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Quantification of the TMS-EEG response in epilepsy

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To my parents.

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"Efforts and courage are not enough without purpose and direction."

- John F. Kennedy

Abstract

Purpose: The purpose of this thesis was to provide quantitative measures of the co-registration of transcranial magnetic stimulation (TMS) and electroencephalogram (EEG). The EEG is used to study changes in the neuronal activity evoked by the non-invasive technique TMS. These effects are determined mainly based on clinical judgment. Current uses in the diagnosis of epilepsy are based only on EEG, not taking into consideration the low sensitivity in the interictal period, in particular if routine recordings are used.
Methods: Patient data was gathered, analyzed and compared to healthy controls. A total of ten patients and eighteen healthy subjects underwent sessions of 75 TMS pulses. The responses to the pulses were filtered and averaged. The use of topographical scalp plots of amplitude and power, and time-series analysis of power in search for late responses provide results which enable separation of epilepsy patients and healthy controls. By investigating the significance of the results it is also possible to determine, in a quantitative way how reliable the methods are for distinguishing between the two groups.
Results: The definition of what is a response is critical in this project, and as such must consider: significant power change, be above a certain amplitude, and be localized. Still, this procedure results in a non distinguishable threshold to separate both groups.
Conclusions: Analysis of the receiver operating characteristic (ROC) curves also led to the understanding the method established is not entirely reliable because it cannot in fact determine differences. Since all patients were under treatment with anti-epileptic drugs (AEDs), it becomes necessary to elaborate a pilot study with recently diagnosed subjects where hyperexcitability is still present.

Keywords: Epilepsy, quantification, TMS-EEG, power, topography, amplitude

Resumo

Objectivo: O objectivo deste trabalho contempla fornecer medidas quantitativas do registo simultâneo da estimulação magnética transcraniana (TMS, do inglês *transcranial magnetic stimulation*) e do electroencefalograma (EEG). O EEG é utilizado para estudar alterações na actividade neuronal evocada pela técnica não-invasiva TMS. Estes efeitos são determinados predominantemente com base na avaliação clínica. A utlização corrente no diagnóstico da epilepsia é baseada apenas no EEG, não tendo em consideração a sua baixa sensibilidade no período interictal, especialmente em procedimentos de rotina.
Métodos: A informação de pacientes foi recolhida, analisada e comparada a controlos saudáveis. O total de dez pacientes e dezoito pessoas saudáveis foram sujeitos a sessões de 75 pulsos a cujas respostas foram aplicados filtros e obtida a sua média. A utilização da representação topográfica do escalpe em amplitude ou potência, e a análise em tempo da potência, na procura de respostas tardias, providenciam resultados que permitem a separação entre pacientes epilépticos e controlos saudáveis. Ao investigar a significância dos resultados é também possível determinar, de uma forma quantitativa, o quanto os métodos são fiáveis para distinguir entre os dois grupos.
Resultados: Em que consiste uma resposta é uma definição crítica para este projecto, e para tal é necessário considerar: alterações de potência significativas, ser acima de uma certa amplitude, e ser localizada. Este procedimento leva a um nível de separação pouco distinto entre os dois grupos.
Conclusões: A análise das curvas ROC (do inglês *receiver operating characteristic*) também conduz a uma compreensão de que o método estabelecido não é inteiramente fiável uma vez que não consegue determinar diferenças. Visto que todos os pacientes estão sujeitos a tratamento com medicamentos anti-epilépticos (AEDs do inglês *anti-epileptic drugs*), torna-se necessário elaborar um estudo piloto com indivíduos recentemente diagnosticados e onde a hiperexcitibilidade ainda se encontra presente.

Palavras-chave: Epilepsia, quantificação, TMS-EEG, potência, topografia, amplitude

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List of Abbreviations

AD	Analog-to-digital
ADM	Abductor digiti minimi
AED	Anti-epileptic drug
C	Central
EEG	Electroencephalography
EMG	Electromyography
EOG	Electro-oculogram
EP	Evoked potential
ERP	Event related potential
F	Frontal
Fp	Frontopolar
FT	Fourier transform
GABA	Gamma-aminobutyric acid
GMFA	Global mean-field amplitude
HFO	High-frequency oscillation
IED	Interictal epileptiform discharge
MCL	Motor cortex left
MCR	Motor cortex right
MEP	Motor evoked potential
MRI	Magnetic Resonance Imaging
MT	Motor threshold
NIRS	Near-Infrared Spectroscopy

O	Occipital
P	Parietal
PCA	Principal component analysis
PET	Positron Emission Tomography
RMS	Root mean square
ROC	Receiver operating characteristic
SOZ	Seizure onset zone
SPECT	Single-Photon Emission Computed Tomography
SREDA	Subclinical rhythmic electrographic discharges of adults
TEP	TMS evoked potential
TMS	Transcranial Magnetic Stimulation
TMS-EEG	Combined use of TMS and multi-channel EEG
WHO	World Health Organization

1

Introduction

1.1 Motivation

Since ancient times, epilepsy has been associated with evil and religious entities. For centuries it has been surrounded by fear and discrimination. Even though there is still some social stigma in certain regions, today, epilepsy is viewed as a neurological disturbance where a high number of nervous cells are excited simultaneously during a seizure. Epilepsy consists of more than seizures for the affected individual, especially because it leads to many interacting psychological, medical, economic, and social repercussions.

Given the current digital advances, it is surprising that major breakthroughs in the clinical use of quantitative electroencephalographic (EEG) analysis are somewhat limited. This situation contrasts with advances in, for example, neuroradiology, where digital signal analysis has greatly influenced imaging techniques. Along with the long learning curves associated with the visual interpretation of the EEG in a clinical environment, there can be several inter- and intra-observer inconsistencies. Furthermore, qualitative information may not always be suited to communicate particular features. Elements of spatio-temporal dynamics are often difficult to translate into the language domain. For this reason, it is necessary to develop alternative presentations which may assist the interpretation.

EEG recordings simultaneously with the application of transcranial magnetic stimulation (TMS) is a new non-invasive tool, which could substantially improve the diagnosis of focal and generalized epilepsy, based on the identification of the hyperexcitable cortex [1]. Most of the potential of this technique is still only hinted and its clinical applications can only be fully explored through research. The main advantage of combining TMS with EEG is the possibility of studying cortical excitability [1] and functional connectivity with high spatio-temporal specificity and enabling the assessment of cortical reactivity with excellent sensitivity [2]. Several studies [3–10] have been undertaken, with only two main objectives. One has been to describe the nature of the TMS-evoked potentials (TEP), so that it becomes possible to understand

the activation mechanisms of TMS. The second objective is to confirm the potential applications of the combined use of TMS and multi-channel EEG (TMS-EEG) as a tool for neurophysiological research and diagnostic purposes.

Functional brain mapping methods such as EEG, functional magnetic resonance imaging (MRI) and positron emission tomography (PET) have, so far, permitted the non-invasive investigation of the functional organization of the human brain by providing maps of the distribution of activity [1, 11]. However, functional MRI and PET have also made it difficult to investigate the dynamical connectivity between neurons in the brain and are of little use in determining cortico-cortical connections [6, 11] because of their low temporal resolution. For these reasons, they would not be adequate for the quantitative study of epilepsy. Using TMS with the mentioned neuroimaging methods expands the applicability of TMS to the study of cortical reactivity and connectivity. The temporal resolution shown by these methods does not permit the establishment of the time course activation of the stimulated area and remote sites [5]. EEG, however, has a very good temporal resolution (in the order of milliseconds), that combined with TMS could provide new information concerning diagnosis and therapies. TMS is different from other *in vivo* methods that show the function of the human brain because instead of observing the brain in operation, neurons are actually triggered into action [1].

Responses to TMS-EEG can be defined as early and late responses. Early responses usually include most, if not all, of the TEP. An epoch surrounding a TMS pulse is defined from one second before to one second after the pulse. A study by Valentín *et al.* [12] identified late TMS-EEG responses in 73% of epilepsy patients (11 out of 15), whilst in 100% of healthy subjects there was no such response. The late response period defined in this study is from 100 to 1000 ms. The finding suggests that late responses are abnormal responses of the epileptic cortex to the TMS. This might indicate the existence of a hyper-excitable cortex under the stimulated area. The sample size was small but these preliminary results introduce the possibility of more certain and earlier diagnosis using the combination of EEG and TMS.

1.2 Objective

The objective of this project is to provide quantitative measures of the co-registration of TMS and EEG activity mapping that currently exists. To achieve this aim, signals will be processed and the tools which analyze and extract information from these signals will be developed. The attention is thus drawn to the EEG signals obtained after TMS. Through the use of topographical plots to evaluate potentials and determination of power spectrum, the amount of information from the collected data is reduced. The techniques developed and applied were used to create a clearer understanding and provide significant information regarding differences between epilepsy patients and healthy subjects. Individually or combined, they were applied to the signals collected. An analysis to evaluate the quality of these results was also done.

When any new medical tool is developed it is important to understand what the new technique offers differently from the methods that already exist in terms of diagnostics, prognostics and therapeutics in clinical practice. Benefits should include: the establishment of an earlier differential diagnosis or determining such diagnosis with greater certainty for a given clinical presentation; better prediction of the likely course of development of the condition; assistance in identifying the most suitable treatment strategy; or even improvement of the clinical outcome when used as a therapy [13].

1.3 Thesis Overview

The study evaluates the combined use of TMS-EEG in the development of quantitative tools for the diagnosis of epilepsy. From the data processing and analysis of eighteen healthy subjects and ten patients with epilepsy, there was an assessment of the occurrence and significance of the TMS-evoked responses. The implemented algorithms were developed using MATLAB, a high-performance interactive software ideal for scientific and engineering computation.

This work is divided into a total of five chapters. In this first chapter the thesis context is exposed, thus giving some insight on the objectives which led to the development of this project and the motivation behind it. In chapter 2 there is an introduction to the theoretical concepts concerning the following topics: epilepsy, EEG, TMS, and the combination of TMS-EEG, with some information on what has been done in these fields. This will provide a better understanding of the overall place were this project will fit into. Chapter 3 focuses on the collection of data from patients and healthy subjects and the methods developed for signal processing and how they can be used for quantification. Techniques include topographical plots for the evaluation of amplitude and power, as well as time analysis of power. In chapter 4 the results are presented with examples of how the methods developed in chapter 3 can quantify the data obtained. A follow-up discussion is presented, which examines how quantification is performed and how the methods compare, in efficiency and value. The last chapter contains the conclusions that can be derived from this project as well as any future developments and recommendations.

2

Theoretical framework

This chapter provides a framework for the main topic of this thesis. Some insights are given regarding the condition of epilepsy and some of its associated mechanisms. In regards to the techniques of EEG, TMS, and the combination of TMS and EEG, more detail is given on how they have evolved and how they are applied today. More focus is given to the co-registration of TMS and EEG due to its importance in the application of the quantification tools that were developed during this project.

2.1 Epilepsy

Rhythmic activity is a fundamental property of neural elements. Its organization is in the form of complex patterns which depend on the state of the brain and on the task that is being executed. Synchronization of oscillations across neuronal elements, either locally or over longer distances, is one of the organizing principles of rhythmic activity [14]. Prevailing rhythmicity and organization could be a sign of abnormality, and disorganized oscillations do not necessarily imply abnormality [15]. Brain oscillations have a range from 0.05 to 600 Hz, where fast wave activity is associated with the awake state and slower oscillations with sleep [16]. This demonstrates that oscillatory activity in distinct frequency bands has been related to specific functions.

The word epilepsy derives from the Greek word *epilambanein*, that could be defined as "to be seized or overwhelmed by surprise". Epilepsy is one of the most common, serious neurological conditions, with a prevalence rate ten times higher than that of multiple sclerosis and 100 times higher than motor neuron disease. In most developed countries, the number of new individuals with epilepsy (incidence) is of 50-70 cases per 100 000 people per year. The number of all individuals affected (prevalence) by epilepsy is five to ten cases per 1000 people, while lifetime prevalence is about five per cent [17]. Epilepsy accounts for 1% of the global burden of disease, where 80% can be found in the developing world (see figure 2.1). In some areas 80 to 90% of people with epilepsy receive no treatment [18], according to the World Health Organization (WHO). Incidence is greatly influenced by the factor of age, with high rates in early childhood and another

peak at the age of 65 and older [17].

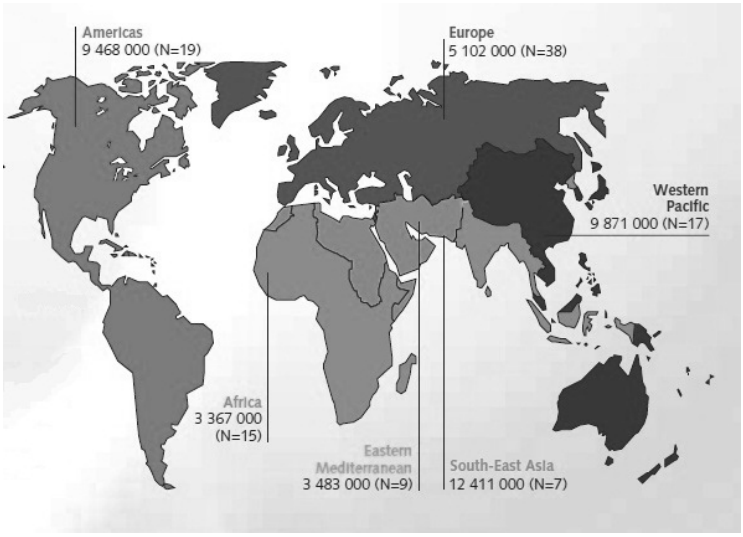


Figure 2.1: Number of people with epilepsy in WHO regions, where N is the number of responding countries in each area. The numbers (N=105) are only based on information provided by respondents to WHO's Atlas. These were not corrected for those countries that did not respond [18].

