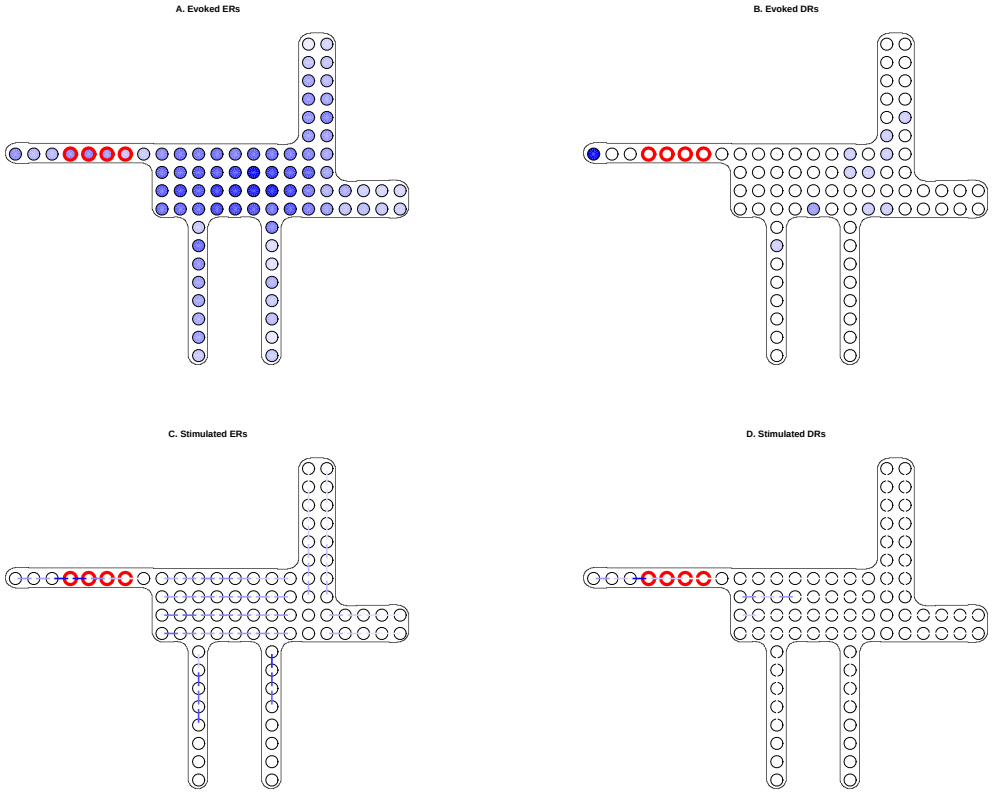


Figure E.11: *Patient 55 is seizure free (Engel class Ia). The red electrodes reflect the SOZ. The SOZ was characterized by gamma onset.*

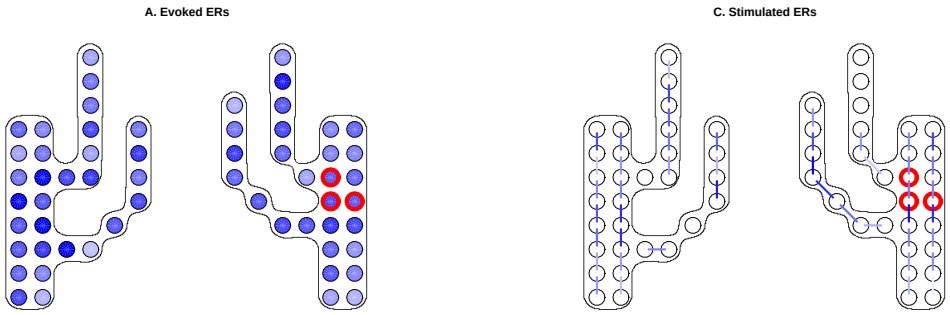


Figure E.12: *Patient 77 is seizure free (Engel class Ia). The SOZ was not characterized by gamma onset.*