The pathophysiological basis for human epilepsy is thought to be a cortical imbalance between inhibitory and excitatory mechanisms involving increased, hyper-synchronous and autonomous activity [12, 19]. In other words, it is a short-lasting occurrence of signs due to the abnormal synchronization of neuronal activity in the brain. Brain cells produce electrical discharges through the use of chemical interactions. Each cell either excites or inhibits other brain cells with its discharges. When the balance shifts too much in the direction of excitation, then a possible outcome is a seizure. Hughlings Jackson, more than half a century before the discovery of the human EEG, defined epilepsy as an "occasional sudden, excessive rapid and local discharges of grey matter" [19].

In fact, epilepsy is known as a seizure disorder, classifying the seizure as the event and epilepsy as the disorder. The diagnosis is usually made after a person has had at least two spontaneous seizures [20] and if the brain has an increased tendency to generate seizures [21]. These seizures may be related to brain injuries or family tendency, also known as symptomatic epilepsy; but often (six out of ten) the cause is unknown, i.e. idiopathic epilepsy.

2.1.1 Types of Seizures

Seizures are, by convention, divided into two basic types: generalized and partial/focal. This division is based on both clinical and electrophysiological terms. The type of seizure depends on several factors, such as where the abnormal electrical discharge occurs in the brain. The temporal lobe is an area prone to generate seizures. Parts of the brain most commonly involved in adult epilepsy, such as the amygdala and the hippocampus, are found in the temporal lobe. Physiological events can range from disruption of the elementary functions of the brain region involved in a well localized discharge; alteration of consciousness and behavior in more widespread discharges [19].

Generalized seizures demonstrate a sudden widespread disturbance of cerebral activity and consciousness will be lost immediately. Depending on the seizure type,

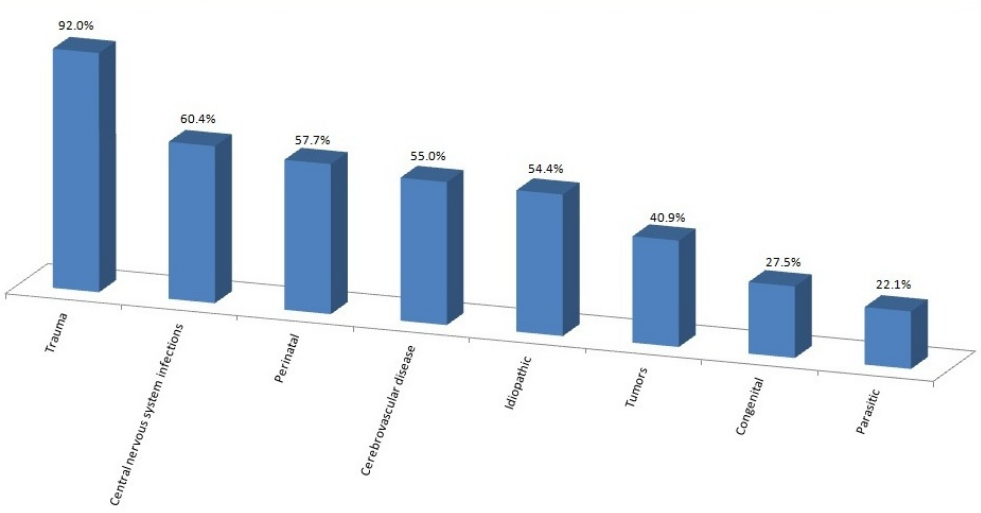


Figure 2.2: Most frequently reported causes of epilepsy, as reported by countries part of WHO (N=149). Adapted from WHO's Atlas: Epilepsy Care in the World [18].

the discharges and clinical manifestations may cease in the same abrupt way that they began or there may be a more or less prolonged post-ictal disturbance accompanied by unconsciousness or confusion. In patients with generalized seizures, the discharges are themselves bilateral, symmetrical, and synchronous. They show greater amplitude in the frontal regions but sometimes posteriorly as well [19].

Partial seizures have a localized onset. In this case, consciousness is usually preserved and clinical manifestations are limited to one disturbance, such as, for example, involuntary movements of the arm. The seizure does not have to remain localized, which means involved neurons can recruit neighboring neurons. If, in this process, consciousness becomes reduced, then these seizures are known as complex partial seizures. The activity can spread even more, which will lead to partial seizures with secondary generalization, where consciousness is rarely present [21]. Patients with partial seizures have abnormalities with a topography that corresponds very closely to where the seizure arose [19]. In many patients, the area responsible for seizure generation, or the seizure onset zone (SOZ), can be difficult to specify. Non-agreeing clinical and laboratory studies in patients with identifiable lesions on brain MRI will often indicate poor localization of the SOZ [22].

If an abnormal electrical discharge originates in the motor cortex, the patient will experience a motor seizure; if it takes place in the sensory cortex, it will be a sensory perception; if it happens in the visual cortex, there will be lights, flashes, or jagged lines [20]. If a seizure occurs in the deep temporal lobe there will be a loss of memory or awareness and stop of all activities. The spreading of a seizure to all regions of the brain leads to a tonic-clonic seizure accompanied by loss of consciousness, stiffening and jerking. When persistent seizure activity is present and consciousness (in the case of generalized seizures) is absent for more than 30 minutes, then patients present a *status epilepticus* [21].

In the interval between seizures it is common to find abnormal wave-like representations. These interictal spikes can be used as electrophysiological biomarkers for the epileptogenic zone, instead of waiting for the spontaneous occurrence of seizures [22, 23]. An epileptogenic zone is defined as the area in the cortex responsible for generating seizures in epileptic patients [12].

Interictal discharges usually occur singly or sporadically. The brain generates its normal rhythms and only now and then interictal epileptiform discharges, limiting the sensitivity of a routine EEG recording. These measurements can be made by surface or intracranial recording; and in short-term (under 30 minutes) or long-term monitoring. The time interval between discharges can vary from minutes to days, which is why in a 20 minute EEG recording, the interictal events may not be present [21, 24]. Intracranial recording is not routinely used due to its invasive nature. A more in-depth analysis of the use of the EEG in the diagnosis of epilepsy can be found in subsection 2.2.3.

2.1.2 Treatment

The goal of treatment is to reduce the likelihood of seizures, ideally to a level where it can be compared to the general population. Options include: treatment with medication - anti-epileptic drugs (AEDs); neurostimulation; and treatment by resection of the epileptic focus [21]. The choice that is made depends on several factors such as the number and severity of seizures that the patient would experience without treatment, the underlying cause, and the age.

Treatment for epilepsy is available and in a majority of cases it can guarantee a normal life. Success of treatment depends on a variety of factors, such as type of seizure, how early the diagnostic is made, the efficacy of medication, compliance with medication, the existence of other associated lesions, and social professional problems. Some epilepsies in children heal always, other types almost always, and only some need permanent anti-epileptic medication. In general, 70 % of patients are free of seizure fifteen years after the beginning of medication [25].

For about 70% of the patients, treatment with medication is suitable; however, for the remaining patients, seizures are not well controlled. In the latter group, if the seizures are focal, with a well defined cortical generator, surgery can often be performed [25].

2.2 Electroencephalography

The outer surface of the cerebral hemispheres, the cerebral cortex, contains neurons (grey matter) and is separated into regions by fissures (sulci). Underneath the cortex are the nerve fibers which connect to other parts of the brain and the body (white matter). Cortical potentials originate from the excitatory and inhibitory post-synaptic potentials generated by cell bodies and dendrites of pyramidal neurons. The scalp EEG represents an average of the electrical activity of a small area in the cortical surface underneath an electrode.

In the brain there are two main classes of cells: neurons and glial cells. In these cells, the resting potential is approximately -70 mV. This difference in potential across the cell membrane comes from the inequality of concentration of cations (potassium and sodium), anions (chlorine), and large organic anions, in combination with a semipermeable membrane. Such a condition is maintained by the active transports of cations, using the energy supplied through metabolic processes. The electric activity of neurons is demonstrated by the generation of action potentials and post-synaptic potentials. Currents are generated along the cell membrane in the intra- and extracellular spaces, producing an electric field that can be compared to a dipole. To observe this electric field, there needs to be a synchronization of electric activity by a large number of dipoles oriented in parallel. The EEG signal comes from the sum of synchronously generated post-synaptic potentials [26]. However, the major contribution to the EEG

signal comes form the pyramidal cells. Pyramidal cells make up 70 to 80% of all cortical neurons and their dendrites with the synaptic inputs are perpendicular to the cortex surface. The current dipoles created in individual pyramidal cells are too small to result in a reliably measured electrical signal at the scalp. Synchronous excitatory and inhibitory post-synaptic potentials can arise from the activity synchronization done by the cortical pyramidal cells.

The amplitudes of the scalp EEG range between 10 and 100 µV. The frequency range of the EEG (scalp and intracranial) has diffuse lower and upper limits (about 0.05 to 600 Hz). Due to the fact that there are ultra-fast and ultra-slow frequency components that seem to play no significant role in the clinical EEG, the frequency response curve of an EEG concentrates on the clinically relevant range (0.5 to 35 Hz) [15]. Ultra-slow oscillations may reflect slow cortical potentials that may occur during spreading depression. The predominant frequencies can be divided into the bands seen in table 2.1 with the corresponding wave oscillations in figure 2.3.

Table 2.1: Common definition of frequency bands in the EEG.

Band	Frequency
Delta	Below 4 Hz
Theta	4-8 Hz
Alpha	8-13 Hz
Beta	13-30 Hz
Gamma	Above 30 Hz

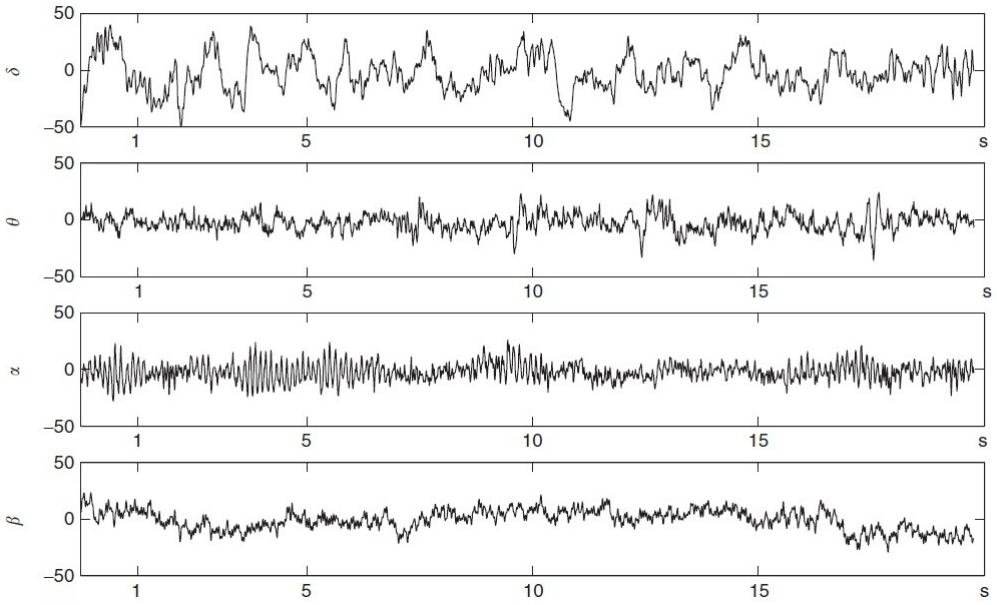


Figure 2.3: Characteristic EEG rhythms - delta, theta, alpha, and beta. As defined in table 2.1. Adapted from Blinowska and Durka [27].

The spontaneous awake EEG indicates that different regions of the human brain tend to work in electrical oscillations at different frequencies. The rhythms are rather variable and their topography can change radically in a second if, for example, the eyes are opened. This means that it becomes quite difficult to interpret the spontaneous EEG as to whether the different cortical circuits are intrinsically dependent on oscillations in specific frequencies [15].

2.2.1 Historical Background

The history of electroencephalography and epilepsy are closely related. Considering descriptions of seizures in ancient literature such as in Akkadian (oldest written language), ancient Egyptian, Indian, and Chinese; epilepsy can be said to be as old as mankind. The first book on epilepsy was "The Sacred Disease", where a large portion of text was written by a number of physicians of the Hippocratic School 2400 years ago. It initially was suggested that epilepsy was due to divine influences or magic [28].

Luigi Galvani is the founder of the field of electrophysiology. With the publishing of his discovery of animal electricity, based on the experiment in figure 2.4, he paved the way to understanding epilepsy. His concept of animal electricity - electricity was generated in the body and channeled through nerves - went against the beliefs of contemporary physicist Volta. Volta stated that electricity was generated by plates of different metals. The acceptance of Galvani's ideas suffered a delay of about three decades due to the dominance of the scientific area by Volta. With the publishing of a book by Du-Bois Reymond, which included an illustrated registration of muscle potential from surface recordings, there was renewed interest in Galvani's work [28]. With this came the establishment of the basis of clinical electromyography (EMG). As Galvani supposed in the 18th century, animal electricity exists in a state of disequilibrium, and it is, therefore, ready to move in response to any internal stimuli or following external influences [29].

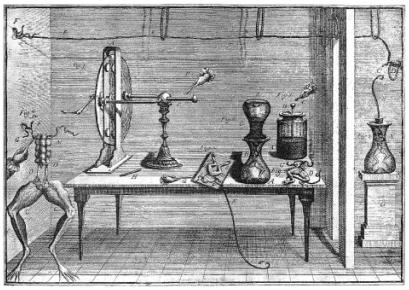


Figure 2.4: Galvani's experiment that pioneered the subject of electrophysiology. This involved the study of muscular contraction in a frog by touching its nerves with electrostatically charged metal [29].

The electrical activity of the brain, through the intact skull, was actually only first measured in 1923 by Hans Berger. At the time, this measurement was a major accomplishment because there were no modern operational amplifiers available, with their high input impedance. Bioelectric signals were usually measured with a string galvanometer. Nowadays, EEGs are recorded with digital equipment, using high-impedance and low-noise amplifiers. The digital recording offers, among others, the possibility of subsequent signal analysis such as filtering [21].

2.2.2 Technical Aspects

When measuring a biological electrical process that is based on the flow of ions, the ionic currents are converted into electronic currents. At the skin-electrolyte-metal interface, originated by the silver/silver-chloride electrodes and a conducting gel, the conversion of ionic to electronic currents takes place.

Each differential amplifier, necessary to calculate the electric potential, has two inputs, and an electrode is connected to one of the inputs. In the other input is an "inactive"

reference electrode or the average signal of all other electrodes [21]. The former is known as common reference and the latter as common average reference.

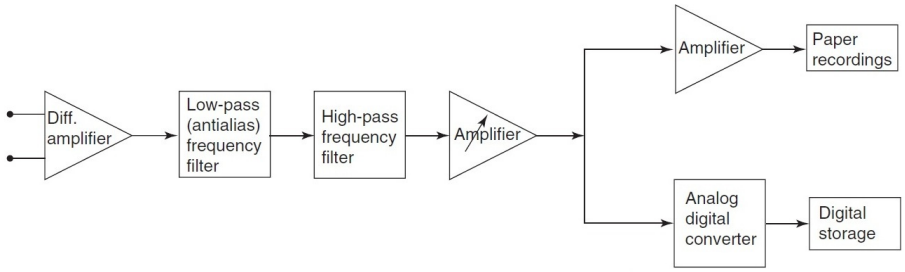


Figure 2.5: Diagram of recording a single EEG channel. The differential amplifier measures the potential between two electrodes, where one of them is treated as the reference. The second amplifier prepares the signal for AD conversion and storage (lower path). Before the development to digital, EEG was stored on folded paper (upper path). Adapted from Blinowska and Durka [27].

In the process of recording EEG data, by measuring voltage differences between the two inputs, the resulting signal will be amplified (typically 60 to 100 dB voltage gain) and displayed as one channel of EEG activity. The amplified signal will be digitalized via a analog-to-digital (AD) converter, as seen in figure 2.5. Sampling rates range from 256 to 512 Hz in a clinical setting and up to 20 kHz in research applications.

2.2.3 EEG in Epilepsy

The potential for the EEG to identify specific interictal and ictal patterns was first demonstrated by Gibbs *et al.* [30] in 1935. In the present-day, the scalp electroencephalogram is the most widely accepted test for the diagnosis of epilepsy [12]. Technology has advanced, especially with the introduction of multichannel recordings, prolonged ambulatory records, spectral analysis, video telemetry, and semi-automated analysis of epileptiform activity [31]. However, the EEG has relatively low sensitivity in the interictal period, not showing clear epileptiform abnormalities in 45% of awake EEGs and 20% of sleep EEGs of patients with epilepsy [12, 21]. A vast majority of patients do not have seizures during the somewhat brief EEG recordings, making it difficult to reach a conclusive diagnosis. Also, a physician rarely has the opportunity to observe a patient's seizure directly, which means that the interictal EEG alterations must suffice in terms of confirmation of the diagnosis of epilepsy but also in classifying the seizure type [24].

The EEG is a procedure ideally performed on all patients with suspected seizure disorders. An example of an EEG that records an epilepsy seizure onset can be found in figure 2.6. The EEG may be normal in a wide number of patients with epilepsy, however, with repeated EEG or long-term recordings, the chance of recording interictal epileptiform discharges (IEDs) will increase due to the enhancement of the diagnostic sensitivity of the EEG [33]. System perturbation techniques such as sleep deprivation, hyperventilation (breathing at 20 respirations per minute for two to four minutes), photic stimulation (with 1 to 50 flashes of light per second), and auditory stimulation (with loud clicks) [24, 34] can be used to evoke specific epileptiform patterns. Epileptiform discharges are sudden bursts in EEG waveforms, acting as a signature for abnormal synchronization of neuronal populations [21]. It is important to note that the interictal EEG should not be used alone to confirm an epilepsy diagnosis; clinical correlation and neurological history need to be taken into account [24].

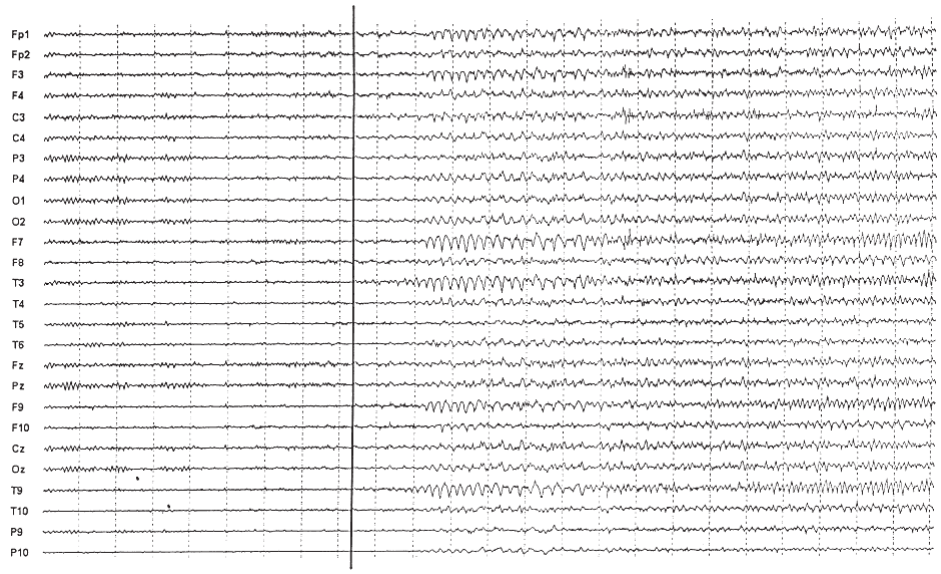


Figure 2.6: 24 seconds of EEG around an epilepsy seizure onset for a patient of a study conducted by Lantz *et al.* [32]. The vertical bar indicates a visually estimated seizure onset.

The specificity and sensitivity of the EEG in patients with epilepsy depend on the type of seizure disorder and the localization of the epileptogenic zone. There can also be attenuation of spike activity by the dura, the bone, and the scalp, therefore altering the diagnostic outcome of the EEG [24]. There are several characteristic EEG patterns associated with well-known and well-defined epilepsy syndromes, as can be seen in table 2.2. EEG can therefore assist in defining certain syndromes, which will influence the decision for therapy and assessment of prognosis [33].

Table 2.2: Typical interictal epileptiform discharges found on the EEGs of patients with characteristic epilepsy syndromes or etiologies [33].

EEG pattern	**Epileptic syndrome/etiology**
Anterior temporal spikes	Mesial temporal lobe epilepsy
Generalized 3-Hz spike-wave complexes	Absence epilepsy
>4-Hz spike-wave complexes, generalized polyspikes	Juvenile myoclonic epilepsy
Generalized slow spike-wave complexes	Lennox-Gastaut syndrome
Regional (extratemporal) polyspikes	Focal cortical dysplasia
Hypsarrhythmia	West syndrome

A normal EEG should not be used to exclude a diagnosis of epilepsy because records have shown that patients with epilepsy can have repetitively normal EEG recordings [21, 24, 33]. Epileptiform alterations may be identified in individuals who do not have epilepsy [24]. This is a rare event but it does mean that an abnormal EEG does not always confirm a diagnosis of epilepsy [24, 33]. Non-specific EEG changes such as focal slowing and asymmetries in amplitude and frequency, and non-epileptiform patterns should not be used to make a diagnosis. Also, revisions of treatment should not only be based on EEG recordings, due to the nature of the technique it only provides a brief sample of brain electrical activity, which means it may not be a good predictor of response to therapy [24].

There is also the possibility of a situation where EEG seizure patterns are recorded in clinically asymptomatic patients. These seizures are called sub-clinical seizures and they probably exist due to involvement of only the asymptomatic cortex in the epileptic

seizure discharge or perhaps if the clinical test did not cover the cortical function that is altered during a seizure. The symptomatogenic zone is defined as the area of the cortex which, when activated by epileptiform discharges, produces ictal symptoms. The spreading of the epileptic activity into the symptomatogenic cortex will eventually lead to symptoms [33].

Specific interictal EEG patterns have a high degree of correlation with the presence of epilepsy, which is why about 90% of patients with epilepsy exhibit abnormal discharges during the intervals between seizures [19]. A great majority of patterns include the sharp wave, the spike, and the spike-wave complex [15, 19, 24]. However, patterns may also include benign epileptiform discharges of childhood, slow spike-wave complexes, 3-Hz spike-wave complexes, polyspikes, hypsarrhythmia, seizure pattern, and status pattern [21,33]. These complexes are much briefer than the ictal discharges [24]. A sharp wave is transient and clearly distinguishable from background activity, having a duration of 70 to 200 ms. A spike is essentially the same as a sharp wave but with a duration of only 50 to 70 ms [21]. The spike and the slow wave are topographically distinct even though the details of their distribution are not exactly clear [19]. In a majority of situations, the slow spike-wave complex consists of a slow spike and a slow wave. Some cases (eg. figure 2.7) consist of true spikes (60 ms or less in duration) followed by a slow wave [15]. The spike-slow-wave complex is a pattern with a spike followed by a slow wave, where the spike has a lower amplitude than the slow wave. It is also possible to have a multiple spike-and-slow-wave complex, which is the same as the spike-slow-wave complex but with two or more spikes associated with one or more slow waves [21].

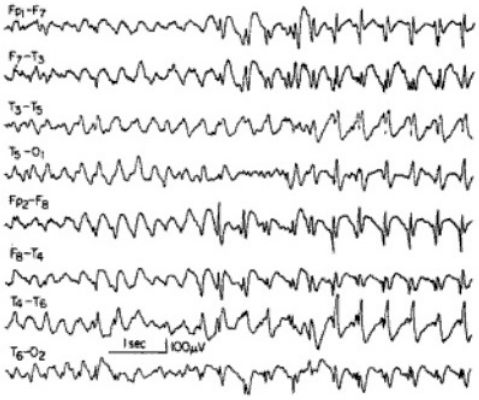


Figure 2.7: An example of generalized slow spike-wave complexes (around 2 s) found in a child with severe epileptic seizure disorder [15].

It is important to keep in mind the possibility of over interpretation, which can lead to a misdiagnosis of epilepsy [35]. In table 2.3 there is a brief summary of several sharp variants, as well as their characteristics, that can easily be mistaken with epileptiform discharges.

The usefulness of the EEG in aiding a diagnosis of seizure disorders is clear; however, what role it plays in monitoring treatment is still uncertain [31]. This is because the EEG may reflect unspecific central nervous side effects of standard AEDs, such as benzodiazepines, phenobarbital, and phenytoin. All standard AEDs can lead to a result in slowing down the dominant rhythm and in increasing the slow activity, while the interictal abnormalities decrease. Intravenous benzodiazepines and phenytoin result in acute seizure control and suppression of IEDs [33].

Table 2.3: Sharp transients representing normal EEG variants, being easily confused with epileptiform discharges [33].

	Frequency (Hz)	**Localization**	**Waveform**	**Level of consciousness**	**Age**	**Duration**
Rhythmical temporal theta	4-7	Temporal	Notched, rhythmic	Relaxed wake sleep stage 1	Young adults	10 s
Wicket spike	6-12	Temporal	Monophasic, similar to μ waves	Wake sleep stage	Adults	0.5 s
Small sharp spike	sporadic (about 50 ms	Frontal maximum	Amplitude <50 μV, duration <50 ms	Relaxed wake, sleep stages 1 and 2	Adults	Single discharges
14- and 6-Hz positive "spikes"	14 and 6	Lateral to posterior temporal	Monophasic	Wake sleep stages 1 and 2	Adolescents, adults	<1 s
6-Hz "spike and wave"	5-7	Generalized	Diphasic, small spike and large wave	Sleep stage 1	Adolescents, adults	<1 s
SREDA	5-6	Generalized	Sudden onset and sudden end	Wake sleep stage 1 / hyperventilation	Elderly	40-80 s

At the moment, EEG interpretation in a clinical setting has been based on visual analysis. This analysis usually includes speculative formulation which serves as a guide for investigating the EEG signal and its various graphic elements [36]. A clinical neurophysiologist, trained in the interpretation of the various EEG rhythms, evaluates the waveforms. This evaluation includes assessing the spatial distribution of various frequencies and the reaction to a variety of stimuli, including eyes opening and closing, hyperventilation, and photic stimulation. These aspects contribute to the mean statistical characteristics of the EEG signal, highlighting their importance as *background pattern*. However, this approach has its drawbacks, such as the long learning curve, the inherent subjective elements, as well as intra- and inter-observer inconsistencies. Due to this, researchers have been motivated into exploring if the computer can assist in extracting relevant EEG features [21, 36]. Yet, it is important to note that the aim is not to replace the classical visual EEG analysis, but quantitative EEG techniques should in fact assist and replace some elements that for now are considered the sole domain of experienced electro-electroencephalographs. The classical visual analysis remains essential for the final interpretation [36].