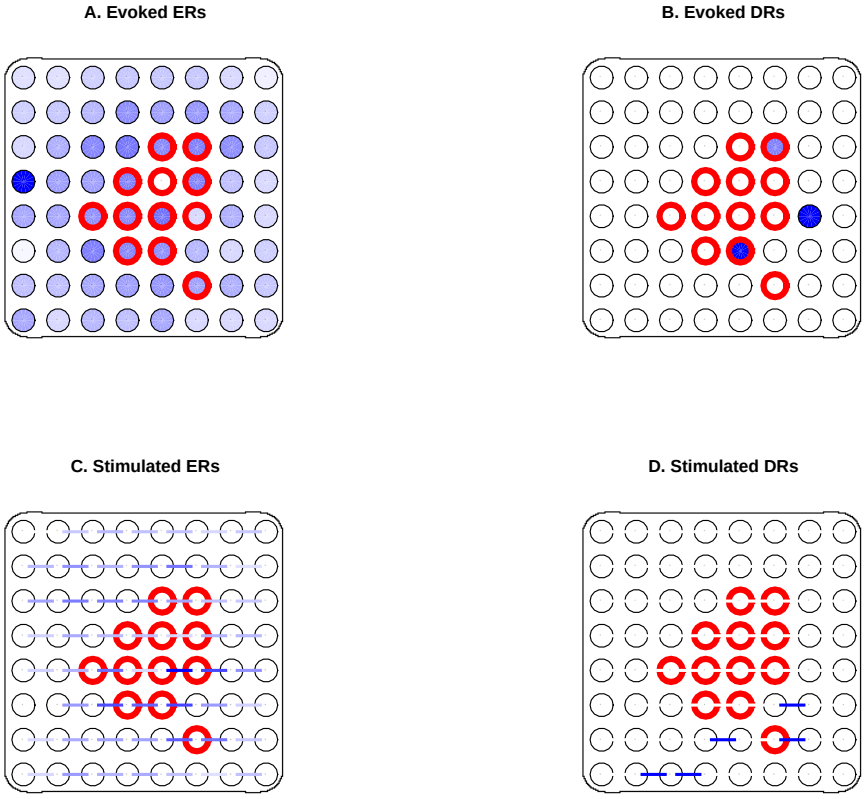


Figure E.13: *Patient 78 is seizure free (Engel class Ia). The red electrodes reflect the SOZ. The SOZ was characterized by gamma onset.*

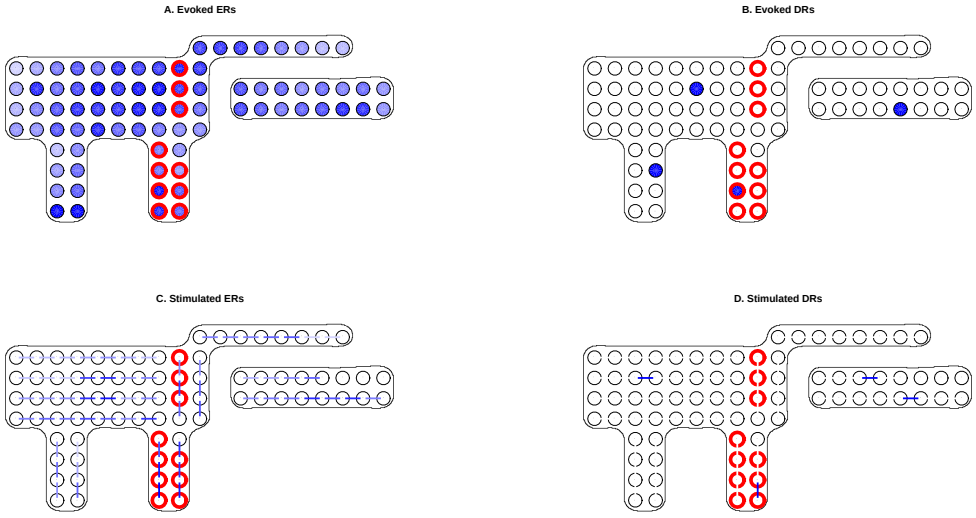


Figure E.14: *Patient 79 is not seizure free. The red electrodes reflect the SOZ. The SOZ was characterized by gamma onset.*

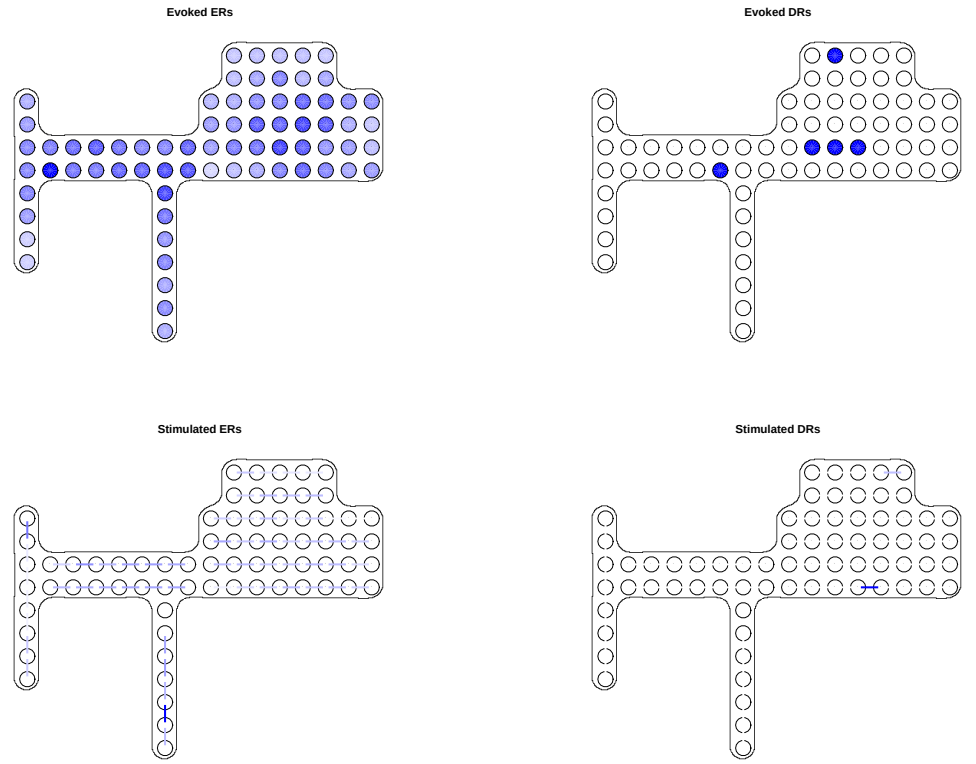


Figure E.15: *Patient 80 is not seizure free. The SOZ was not determined due to diffuse onset.*

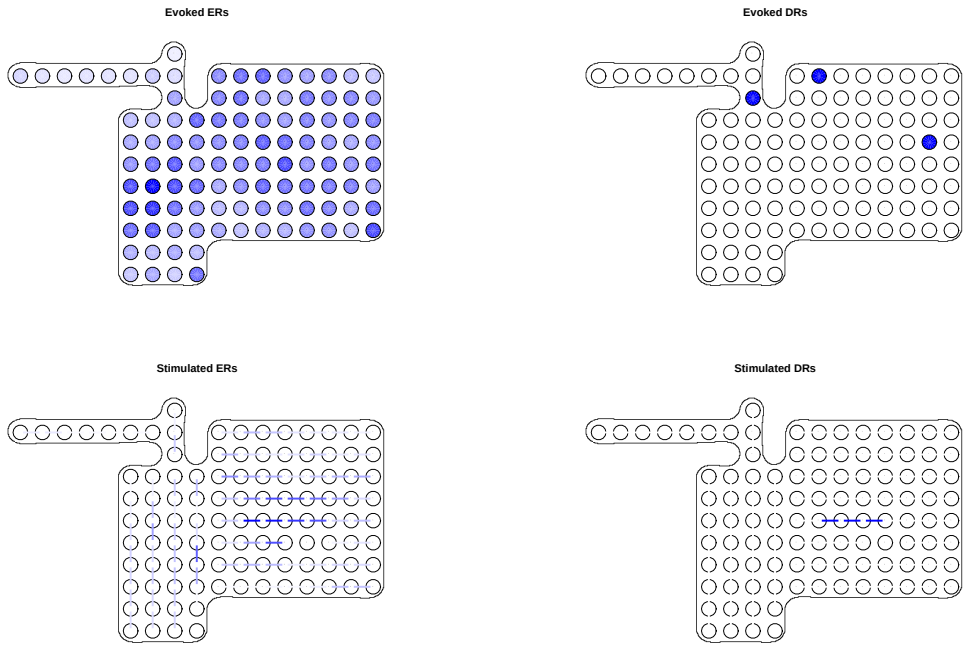


Figure E.16: *Patient 90 is not seizure free. The SOZ was not determined due to diffuse onset. This patient underwent a frontal disconnection.*

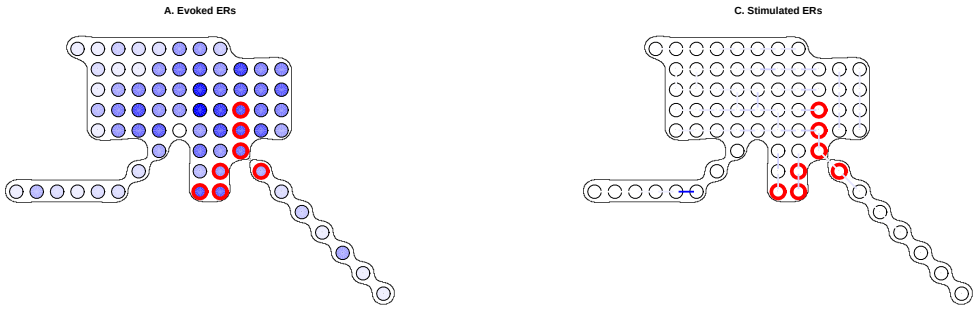


Figure E.17: *Patient 95 is seizure free (Engel class Ia). The red electrodes reflect the SOZ. The SOZ was characterized by gamma onset.*