2.3 Transcranial Magnetic Stimulation

Presently, the clinical value attributed to transcranial magnetic stimulation (TMS) is found in its ability to reveal flaws and miscommunication of the central motor system. However, TMS also holds the potential for sophisticated uses, especially when applied in combination with contemporary neuroimaging techniques [2]. TMS enables the cognitive neuroscientist to manipulate cortical activity in a direct manner and to, consequently, study its influence on behavior [37].

2.3.1 Historical Background

In 1985, Barker *et al.* [38] described a novel method for the direct stimulation of the human motor cortex - TMS. Previously, only electrical stimulation had been used, with applications in the human brain and spinal cord [39]. However, due to the activation of the nerve endings in the scalp, this method was quite painful for the subjects and, in many situations, failed to evoke a response. With the existence of the magnetic field induction of currents in TMS, activation of nerve endings does not occur, thus avoiding pain [40]. Nevertheless, electrical brain stimulation is possible today in a non-invasive form and with less discomfort, using scalp electrodes.

The first description of electromagnetic induction was done by Michael Faraday in 1831 at the Royal Institution of Great Britain. His experiment consisted in winding two coils in an iron ring and showing that when the coil on one side was connected or disconnected from a battery, there was an electrical current passing through to the coil on the other side. When the experiment was repeated, a few weeks later, the same effect was produced but this time with two coils closely positioned in air [41]. In fact, if a pulse of current that passes through a coil placed over a person's head has enough strength and is short enough in duration, there will be a production of rapidly changing magnetic pulses. These pulses penetrate scalp and skull with negligible attenuation, therefore being able to reach the brain [13]. Presently, the stimulating coil acts as the first coil, air is the medium for the magnetic field flow, and the second coil is in fact the electrically conductive living tissue in the area being stimulated [41].

Recordings of experiments related to magnetic stimulation of the brain date back to 1896, when d'Arsonval [42] reported seeing flickering lights in the visual field when he placed his head between two coils with a 110 V supply at 30 A, which involved a direct stimulation of the retina. His report included a description of "phosphenes and vertigo, and in some persons, syncope". In 1959, magnetic nerve stimulation was accomplished by Kolin *et al.* [43] in a frog and then in 1965, Bickford and Fremming [44] demonstrated the stimulation of human facial nerves. Due to the long-lasting activation interval, after using an oscillatory magnetic field that lasted 40 ms, it was impossible to record nerve or muscle activation potentials, leading to the non pursuit of the technique for some time. Using 2-ms-duration pulses, Polson, Barker and Freeston [45], in 1982, recorded, for the first time, motor evoked potentials (MEPs). However, in 1985 came the real success as the group made the first clinical examinations with TMS [38].

Since 1985, there have been major improvements regarding equipment reliability and the development of stimulators with differing output waveforms. Coil design, specifically with multiple windings for precise stimulation of nerves or cortical neurons, has been an area of investment [41]. Devices are usually equipped with figure-of-eight coils, which induce a more focused electrical field in the circular coil [1, 13, 46]. This leads to a better control of the excitation produced by the field, and allows a somewhat detailed mapping of cortical representation [13, 46]. The circular coil induces a more widely distributed electric field which allows bi-hemispheric stimulation, important in the study of central motor conduction [13, 47]. An important development in 1988 was repetitive TMS (rTMS), where sequences of stimuli at 1 to 50 Hz [46] are delivered.

Since the introduction of TMS, its use in clinical neurophysiology, neurology, neuroscience, and psychiatry has spread, even though most of its applications have been in research [13].

2.3.2 Technical Aspects

TMS is a technique that stimulates the human motor cortex in a pain-free, non-invasive and contactless form [37, 38, 48, 49]. A pulsed magnetic field is applied through the use of a coil which is placed above the subject's head. A common measurement is placing the coil over the region of interest in the motor cortex and observing movements in the hand or leg on the opposite side of the stimulation [5,38]. It provides a safe and sensitive measure of both inhibitory and excitatory functions of motor cortical neurons [3].

TMS is defined by the passage of a brief, single, high intensity current pulse in a coil of wire, producing a magnetic field. As the magnetic field penetrates skin and bone it is able to reach the brain with negligible attenuation, and creates an electric field [13, 14, 37, 48]. The physical foundation of TMS can be described by Maxwell's equations. The time-varying current pulse in the stimulation coil will produce a magnetic field, in accordance with the Biot-Savart law. The time-varying magnetic field will induce an electric field, following Faraday's law. This induced electric field will move charges in the direction of its field lines. The coil can be parallel to the surface of the conductor (in this case, the head) or not. Depending on its position, surface charges will appear due to induction or they will accumulate at the conductor surface and in interfaces between tissues with different conductivity, generating a secondary electric field [50].

The total induced electric field inside a conductor (E) is better represented by the general written form of Faraday's law, where the left side of the equation mathematically describes the curl ($\vec{\nabla}$) of the electric field (E) and the right side represents the rate of change of the magnetic field (B) over time.

$$\vec{\nabla} \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t} \tag{2.1}$$

The pulse-generating circuit of the magnetic stimulator produces monophasic or damped sinusoidal (biphasic) current pulses. The decaying current oscillation (I), because of resistive losses in the circuit, obeys the form in equation 2.2.

$$I(t) = \left[\frac{U_0}{L\omega}\right] e^{-(\frac{R}{2L})t} \sin(\omega t) \tag{2.2}$$

where $\omega = \sqrt{\frac{1}{LC} - \frac{R}{2L}^2}$, C is the capacitance, U_0 is the capacitor's initial voltage, L is the inductance of the coil, and R is the common resistance of the components in the circuit represented in figure 2.8.

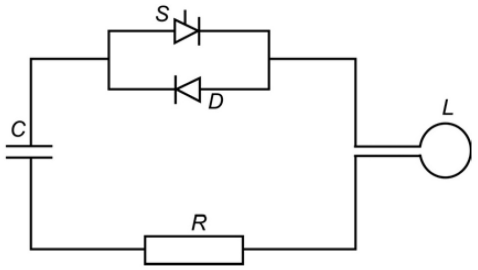


Figure 2.8: A circuit with a sinusoidal current pulse passing through the coil (L). A gate signal from the stimulator opens the thyristor switch (S), thus discharging the high-voltage capacitor (C) through the coil. From then on, the current will flow in the opposite direction through the diode (D), forming the negative half wave of a biphasic pulse. The common resistance of the components in the circuit is represented by R [50].

No contact to the head is necessary; the scalp and the skull have almost no effect on the magnetic field [1]. However, due to the fact that the strength of the magnetic field falls off very rapidly with distance (square of the distance from the stimulating coil) [41], it will only penetrate a few centimeters, meaning that only superficial areas of the brain are most effectively stimulated [14, 50]. The electrical currents will depolarize cell membranes so that voltage-sensitive ion channels are opened and action potentials are initiated [51]. The cortex is activated to a depth of two to three centimeters and with a surface area of several square centimeters, considering the commonly used stimulation intensity and coils (see figure 2.9) [52]. Excited neural structures [48] will then stimulate muscles, peripheral nerves and cortical neurons [14] without requiring surgical access or anesthetic agents [1]. Its uniqueness relies on the fact that it activates all its primary target neurons at the same time [51].

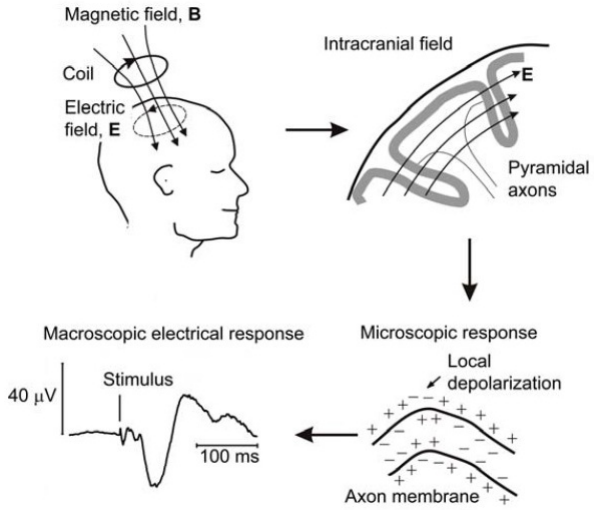


Figure 2.9: Principles and chain of events in TMS. The current pulses in the coil generates a magnetic field *B* that, in turn, induces an electric field *E* that is strongest near the coil. The electric field aligns tangentially to the head surface (closed circles) The pyramidal axons depolarize at their bends, affecting transmembrane potential, and consequently leading to the firing of the neuron. Scalp recorded EEG reflects synchronous activity evoked by TMS [46, 50].

Cortical stimulation can activate, inhibit or interfere in other ways with the activity of cortico-subcortical networks [48]. This will depend on the stimulus frequency and intensity, current polarity, coil orientation, and the configuration of the induced electric field [48, 51].

A magnetic stimulator typically consist of two parts: a high current pulse generator that produces discharge currents of 5000 A or more; and a stimulating coil that produces magnetic pulses with field strengths up to 4 T, and with a pulse duration from 100 μs to 1 ms [41]. During TMS, the operator can control the intensity of the stimuli. This is done by altering the intensity of the current flowing in the coil, which will change the magnitude of the induced magnetic field and of the secondarily induced electrical field [13]. The field intensities found in TMS are lower than or equal to the field of a typical modern MRI scanner. No reason exists to believe that the effect of the magnetic field of a brief pulse would be more harmful than the static one, which itself poses no danger [1].

The coil can be moved until the adequate stimulation site is reached [38]. This is important because the currents generated by TMS and their physiological effects can be modulated by coil construction and positioning, and even by brain conductivity and neuronal orientation. Distribution of field strength and flux is difficult to predict [49]. The overall response amplitudes are highest right underneath the coil, decreasing as the distance from the stimulation point increases [51]. Also, stimulation of the motor cortex with different current directions in the circular coil will yield different responses [53].

There are several parameters that can be measured with the use of TMS, these include motor threshold (MT), motor evoked potential (MEP) amplitude, stimulus-response curve, "phosphene threshold", cortical silent period, intra-cortical inhibition, and intra-cortical facilitation. These are addressed in table 2.4.

Table 2.4: TMS parameters that can be obtained by its use in several forms depending on the objective of the experiment [13, 49, 54]. The physiological significance of measuring these parameters is also shown.

Parameter	Measurement	Physiological significance
Motor threshold	Single pulse: it is the threshold for motor response	Cortical neuronal membrane excitability; corticospinal system threshold excitability
MEP amplitude	Single pulse: average of maximal amplitude	Excitable proportion of neuronal pool
Stimulus-response curve	Single pulse: refers to the increase in peak-to-peak MEP amplitude as a function of TMS intensity	Assesses the neurons that are away from the core region which is activated at MT
"Phosphene threshold"	Single pulse: it is the threshold for visual response	To study the occipital cortex and the visual pathways
Cortical silent period	Single pulse: observation of reduced post-MEP background activity during muscle contraction	Cortical inhibitory mechanisms
Intracortical inhibition	Paired subthreshold conditioning and suprathreshold pulses 2- to 5-ms delay	Possibly GABAergic
Intracortical facilitation	Paired subthreshold conditioning and suprathreshold pulses 7- to 20-ms delay	Uncertain

2.3.2.1 Motor Evoked Potentials

Motor threshold refers to the lowest TMS intensity necessary to evoke MEPs in the target muscle when single pulse stimuli are applied to the motor cortex as mentioned in table 2.4. The MT should relate to the activity of neural inputs into pyramidal cells that will ultimately affect their membrane excitability. This provides an insight into the efficacy of a chain of synapses from pre-synaptic cortical neurons to muscles. There is an alteration in the threshold if a certain disease affecting the pathways from neurons to muscles, is present [13]. EEG activity at low TMS intensities, which means below MT, probably has different distributions than at higher intensities [55].

The generation of MEPs provides important information about the functionality of the central motor and sensory pathways. This is of particular interest when studying diseases of the motor system [56]. MEP amplitudes vary significantly between responses triggered by identical consecutive stimuli. Suggestions as to why this variability occurs include the fluctuations in cortical and spinal motor neuron excitability [57], as well as motor neuron response desynchronization [58].

Selecting targets for TMS according to anatomical brain structures in different subjects does not always lead to the stimulation of the same area. This is due to the inter-individual differences in structure-function relationships, in other words, between brain anatomy and functional architecture [51]. The variable amplitude of the muscle response to TMS of the motor cortex is due to the easiness in producing large MEPs in some healthy subjects. In others, the cortico-muscular pathways can be barely excitable, therefore producing low MEPs. It is, therefore, important to keep in mind that differences found among healthy subjects and patients are important sources of data.

Disparities between individuals include age, genetic factors, physiological differences associated with behavior, and other traits. Intra-individual variances are strongly influenced by time and external factors [59]. Experimental groups should attempt a construction as balanced as possible in sex and demographic factors, such as age and education. Thus, the different coil positioning approaches do not necessarily imply a huge qualitative difference in the TMS-induced effect, but in the magnitude of the respective effect size [60]. Responses therefore depend on the exact coil location and orientation, on the state of the cortex and on the state of vigilance of the subject [8,61,62].

2.3.3 TMS in Epilepsy

TMS delivered at different levels of the motor system can provide information regarding cortical excitability; the functional integrity and efficacy of area-to-area neuronal connections [1]; the conduction along corticospinal, corticonuclear, and callosal fibres; the function of nerve roots and peripheral motor pathway to the muscles [13]. It is also possible to perturb on-going neuronal signal processing in the brain with the purpose of finding cortical areas that are important for specific tasks. With this it becomes possible to treat patients using repetitive stimulation by targeting specific cortical areas [1]. This can help localize the level of a particular lesion within the nervous system or even to predict the functional motor outcome after an injury. An important aspect is the fact that the abnormalities revealed by TMS are not disease-specific, so results should be interpreted with other clinical data. Some findings can be useful for an early diagnosis and prognostic prediction [13].

Since TMS is a measure for excitability (i.e. how easily a response can be evoked) it becomes clear how it should be applied in the diagnostic process of epilepsy. As previously described, epilepsy is characterized by an increase in excitability. With a tool to measure this increase, there has been a rise in the studies that are investigating the use of TMS in epilepsy research.

Applications include investigation of the underlying cortical excitability, determination of the effects of AEDs, pre-operative localization of the epileptic foci and even functional mapping [49]. The ability of a short-lasting magnetic field inducing an electrical current within body tissue allows the researcher to influence or monitor the neuromuscular system. It can also be used to influence sensory neurons in the brain [41].

TMS is an attractive tool for the study of seizure disorders due to its simplicity; it is relatively inexpensive and generally safe. So far, results obtained from TMS studies suggest that patients with generalized epilepsy syndromes have increased cortical excitability, which makes this technique an adequate mean for clinical and research applications [49]. As a diagnostic tool, single-pulse and paired-pulse TMS may be used to map cortical function and also to measure cortical excitability [63].

Assessment of non-invasive pathophysiological mechanisms and effects of AEDs in patients with epilepsy is necessary. Due to the influence that AEDs can have on TMS parameters the ideal approach to investigate epileptic processes would be to evaluate

patients not undergoing any treatment [54]. A recent study by Badawy *et al.* [3] indicates that AEDs suppress seizures by modulating their cellular target in a ways as to change the pattern of the pre-existing cortical hyperexcitability in epilepsy. This study involved the use of TMS, like previous studies before it, with the objective of studying the effect of prolonged AED use and how it affects cortical excitability in epilepsy.

2.4 TMS-EEG

Until a few years ago, most TMS experiments and applications were limited to the stimulation of the motor cortex, because the only observable effects were those that reflected peripheral muscular activity [1]. However, in the past years, it has been demonstrated that the effects of TMS can also be observed by means of SPECT, NIRS [2], functional MRI, PET, and EEG [1] in a more direct manner. The first four techniques have poor temporal resolution because they use the variation in blood flow and oxygenation to detect changes in neuronal activity. Since EEG directly measures the electrical activity of neurons, it has an excellent temporal resolution.

The ability of the EEG to measure direct cortical activation that is induced by TMS shows the importance of using EEG and TMS simultaneously. Unlike any other available brain imaging method, the EEG is able to provide a mean to study the instantaneous neuronal effects of TMS in the brain, and thus probe the brain's excitability [2, 51]. This can provide information regarding the state of the stimulated area as well as the functional connectivity to other regions and their state [55]. TMS-EEG can access any cortical region (primary and associative) in any category of patients, providing a straightforward and flexible way to monitor the state of corticothalamic circuits [64]. Detection and monitoring of the state of corticothalamic circuits therefore becomes more straightforward and flexible [51]. A variety of information can be obtained by altering the TMS intensities, inter-stimulus intervals, induced current direction, and cortical targets [55].

The temporal resolution of TMS is, in theory, only limited by the duration of the TMS pulse (about one millisecond). In the EEG the temporal resolution is limited by the sampling frequency. However, the combination of the two techniques is not determined only by their nature, but also by their interaction. It can take the amplifier several milliseconds to reset after the TMS is applied and the emergence of neural activity, that can generate a detectable signal, can also take some time. EEG is sensitive not only to the firing rates of the underlying neural activity but also to the synchrony of the activity, and the geometry of the active neural elements [37], which means there can be a slight delay in obtaining a signal.

2.4.1 Analysis Methods

Magnetic stimulation is normally repeated several dozens of times to increase the signal-to-noise ratio. In order to extract the part of the response that is related to the experimental conditions, it is necessary to use one or more of the following methods: averaging, independent component analysis, subtraction methods, projection operators [51], principal component analysis, modeling of sources, etc. [12]. Unrelated events that need to be removed include instrumental noise, background cerebral activity, muscle activation, eye movement, movement of electrodes, and the decay of TMS-induced electrode polarization [51]. TMS can induce tactile and auditory artifacts which also need to be accounted for. This means that at the same time that it affects neural activity,

the TMS pulse also activates the muscles in the underlying region of the scalp for a short period of time, creating a light twitching sensation. The rapid movement of the component wire within the coil will cause a loud click, heard every time a pulse is given [37]. A way to deal with the artifact problem is to exclude the channels that are strongly affected by said artifact. However, there is a problem with this solution because these channels are usually the ones closest to the stimulation site, thus they are usually the most informative about the early stages of response [5].

In the use of multi-channel EEG recordings, it is necessary to start artifact removal and data analysis during acquisition. This requires appropriate technological solutions for the recording environment, electrodes, amplifiers, a careful methodological approach, and suitable analysis methods should be used to eliminate de effects of the TMS pulse. The pulse is strong enough to cause significant and visible disturbances in the EEG [51]. However, filters should not be used during recording because these interact with the residual spike-shaped artifact which leads to a ripple in the signal after each TMS pulse that can last up to one second. Filters can be used after recording, once the discharge artifacts from the TMS have been removed [37].

There are several reasons that can account for the long-lasting TMS artifact. The major influence could be due to the fact that electrodes and skin have magnetic properties, which may therefore be affected by the TMS pulse and generating an extra-cortical signal in the recording [65]. Re-positioning of the coil or even due to head movements in one experiment can be a source of artifact. Reasons could include the fact that while the coil position is optimized by examining motor evoked responses, the angle of the coil with respect to the electrodes depends on head size and shape, and local skull curvature under the coil. Even the smallest difference in coil orientation can have major effects on the effective magnetic field strength near a specific electrode [5]. On the other hand, perhaps the exact angle of the electrode with respect to the spatial gradient of the field can also make a difference in the amount of charge that can be accumulated at the skin-electrode junction as a result of the TMS pulse [5].

2.4.2 Responses

EEG can be used to locate the neuronal activity evoked by TMS, and how it spreads to other regions, in order to determine reactivity and connectivity patterns [2]. It is also possible to develop studies about how the brain processes information from the periphery, by determining, temporo-spatially, the effects of TMS on evoked potentials (EPs) and event-related potentials (ERPs). EEG can also be used to monitor abnormalities or to control the efficacy of the use of TMS as a treatment [46].

ERPs are the measured brain response by EEG to external stimuli. Short-latency ERPs are influenced mostly in the physical characteristics of the stimulus while longer-latency ERPs predominantly depend on the conditions of how the stimuli is presented [34]. The causality between the detected activations is evident through their temporal sequence, as long as the EEG's temporal resolution is sufficient to identify the neural phenomena [2]. An important aspect of TMS-evoked EEG topography is that even though only one cortical hemisphere is stimulated, bilateral EEG responses can be evoked and with different features because of inter-hemisphere connections [51].

The TMS response in the EEG is an evoked potential, which means it is an electrical potential recorded from the brain after stimulation. This response has distinct characteristics, after averaging multiple measurements, because the response is much smaller than the ongoing EEG.

A typical scalp-recorded averaged TMS-evoked EEG signal can be seen in figure 2.10. There are several deflections, first as rapid oscillations and then as lower frequency waves. The responses depend on the state of the cortex in that instant [61, 62] and on the location of the stimulation [8]. The TMS-evoked average responses are usually highly reproducible, as long as the delivery and targeting of TMS is controlled and stable from pulse to pulse and between experiments [51].

Several peaks are identifiable in the typical TMS-EEG response in figure 2.10, such as P30, N45, P55, N100, and P180. These represent the time, in milliseconds, after the TMS pulse, at which they occur. While N stands for a negative peak, P is a positive peak. The N100 is the most pronounced, reproducible and long-lasting component in response to motor cortical TMS, according to reports [7, 62, 66]. There can be some small deviations in the time at which these peaks occur. Recording EEG during TMS can be a technically challenging task because TMS induces a very strong electrical field which could saturate recording amplifiers for quite some time [65].

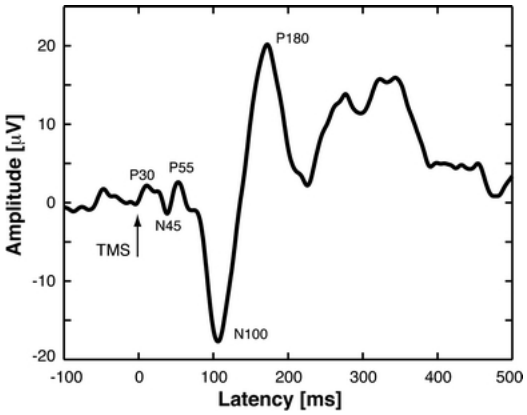


Figure 2.10: TEP: TMS-evoked potential. There is a clear identification of the major peaks and their polarities, P30, N45, P55, N100, and P180. P=positive, N=negative and the number represents the time at which said peak occurs in milliseconds. This is a single-channel response. The structure and latency of these peaks may vary between subjects and measurements [51]

The sub-millisecond synchronization, observed initially, is soon lost due to the conduction from the site of stimulation to the first synapses and further along the neuronal network, initiating a cascade of serial and parallel effects. The stimulated cortex assumes an inhibitory state for a period of 100 ms or more, because of the activation of inhibitory cells as well as excitatory cells [51]. This is known as the cortical silent period, evidenced by a period of EMG silence following each MEP when the subject tries to maintain spontaneous muscle activity during the whole measurement. Most of the silent period is believed to be due to inhibitory mechanisms at the motor cortex [13, 67].

Even though there have been many studies regarding simultaneous TMS and scalp EEG [6–9], none addressed a comparison between healthy controls and individuals suffering from a neurological condition. In the study by Valentín *et al.* [12], EEG responses to TMS are described as well as how they can be used to evaluate focal epilepsy. This evaluation can be for diagnostic purposes or to identify the epileptogenic cortex during presurgical assessment. Prior to this study, TMS-EEG responses had not been evaluated for the diagnosis of epilepsy.

For diagnostic purposes, a useful measure of cortical excitability can be obtained at baseline or after anti-epileptic treatment in case of patients with epilepsy. The use of TMS-EEG may also include real-time monitoring of epileptiform activity in vulnerable

populations, or even as component of a responsive neurostimulation set up in which TMS timing is determined by underlying EEG activity [68]. It could also become an alternative method to identify epileptogenic cortex non-invasively in patients with epilepsy [12].

Valentín *et al.* [12] saw several types of responses to TMS in the EEG. Early responses are in seen in both groups of individuals being studied: patients and healthy controls. With this outcome, the focus was given to late responses, where they saw a difference. Their results suggested that the use of TMS can increase the diagnostic sensitivity of the EEG in epilepsy. What they defined as delayed responses appear to be equivalent to the ones that were described in their previous studies by patients with intracranial electrodes. Due to the fact that late TMS-EEG responses were seen in zero of the 15 healthy subjects and in 11 of the 15 patients, they consider these responses as abnormal. This could be related to the hyperexcitable cortex existing between the stimulated area.

The objective of this project is to attempt to reproduce the results by Valentín *et al.* and present these results as a measurable quantity. This will require the development of tools that can quantify the response obtained in an EEG time-series. To improve the diagnosis of epilepsy, two groups of individuals (patients and healthy controls) will be classified according to the quantitative value obtained. Representing the data by focusing on the channel information, instead of time, will allow the identification of the hyperexcitable cortex.

3

Methods

In this chapter the characteristics of the included participants are presented. A description of how the raw data was acquired and the subsequent data analysis is also provided. This is where the quantification tools, that were applied in order to show the results present in chapter 4, are outlined and an explanation is given as to why they were chosen.

For this particular study, there was the participation of patients suffering from epilepsy and healthy controls for comparison. This means that part of the work developed was related to the preparation, assistance and work with people who volunteered to take part. All of aforementioned points contribute to a project which considers all aspects of clinical operations, because there is the recruitment of individuals, the collecting of the experimental data and its analysis through the tools which will enable a quantifiable result.

3.1 Subjects

Patients participated after contact through the Department of Neurology and Clinical Neurophysiology at the Medisch Spectrum Twente in Enschede, The Netherlands. The diagnosis of epilepsy and its sub-syndromes was made by the clinical neurophysiologist or the neurologist, based on clinical history, imaging, and EEG findings.

3.1.1 Inclusion Criteria

Inclusion criteria for this study in the population of healthy subjects consists of:

- Subject is between 18 and 60 years of age;
- Subject obtains a minimum score of 9 in the Dutch Handedness Questionnaire [69].

The Dutch Handedness Questionnaire can be found in appendix A and will be used to confirm right-handedness. There was no minimum score for the patients included.

Inclusion criteria for this study in the population of patients consists of:

- Subject is between 18 and 60 years of age;
- Subject is diagnosed with epilepsy (focal or generalized);
- Subject is able to understand and comply with the instructions for the TMS experiment.

3.1.2 Exclusion Criteria

Subjects are not eligible for inclusion [70] if they have any of the contraindications according to the TMS screening questionnaire, found in appendix B:

- have hearing problems;
- have implanted metal structures in their brain/skull;
- have a cochlear implant, depth or subdural intracranial electrodes, other electronic implants such as vagus nerve stimulators, or cardiac pacemakers;
- had spinal surgery, or have drains in their spinal cord or ventricles;
- have used any illegal drugs in the last month;
- might be pregnant;
- suffer from a severe medical condition other than epilepsy;
- use medication that forms a relative problem for application of TMS because it lowers seizure threshold potential.