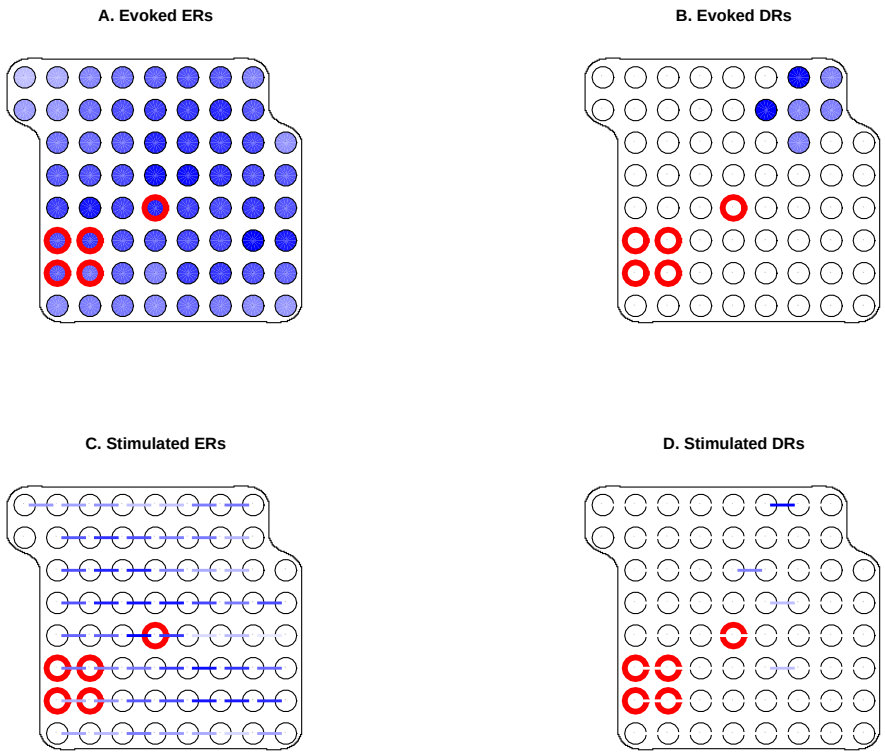


Figure E.18: *Patient 96 is not seizure free. The red electrodes reflect the SOZ. The SOZ was not characterized by gamma onset.*

Appendix F

Instructions GUI

F.1 Why this GUI?

This Graphical User Interface (GUI) was built to enable visualisation of the ER and DR networks. It facilitates getting insight in the relation between ERs and DRs and could give new questions to be investigated in further research.

F.2 Ingredients

- grid lay out in Excel. Make sure each electrode has a unique number
- trc-file with SPES stimulations
- matlab script "mainfile.m"

F.3 Pre-processing before usage

1. Pre-process the trc-file with the steps mentioned in Appendix A, using Matlab script "mainfile.m".
2. Convert the grid lay out into matlab
3. Run the ER detector
4. Run the DR detector
5. Convert the found ERs into an adjacency matrix
6. Convert the found DRs into an adjacency matrix
7. Place the adjacency matrices and grid lay out under the specific patient
8. Run the GUI

F.4 How to use the GUI?

In Figure F.1, the lay out of the GUI is displayed. On the left side, the grid lay out is displayed for the specific patient. On the right side, several options can be chosen.

Patient You can select the specific patient for visualisation of the ERs and DRs.

Connection You can select "Evoked" or "Stimulated". When you select "Evoked", the electrode pairs which evoke a response in a specific electrode are displayed. The specific pairs are connected by a small line. When "Stimulated" is selected, it is displayed in which electrodes a response is evoked when the specific electrode/electrode pair is stimulated.

Electrode or stimulation "Electrode" or "Stimulus" can be selected. However, when the connection is set on "Evoked", only the option "Electrode" will result in a visualisation. When the connection is set on "Stimulated", both options are available. When "Electrode" is selected, the responses in other electrodes are displayed when a specific electrode is stimulated. Some electrodes are stimulated in two different electrode pairs (f.e. 1-2 and 2-3). The electrodes in which once a response is evoked are light green. The electrodes in which twice a response is evoked are dark green. When "Stimulus" is selected, the responses in other electrodes when a specific electrode pair is stimulated are displayed.

Number When "Electrode" is selected in the previous pop-up menu, the number of electrode can be chosen. When "Stimulus" is selected in the previous pop-up menu, the number of electrode pair can be chosen. This number is depending on the order of stimulation. Usually, the electrodes are stimulated in rows from left to right, starting at the upper electrodes.

Response When "ERs" is pushed, the ERs are visualised in the grids with a green color. When "DRs" is pushed, the DRs are visualised in the grids with red. When "Both" is pushed, ERs, DRs are displayed with respectively green and red. When both an ER and DR are found in an electrode/electrode pair, it is displayed with a blue line or electrode.

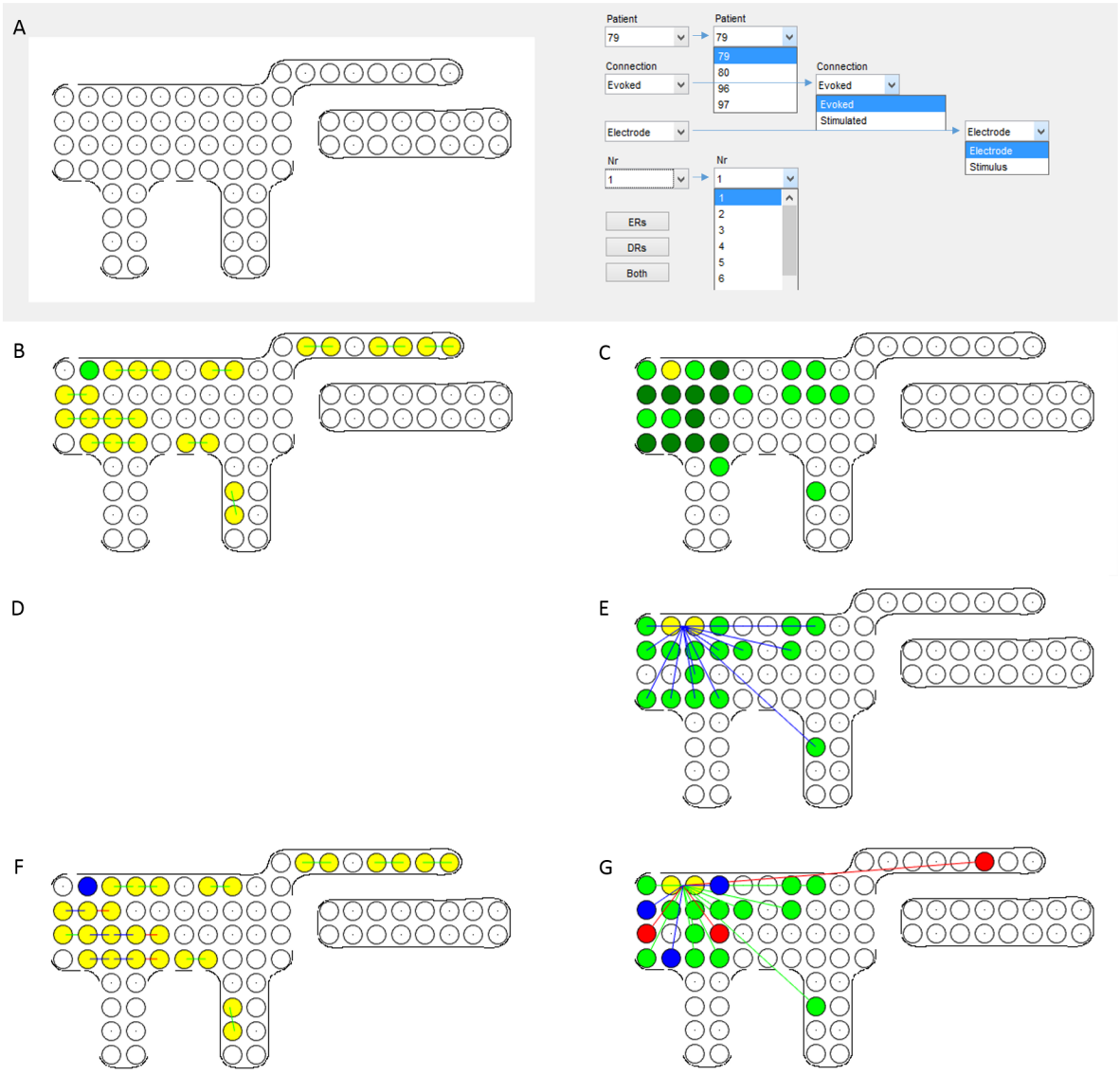


Figure F.1: *The possibilities in the network GUI in patient 79. A: The plain grid reconstruction. On the right side, the specific possibilities are displayed. First, you have to select a patient which is loaded into Matlab. Then, you select whether you want to display the evoked (B, F) or stimulated connections (C, E, G). Then, you select whether you want to display connections in single electrodes (B, C, F) or in stimulus pairs (E, G). Then, you select the number of electrode or stimulus you want to display. At last, you select whether you want to display ERs (B, C, E), DRs or both (F, G). B: It is displayed when an ER is observed in single electrode number 2 (green) when stimulated elsewhere (yellow) in patient 79. C: It is displayed in which electrodes ERs are observed (green), when electrode number 2 (yellow) is stimulated. In the light green electrodes, ERs are observed once (in stimulus pair 1-2, or 2-3). In the dark green electrodes, ERs are observed twice (in stimulus pair 1-2 and 2-3). D: It is not possible to display which electrode pair is evoked after stimulating elsewhere. E: It is displayed in which electrodes ERs are observed when electrode pair number 2 (yellow) is stimulated. F: It is displayed when an ER (green), DR (red), or both (blue) is observed in electrode 2 (blue), when other electrode pairs are stimulated. G: It is displayed in which electrodes ERs (green), DRs (red), or both (blue) are observed in other electrodes, when electrode pair 2 (yellow) is stimulated.*