Additionally, healthy individuals were not included if they had a personal history of epilepsy or have a lesion in the brain, whether it is vascular, traumatic, tumoral, infectious, or metabolic.

All patients and healthy subjects received fully disclosed information concerning the nature of the research, which was followed by their informed consent, according to the 1964 Declaration of Helsinki. The experimental procedures were approved by the local ethical committee. The healthy subjects were included in the second half of 2010, while measurements involving patients were performed until August 2012.

TMS-EEG responses were acquired in 20 healthy subjects (12 males and 8 females, mean age 28.1 years, range 20 to 54 years) and ten patients (3 males and 7 females, mean age 24.3 years, range 19 to 50 years). There were two healthy subjects excluded from this study. One individual fainted and the other could not be scheduled for the MRI. No patients were excluded from the study. All participants but six of the patients had structural MRI studies. All, except one (number 2), patients were taking AEDs prior to and during the study.

3.2 Data Acquisition

This thesis is found within the scope of a wider research project which studies TMS-EEG in a variety of applications and conditions. Since part of the objective involves comparing

healthy controls to patients with epilepsy, we used data from healthy subjects that had been previously acquired, in order to have data from the groups being analyzed.

Each healthy subject participated in five separate sessions which targeted four cortical sites: motor cortex right (MCR) and left (MCL), and left and right Brodmann's Area 19; at different stages during the day (8:00 am, 10:30 am, 1:00 pm, 3:30 pm and 6:00 pm). The effect of the daytime is beyond the scope of this project, so only one session for each site and each healthy control was considered. For the patients six cortical sites were targeted - motor cortex, temporal lobe and Broadmann's Area 19 in both hemispheres. During one session, an average of 75 trials were collected with approximately four seconds between each pulse.

3.2.1 Protocol

Subjects were seated in a comfortable armchair with their elbows flexed at 90°, hands pronated in a relaxed position, and eyes open. Room conditions were also kept standard with an attempt to minimize distraction during the procedure. The chair is placed in front of an infra-red camera, the NDI Polaris Vicra (Northern Digital Inc., Waterloo, Canada), that is responsible for tracking the position of the subject in space. This system can locate the position of the coil with 1 mm accuracy. The camera works by tracking the reflecting balls on a headband that the subject is wearing, as it can be seen in figure 3.1.

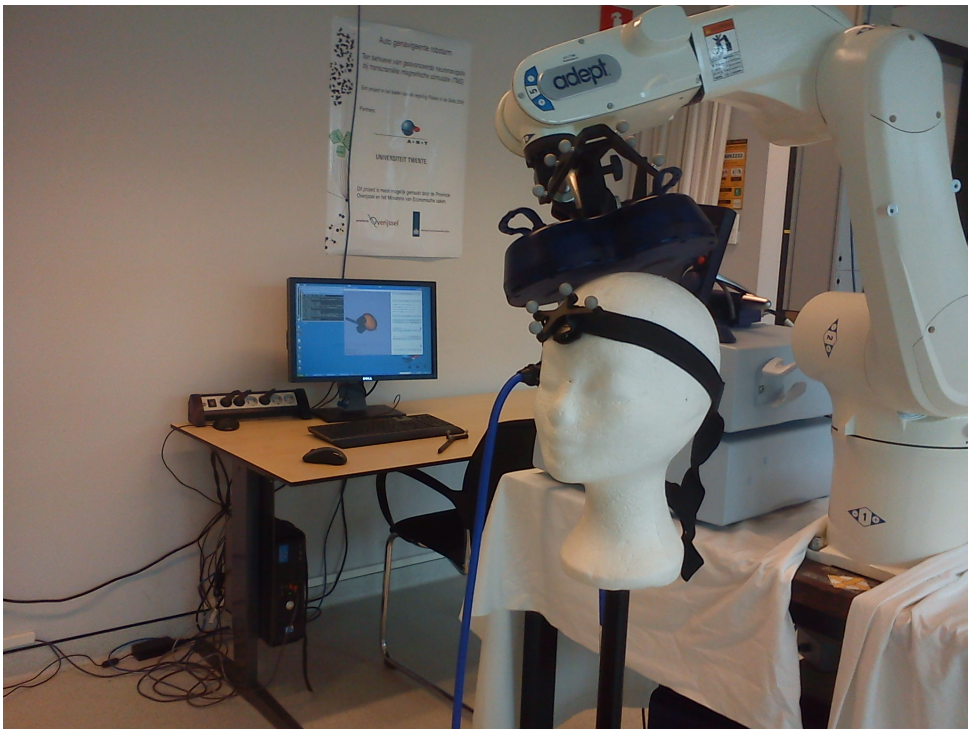


Figure 3.1: A Styrofoam head is used to simulate a subject, with the reflecting balls placed on a headband. In the background is a 3D model of a head constructed using MRI scans.

By identifying certain digitized skull landmarks: nasion, nasal tip and outside corner of the eye, and by creating an outline consisting of about 300 additional points on the scalp with a tracking pointer, the software will create a subject-specific head model. From there it is possible to map several other points in the skull and relate it to the cerebral anatomy. Some patients (four) and all of the healthy subjects included had an

MRI scan which could be used. If there was no MRI scan available, a MRI-constructed three-dimensional frame of reference was used.

Calibration of the coil is also an essential step. This is so that the computer knows where the coil is, in relation to the camera. For this calibration the pointer is placed in three pre-defined places on the coil and the computer acknowledges each identification. The dimensions of the robot-arm and the angles of the different joints are known, thus the computer can calculate the orientation and the position of the coil. There is some movement compensation from the robot-navigated system in order to "follow" the participant's movements.

3.2.2 TMS

TMS was carried out with a Magstim Rapid^2 magnetic stimulator (Magstim, Dyfed, UK) and delivered through a 70 mm figure-of-eight air film coil. This stimulator can operate from 1 to 100 Hz, with a maximum output of 1.5 T. The pulse has a biphasic single cosine cycle with a period of 400 µs, which is more suited for bilateral cortical stimulation [41]. Its shape is shown in figure 3.2. The system functions based on a touch screen interface which assists in the control and operation, as can be seen in figure 3.3.

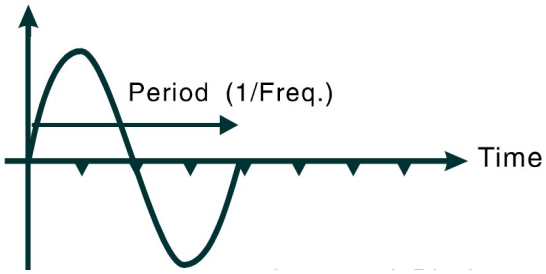


Figure 3.2: The shape of the TMS pulse used when acquiring the EEG data for every individual [41].

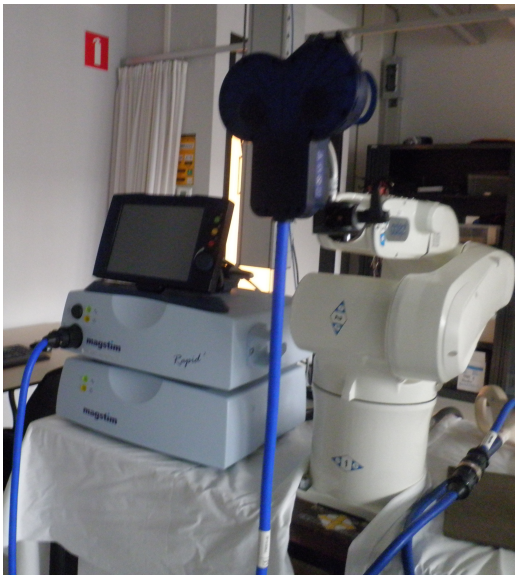


Figure 3.3: The magnetic stimulator with the touch screen interface. The robot-navigated system is used for accurate positioning of the figure-of-eight coil.

The pulse waveform and relative current direction will influence the stimulation threshold of different neurons [71]. This is important to keep in mind because using different stimulation parameters will determine the type of response obtained by applying a TMS pulse to the motor cortex.

3.2.2.1 MEP and EMG

For the motor evoked potential (MEP), the stimulation coil was held in a fixed position by means of a mechanical support, that consisted of an articulated mechanical holding arm (ANT Neuro, Enschede, The Netherlands), over the area where the lowest motor threshold (MT) was obtained. The mechanical arm reduces variability of the induced artifacts over time and allows maximum flexibility for positioning the coil at the desired location, orientation, and with maximum stability. A small alteration in stimulation spot (e.g. 5 mm) can cause significant differences in responses [8]. The coil was placed tangentially on the scalp with the handle pointing backwards and laterally at a $45°$ angle away from the mid-line, which means it was approximately perpendicular to the line of the central sulcus (the fissure separating the frontal from the parietal lobes of the brain) [4, 6, 8].

In order to stimulate the motor cortex in an adequate way, it is necessary to know if the site of stimulation is correct. For that confirmation, the use of EMG surface recording is essential. We recorded the abductor digiti minimi (ADM) muscle, contralateral to the site of stimulation, in order to determine the best position for inducing maximal MEPs. The active electrode is placed on top of the ADM muscle, while the reference electrode is placed on the little finger.

In all subjects TMS intensity was above the threshold for triggering a significant EMG response in the motor cortex, 110% of the motor threshold. The ADM has a small cortical representation, which means that it requires high reproducibility of coil placement. The intensity setting with the Magstim Rapid^2 is set in a percentage form. A number of controls (number 1) and patients (number 6, 7 and 8) had a MT higher than 90%, which meant that the intensity of the stimulation was of 100% (which is the highest value).

3.2.3 EEG

The electroencephalogram (EEG) was recorded and manually reviewed with the use of the software Advanced Source Analysis-Lab (ANT Neuro, Enschede, The Netherlands). There were no filters set for the acquisition. Sampling frequency was set at 2048 Hz and cut-off frequency (for the low-pass filter) was defined by the software as 550 Hz for anti-aliasing purposes. The data using this software was stored as a *.cnt* file, which included all EEG data per subject, target and session.

The clinical EEG is commonly registered using the International System 10/20. This is a standard system for the uniform placement of silver/silver-chloride electrodes on the scalp surface, which comprises of 19 active electrodes. The contact is guaranteed by the use of a conductive Electro-Gel (ANT Neuro, Enschede, The Netherlands), in order to decrease impedance. However, in this work it was decided to use a TMS-compatible 64-channel true-DC EEG amplifier (ANT Neuro, Enschede, The Netherlands) to record the TMS-evoked potential (TEP). This was done recurring to a 64-channel TMS-compatible WaveGuard cap due to its very short recovery times in the EEG (ANT Neuro, Enschede, The Netherlands). A scheme of the placement and designation of the electrodes can be found in figure 3.4 and FPz is defined as the ground electrode.

The acronyms used in the scheme give information regarding the localization of the electrode. The first part indexes the array (in rows) of electrodes from the front of the head to the back: Frontopolar (FP), frontal (F), central (C), parietal (P), temporal (T), and occipital (O). The second part consists of numbers: even on the left side and odd on the right, while in the center there is a z.

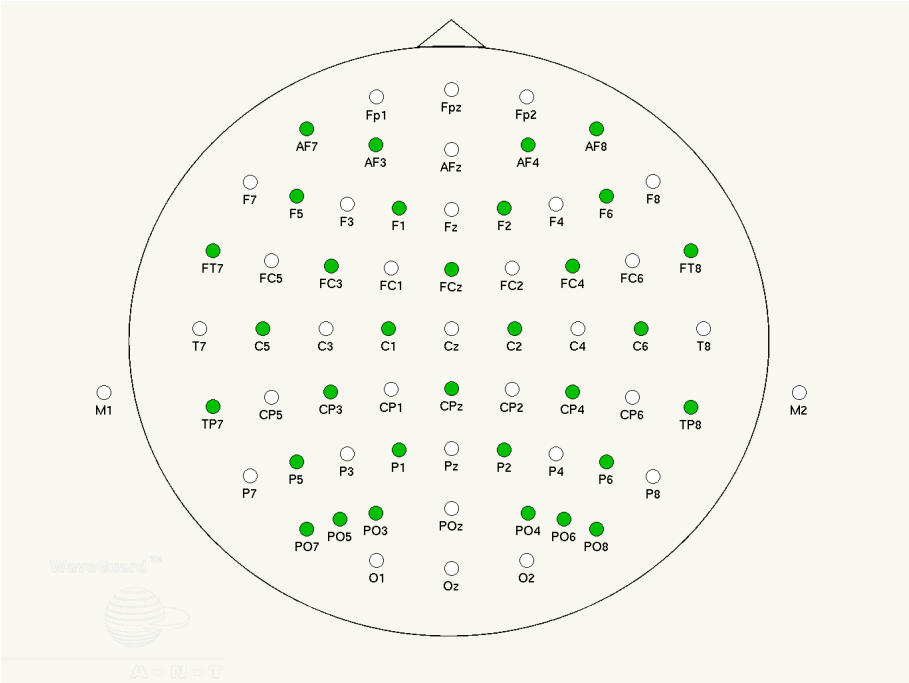


Figure 3.4: Representation of the 64 channels used to acquire the EEG signal. Highlighted in green are the first 32 electrodes. Note: FP = Frontopolar, F = Frontal, C = Central, P = Parietal, T = Temporal, O = Occipital. Even numbers can be found on the right side of the skull and odd numbers on the left, while z represents the mid-line of the skull.