Appendix G

Patient specific SPES sessions

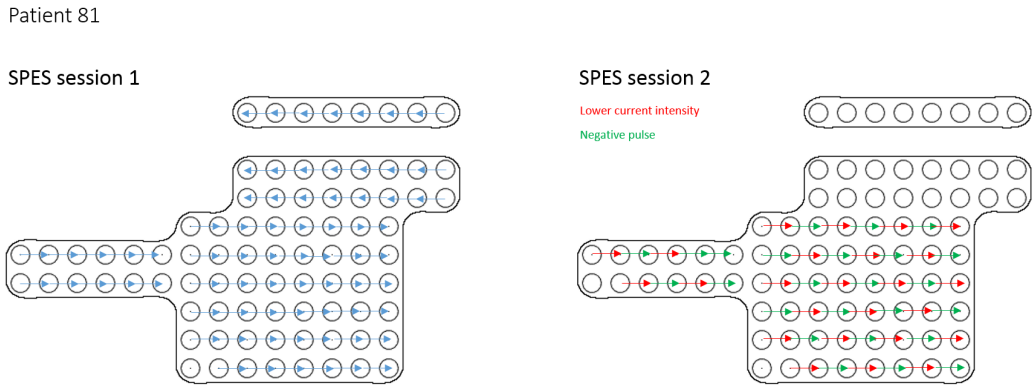


Figure G.1: *SPES session 1 and 2 in patient 81*

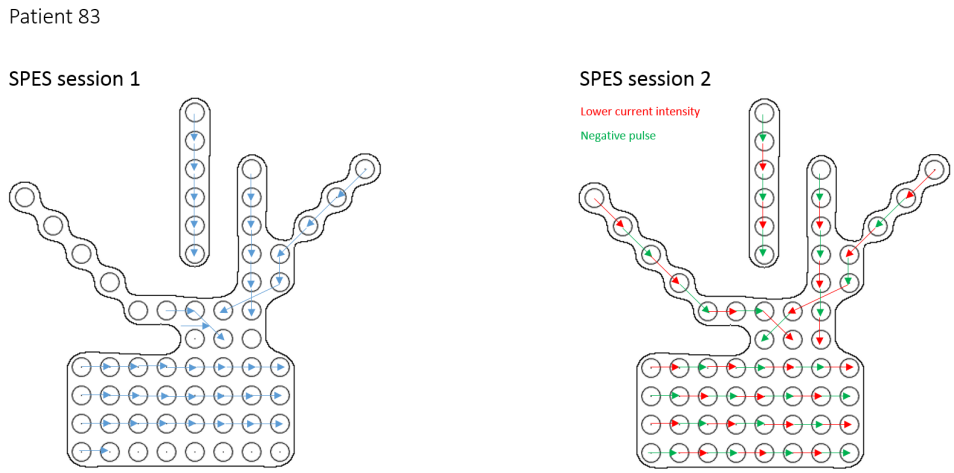


Figure G.2: *SPES session 1 and 2 in patient 83*

Patient 88

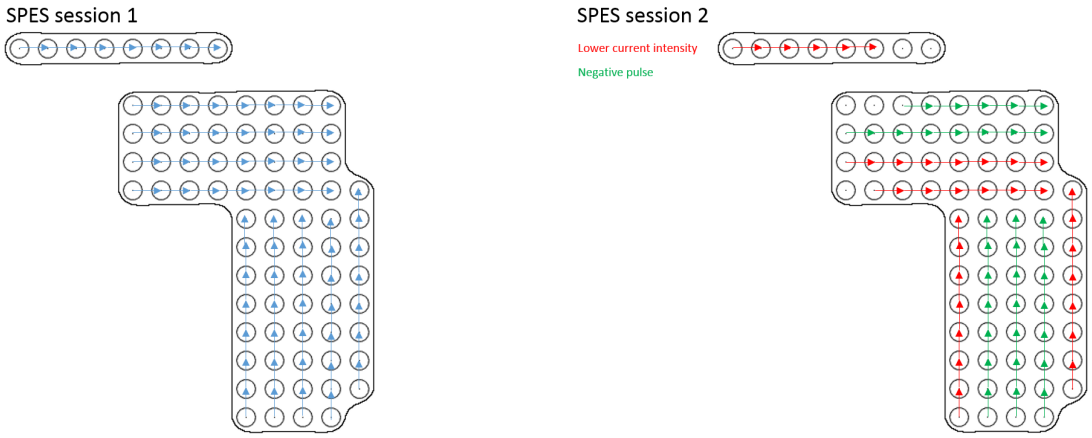


Figure G.3: *SPES session 1 and 2 in patient 88*

Patient 89

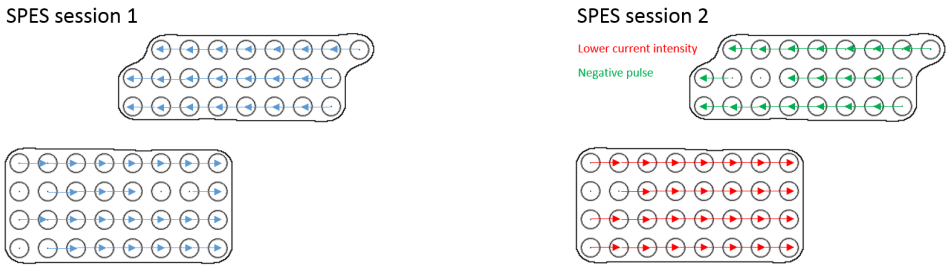


Figure G.4: *SPES session 1 and 2 in patient 89*

Patient 91

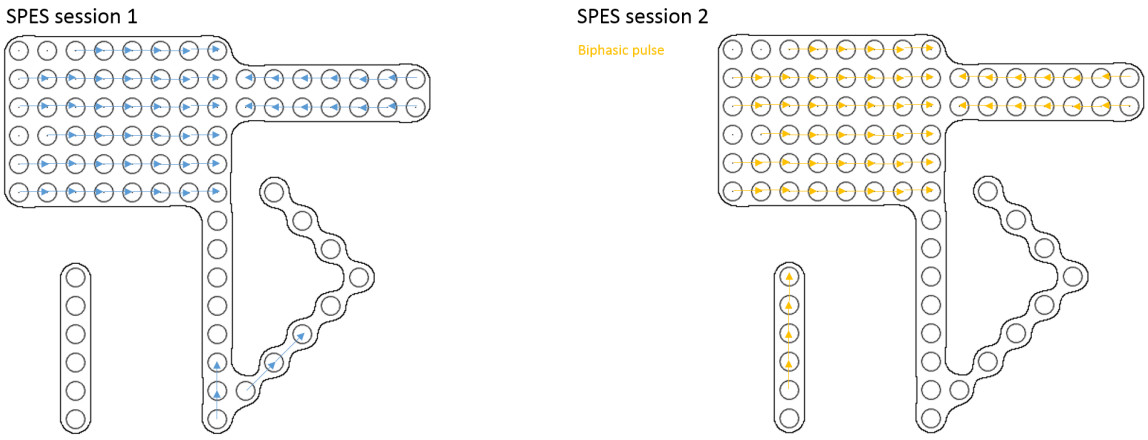


Figure G.5: *SPES session 1 and 2 in patient 91*

Patient 93

Figure G.6: *SPES session 1 and 2 in patient 93*

Patient 96

Figure G.7: *SPES session 1 and 2 in patient 96*

Patient 97

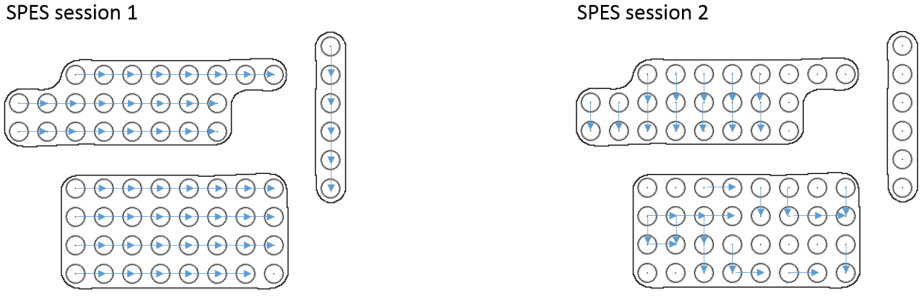


Figure G.8: *SPES session 1 and 2 in patient 97*

Appendix H

Abstracts

H.1 The spatial relation between early and delayed responses evoked by single pulse electrical stimulation in pre-surgical evaluation of epilepsy patients