The impedance at all electrodes was kept under five kiloohms. The reference electrode is, usually, placed at a relatively inactive position; forehead, nose, and linked-mastoids references have been used in TMS-EEG experiments [64]. In this work, the common average reference was chosen as reference in order to avoid over-weighing of the signal from a single reference point on the skull.

3.2.4 Safety

The safety of the individual is guaranteed by some movement limitations of the articulated mechanical holding arm, because it cannot make a turn of more than $90°$ or move along a trajectory where the subject's head is located. The robot also stops if the camera loses information on the location of the head tracker. A major safety advantage is the speed limitation and also the latency with witch the mechanical holding arm moves to the desired location.

3.2.5 Other Considerations

As discussed previously, the site for stimulating the motor cortex is determined through the measure of the ADM muscle response in the EMG. The site for stimulation of Brodmann's Area 19 was selected based on an atlas of brain regional anatomy, being

identified on a T1-weighted individual MRI (resolution 1 mm) acquired with a magnetic field of 1.5 T. Stimulation of the temporal lobe is done by selecting the temporal region through the electrodes of the cap.

To avoid contamination of the TEP by auditory potentials evoked by the click associated with the TMS discharge, participants wore inserted earplugs as well as headphones which continuously played white noise (90 dB) capturing the specific time-varying components of the TMS click. Bone conduction was attenuated by placing a thin layer of foam between coil and scalp.

3.3 Data Analysis

Offline data analysis was performed using the software MATLAB (The MathWorks, Inc., Massachusetts, USA). MATLAB is a programming environment ideal for algorithm development, data analysis, visualization, and numerical computation. By using it, technical computing problems can, usually, be solved faster than traditional programming languages.

All *.cnt* files are loaded into MATLAB using specifically designed scripts. The first analysis were performed on the already existent scripts, that were developed prior to the beginning of this project, with some small alterations. However, these scripts were improved throughout the project, and in such circumstances refined through trial and error. For each subject and target a data set was constructed containing about 75 epochs of 8194 samples for the 64 channels.

All quantification analysis was preceded by common-average referencing. This means all electrodes will be referenced to the average of the accepted 61 channels - the three FP electrodes will be excluded. There is a pulse detection cycle using a threshold which allowed for clear identification of where the TMS pulses were given. When figure 3.5 is constructed, the user needs to define a threshold. If the value of the signal is higher than the imposed threshold, then there will be a mark placed on the signal, such as can be seen in figure 3.6. This identifies the TMS pulses and stores them for posterior analysis.

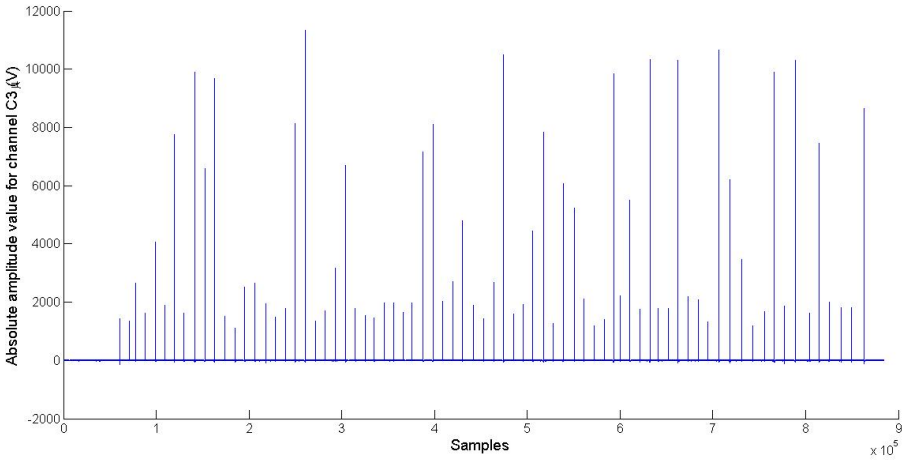


Figure 3.5: Auto-scaled image of the EEG signal, including the TMS pulses. In this image, it will be necessary to select a threshold for detecting all the pulses.

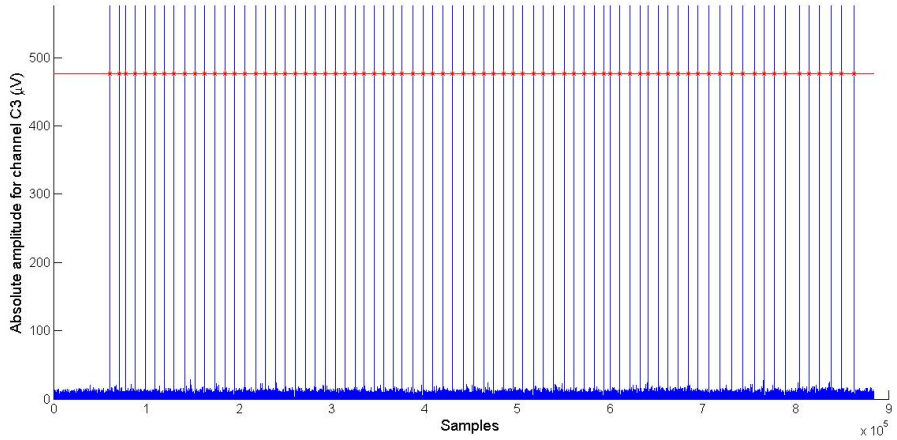


Figure 3.6: This image represents the same EEG signal (blue) in the previous figure, with an adapted scale and with the trigger (red) method identifying the peaks of TMS pulse.

The EEG data was divided into epochs of one second before the pulse and one second after the pulse, as there are on average four seconds between pulses. A baseline correction of the signal is done. This is followed by an interpolation which will replace the TMS pulse for about five milliseconds before and ten milliseconds after the pulse. This interpolation removes the contamination that the pulse causes in the signal response. However, the method is not ideal because there is a clear replacement of a measurement by a *fabricated* piece of data. A way to eliminate the pulse effect and maintain the original data would be to use principal component analysis (PCA), but such an alternative will not be explored in this work.

The corrected data was band-pass filtered with a fourth-order digital Butterworth filter established for frequencies 1 to 80 Hz and band-stop filtered, also with a fourth-order digital Butterworth filter for the removal of the 50 Hz contamination due to electricity, machines and lights. A Butterworth filter is designed to have a flat (no ripples) frequency response in the band-pass and to approach zero at the band-stop. The average response is then constructed with the mean of the trials that were accepted for analysis.

The three FP electrodes were removed from the baseline and were not considered throughout the assessment of the signals. This was done in order to avoid contamination of data due to eyeblinks clearly present in the signal.

For all analysis, we studied the TEP. An example of the TEP is plotted in figure 3.7 for patient number 4 with stimulation site MCR. This evoked potential was studied and plotted in topographical scalp plots. For all patients and healthy subjects we conducted three studies. These included the amplitude, the global mean field amplitude (GMFA), and the root mean square (RMS). By studying the TEP for all channels in the volunteer data, results will be obtained in order to be interpreted.

The choices made for data analysis were done based on what would help in representing the data that is portrayed in the time-series of the acquired EEG. This can be done by improving the space resolution in studying the amplitude in a topographical scalp plot. The time resolution decreases because it is not practical to create a plot for every millisecond.

In order to enhance any responses that may be visible, the power will be calculated in two different ways. Analysis with the use of the GMFA will determine the potential

differences between all possible electrode pairs in the field. This method will only enable a representation in regards to time, which means that this resolution will increase but the sum will be performed for all channels, thus decreasing space resolution.

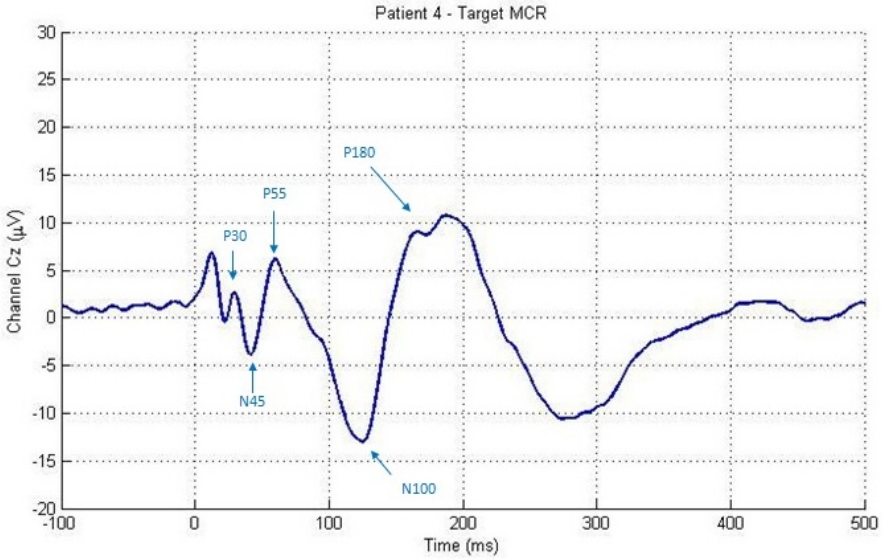


Figure 3.7: TMS-evoked potential obtained for patient number 4 with stimulation site MCR. The peaks P30, N45, P55, N100, and P180 are identified.

By combining both methods, we calculated power and represented it in a topographical plot. Time resolution is not present because the values are averaged throughout a time interval, but spatial resolution enables a clear identification of where the response is located.

Since the goal is to obtain a quantification value for the TMS-EEG response, this last method will enable to determine how high the increase is and its localization, in a time interval that is of our interest.

3.3.1 Amplitude

The MATLAB function *topoplot* allows the plotting of a topographic map of scalp data. This is done in a two-dimensional setting. A circular view from the top of the head is the result. The information is presented through a map that codes the amount of activity in several tones of color, where the predefined colors are blue - low amplitude and red - high amplitude. Through interpolation it is possible to calculate the spatial points between the electrodes, obtaining a smooth gradient.

This approach represents the location of alterations of magnitude and changes in activity. The method for quantification of responses is close to ideal because it will easily enable an objective viewer to identify in which channel the highest activity can be found, how that quantity correlates to the surrounding channels, and how it compares to other instants.

Determination of the amplitude, either at a specific time t or within a time interval, where the average amplitude is used, enables the analysis of significant aspects gathered from the EEG such as localization. This type of information can highlight the areas which demonstrate activity at either different time intervals or at a given time, and can represent the TEP.

In figure 3.8 are presented some examples of topographical maps for several instants. However, it is important to keep in mind factors that might mislead the interpretation of results, such as artifacts that can still be present even after the band-stop filtering. An example of using topographical plots to remove specific artifacts can be found in the study done by Mäki and Ilmoniemi [72] in 2011. Here, the authors worked to remove muscle artifacts from TMS-evoked EEG.

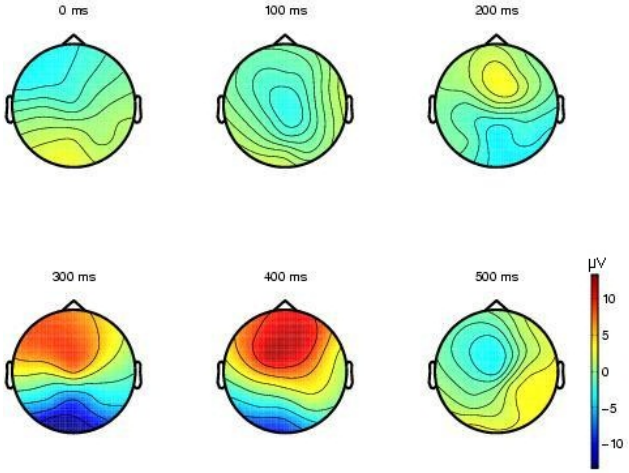


Figure 3.8: Example of topography illustrations given by the developers of EEGLAB. The several images indicate the time at which the calculation was made [73].

3.3.2 Global Mean Field Amplitude

The power of a signal is defined as the average of the square magnitude of the signal - the energy - over a given window. With this definition, it is possible to reach an average reference power measurement of the field, which uses the root of the mean of the squared potential differences between all possible electrode pairs within the field.

The global mean field amplitude [9, 72] was calculated as a function of time using equation 3.1 and is used for calculations with the original data. This equation reflects the overall EEG response, for all channels. GMFA values are directly related to power measurements, and the term power will be used for the results obtained with equation 3.1.

$$GMFA(t) = \sqrt{\frac{\sum_{k=1}^{d}(x_k(t) - x_{mean}(t))^2}{d}} \tag{3.1}$$

where $x_{mean}(t) = d^{-1}\sum_{k=1}^{d} x_k(t)$ is the mean signal over the channels, also defined as the baseline.

The scale of these variables will be significantly reduced, unlike what would happen if the amplitude of the signal was simply squared. Squaring would make the differences larger and easier to see; however, the scale would have to be adapted for each signal. Using this equation makes it easier to compare the graphs that are constructed. The first analysis included all electrodes, except, as previously mentioned, the three FP electrodes.