D. van Blooijs[1,2], G.J.M. Huiskamp[2], M.A. van 't Klooster[2], H.G.E. Meijer[1], S.A. van Gils[1], G.J. Hebbink[1,2], P.H. Gosselaar[2], F.S.S. Leijten[2]

1. MIRA, University of Twente, Enschede, the Netherlands

2. Brain Center Rudolf Magnus, University Medical Center Utrecht, the Netherlands

Purpose: Single pulse electrical stimulation (SPES) assists delineating epileptogenic tissue during long-term intracranial monitoring for pre-surgical evaluation. The SPES protocol consists of systematic stimulation of neighbouring electrode pairs and analysis of responses in all other electrodes. SPES evokes early (ER) and delayed (DR) responses. DRs occur in epileptogenic tissue, but are stochastic: 10 stimuli are needed to reliably establish a response. ERs occur in healthy and diseased tissue and require only a single stimulus. We studied the spatial relation between ERs and DRs. If it is possible to predict DRs from ERs, this might improve the efficiency of SPES.

Method: Data of one patient with TLE recorded in 84 grid electrodes were used. We counted per electrode the number of stimulus pairs that evoked ERs (eER) and per stimulus pair the number of ERs that were evoked (sER). Similarly, for DRs, eDR and sDR were determined. Also, per electrode we counted the number of stimulus pairs that evoked both ERs and DRs, eERDR and vice versa for sERDR. We selected electrodes with eER or sER > median as eER50 or sER50. We tested whether values for eDR and sDR were significantly more in the eER50 and sER50 groups. Finally we tested whether the ratios eERDR/eDR and sERDR/sDR differed significantly from 0.5.

Results: eER values ranged from 0-41, median=8; sER: 3-27, median=17; eDR: 0-28, median=17 and sDR: 0-26, median=9. eDR and sDR values were higher in eER50 ($p<0.001$) and sER50 ($p=0.1$) electrodes. The ratios of eERDR/eDR and sERDR/sDR ranged from 0-1, median=0.24 and 0-0.64, median=0.25 respectively, with the average significantly lower than 0.5 ($p<0.001$).

Conclusion: The correlation between eER50 electrodes and high values of eDR indicates that ERs and DRs seem to occur within the same network. The low ratio eERDR/eDR suggests that ERs and DRs are not directly related. We will analyse more patients to confirm these findings.

This abstract was submitted to the IEC2015 in Istanbul. A poster is recently presented during the congress.

H.2 From Single Pulse Electrical Stimulation data to a computational network model

van Blooijs D.[1,2], *Hebbink G.J.*[1,2], *Huiskamp G.J.M.*[2], *van Gils S.A.*[1], *Leijten F.S.S.*[2], *Meijer H.G.E.*[1]

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In patients with refractory epilepsy, Single Pulse Electrical Stimulation (SPES) assists in delineation of the seizure onset zone during long-term intracranial EEG monitoring for pre-surgical evaluation. Prior to intracranial EEG monitoring, a subdural electrode grid is implanted. During SPES, brief electrical stimulations are applied between two adjacent electrodes of this grid. This evokes early responses (ER) and delayed responses (DR) in the other electrodes. Appearance of DRs correlates with epileptogenic tissue, while ERs are considered to have a physiological origin. In this study we consider ERs to construct a patient-specific network.

Detection of ERs in SPES data is often done visually, which is a laborious task. We develop a systematic method to detect ERs in the time domain automatically. This method detects an ER in an electrode if the extremum of the activity observed in a short interval after the stimulation is larger than six times the standard deviation of the baseline activity just before stimulation.

Next, we build a network model of coupled nodes where the connectivity is based on the detected ERs. The dynamics of each node represents the activity of a large population of neurons. We simulate this model and based on the seizures seen in this simulation we try to determine the seizure onset zone. We test the removal of this seizure onset zone in the model. We illustrate this process from data to model with an example.

Our method provides a consistent and fast way to detect ERs in SPES data. We used ERs to build networks of coupled neural masses. Simulations of these networks can sometimes delineate the seizure onset zone. However, future refinements in the model are needed to improve the prediction.

This abstract was submitted to the BACI2015 in Utrecht. A poster is recently presented during the congress.