A plot showing one second after the pulse and 100 ms before the pulse was constructed for each set of data, just like the plotting of the time-series, in patients and healthy controls. A baseline will also be included in order to provide a reference for the

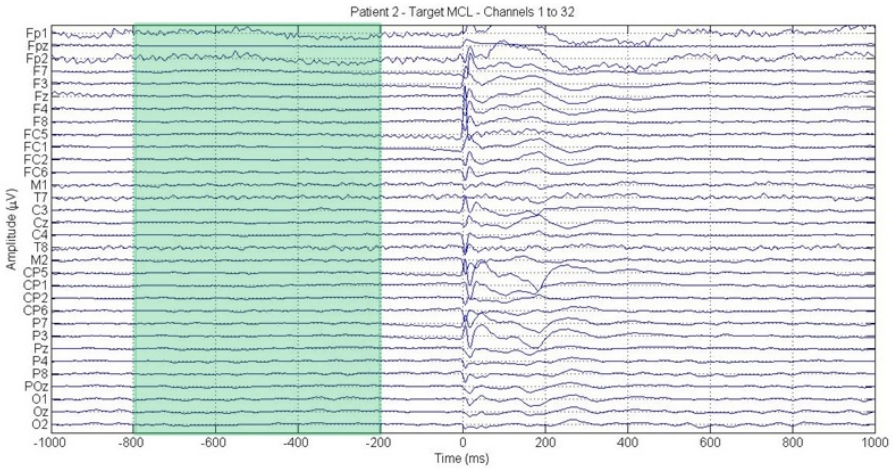


Figure 3.9: Identification of what was considered the baseline in the time-series, from 800 to 200 ms before the pulse.

results obtained. This baseline will be determined from the signal prior to the pulse, having been determined as 800 to 200 ms before the pulse, as can be seen in figure 3.9.

A clear view of what is happening around the stimulation site is also necessary. For this reason the signal power was calculated, using equation 3.1, but with only $d = 9$ electrodes. For MCR, the area around the stimulation site thus the electrodes considered were FC2, FC4, FC6, C2, C4, C6, CP2, CP4, and CP6. For MCL the idea was applied to the corresponding opposite electrodes in the skull, FC1, FC3, FC5, C1, C3, C5, CP1, CP3, and CP5. A spatial representation of these electrodes can be seen in figure 3.10.

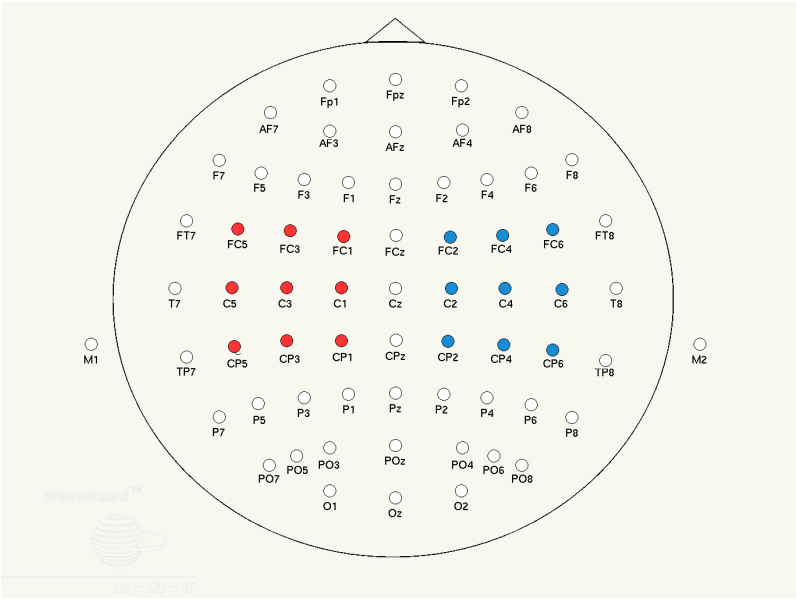


Figure 3.10: Setting used to determine response changes (increase or decrease) in patients and healthy controls. Electrodes FC2, FC4, FC6, C2, C4, C6, CP2, CP4, and CP6 highlighted in blue, while the corresponding opposite electrodes on the left side are in red.

In order to better understand the contribution of certain channels, the calculation of how many channels, overall, have a certain amount of power is performed. For each channel, this was done by doing a form of integration which consisted in adding the

power throughout the whole epoch. In the time prior to the stimulation, the channels have a value close to zero which means that the sum for this time interval will be similar for all channels. What separates the channels is what happens after the stimulation. Normalizing these results - to facilitate analysis - is the next step. The number of channels that have their total power between 0 and 0.1 are plotted at 0.1. This is done in intervals of 0.1, as in a histogram. This helps to determine if there is a bigger number of channels with high energies in patients or in healthy subjects. Even though this may not give specific details of where the response is located, it provides information regarding the power present throughout the whole response period (early and late).

3.3.3 Root Mean Square

The root mean square (RMS), in mathematics, is also known as the quadratic mean and has the purpose of providing a statistical measure of the magnitude of a varying quantity. This measure is particularly useful when the magnitude varies both positively and negatively. Such a calculation would make sure that the average of the signal over a time interval would not be null. It can be used in a series of discrete values or for a continuous function. Its name clearly states what it does, the square root of the arithmetic mean (or average) of the squares of the original values (or function).

A reference-free power measurement of the field, which uses the root of the mean of the squared potential differences between all possible electrode pairs found in the field is an option for calculation. However, equation 3.2 represents the scaling down by the square root of the number of electrodes that were used, the outcome will be the root of the mean of the squared voltage deviations at all electrodes from the average reference. This is done for an interval of time previously established.

$$RMS(k) = \sqrt{\frac{1}{t_d} \sum_{k=t_1}^{t_2} x_k(t)^2} \tag{3.2}$$

where the $x_k(t)$ is the signal of channel k after filtering and interpolation, t_1 and t_2 represent the time limits of the interval, and t_d is the length of the time interval. The calculation is performed taking into account the samples acquired for each data set; however, when plotting, the samples will be converted to time. RMS values are directly related to power measurements, and the term power will be used for the results obtained with equation 3.2.

An important aspect to evaluate is also how much of the signal is an actual response. Subtracting the background data that can be found prior to the stimulation will allow this calculation. Thus, a baseline was, once again, calculated from the data recorded before the TMS pulse. The time was determined to be within these limits (800 and 200 ms before the pulse) in order to avoid possible influences from previous data and from the pulse itself.

Knowing that early responses are usually present, as can be seen in the time-series, a closer analysis was performed to determine the existence of late responses. Assuming that at 400 ms the presence of initial responses is no longer present [12], it was decided that this would be the starting point of the analysis period. This means that a post-stimulus time period from 400 to 950 ms was chosen. From this moment, analysis will only be done concerning this time interval. An outline of intervals for the baseline period, the early response period and the late response period defined in this project can be found in figure 3.11. To evaluate if TMS induced an increase in activity for each channel, the baseline period is subtracted from the response period. This subtraction is

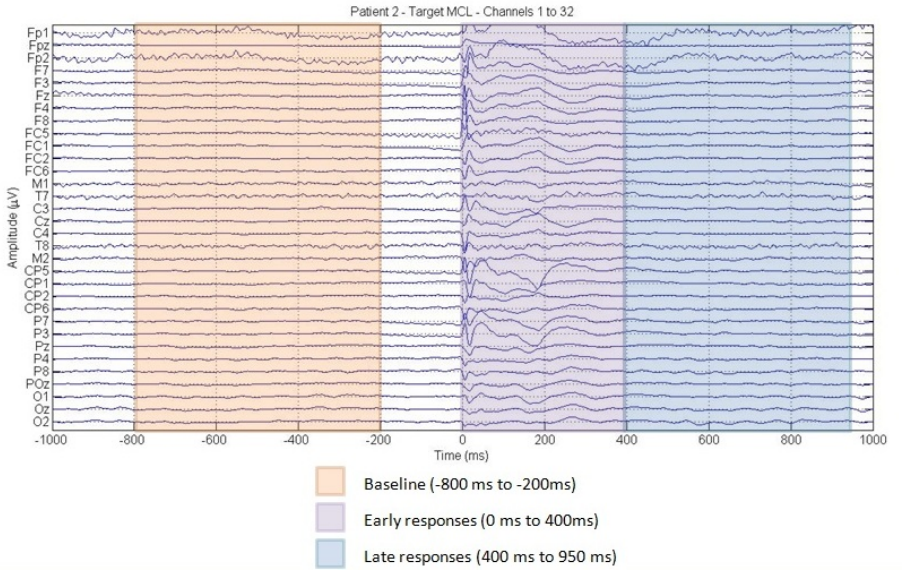


Figure 3.11: Identification of the time intervals determined as the baseline period (800 to 200 ms before the pulse - orange), early response period (0 to 400 ms after the pulse - purple) and late response period (400 to 950 ms after the pulse - blue).

done in absolute values in order to obtain the net effect of the stimulation and not the relative response.

By returning to the topographical plots, thus combining the two methods described in the previous subsections, will enable a better understanding of what is present in the late response period of the TMS-EEG. The two-dimensional setting that codes data through tones of color allows a better spatial representation of the power change from the baseline to the late response period.

3.3.4 Statistics

To determine if the difference noted from the increase or decrease in activity is statistically significant or not, a t-test is performed. Any statistical hypothesis test in which the test statistic follows a Student's t distribution is the definition of t-test. Its application is most common when the test statistic follows a normal distribution (if the scaling term is known). The formula is a ratio, where the numerator is the difference between the two means or averages and the bottom part is a measure of the variability between the two groups of data being studied, as seen in equation 3.3.

$$t = \frac{\overline{X} - \mu}{\frac{s}{\sqrt{N}}} \tag{3.3}$$

where $\overline{X}$ is the sample mean of the data, μ is the data under analysis, s is the sample standard deviation of the sample, and N is the sample size.

3.3.5 Modification of Initial Definitions

Throughout the development of the project important changes were made on the script which evaluates the EEG signals. These alterations were made in order to confirm if the responses that are visible in the first set of results are really present or are an influence of unknown or unclear artifacts.

For this, the size of the epoch intervals was subjected to a modification, increasing the epoch size by one second in each direction. Now we consider a time interval from two seconds before the pulse to two seconds after the pulse. This change arose from the analysis of some of the channels, one at a time. When visualizing the time-series with all 64 electrodes or just 32 electrodes, it is not clear if there is any artifact due to the filters. However, with a closer analysis, these filter artifacts are at the endpoints of the epochs. The effects are still present after the increase of epoch length, however, they no longer affect the data that is being subjected to analysis. Topographical plots were produced with this new setting, while maintaining the filter definitions.

To further ensure that the data is as *clean* as possible, we used NeuroCenter Viewer (Clinical Science Systems, Voorschoten, The Netherlands). Through the use of this software it is possible to include the MATLAB identification (after conversion to *.edf*) of the TMS pulses and also annotations regarding the TMS pulses which should be excluded due to eyeblinks and some other artifacts. Example of a healthy subject under analysis can be seen in figure 3.12 and of a patient in figure 3.13.

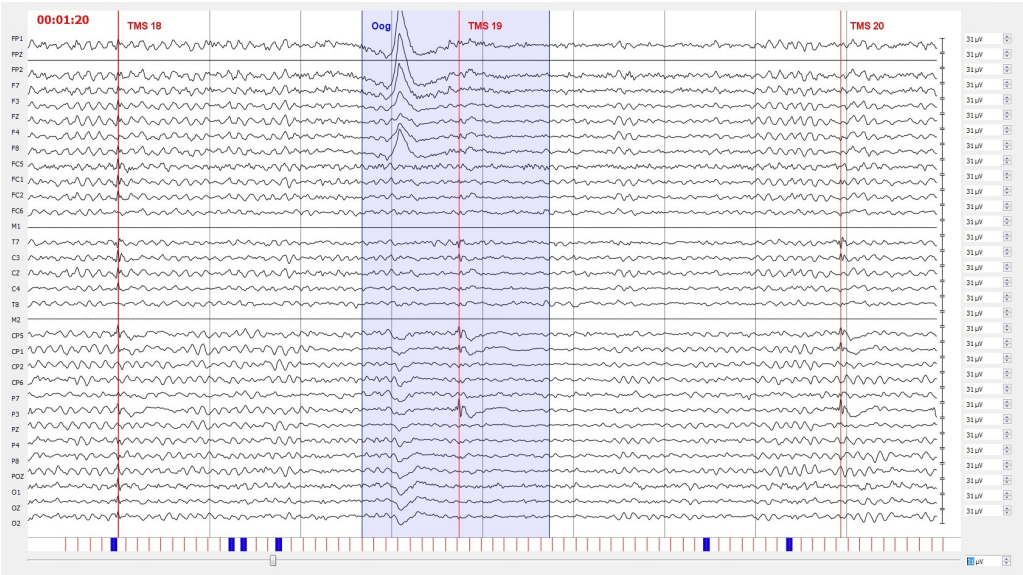


Figure 3.12: Use of the software of NeuroCenter Viewer to identify the trials which contain artifacts and which will be removed from the follow-up analysis for healthy subject 17, stimulation site MCL.

From previous analysis it is known that some channels contain artifacts throughout the whole period of recording or even have connection problems. This software also enables a close identification of these channels so that they can be excluded from the common average reference used in posterior evaluation. The excluded channels and trials for both stimulation sites (MCL and MCR) associated with the patients and healthy controls can be found in appendix C. This was a visual analysis and was thus performed by two individuals to confirm the channels and the trials that should be excluded.

Maintaining the rest of the settings of analysis, a manner of evaluating the quality of the method developed is established. This will ensure that there is a smaller dependency on the subjective opinion that an observer makes.

After obtaining these results for each of the 61 channels, the information will be reduced to the area surrounding the stimulation site. These two areas are defined by figure 3.10 and include FC5, FC3, FC1, C5, C3, C1, CP5, CP3, and CP1 for stimulation site MCL. These channels were chosen by looking at the topographical plots with the

representation of the power, and around the area of stimulation. This involved a search for a significant difference between the baseline and the late responses, just like it was explained in subsection 3.3.3.

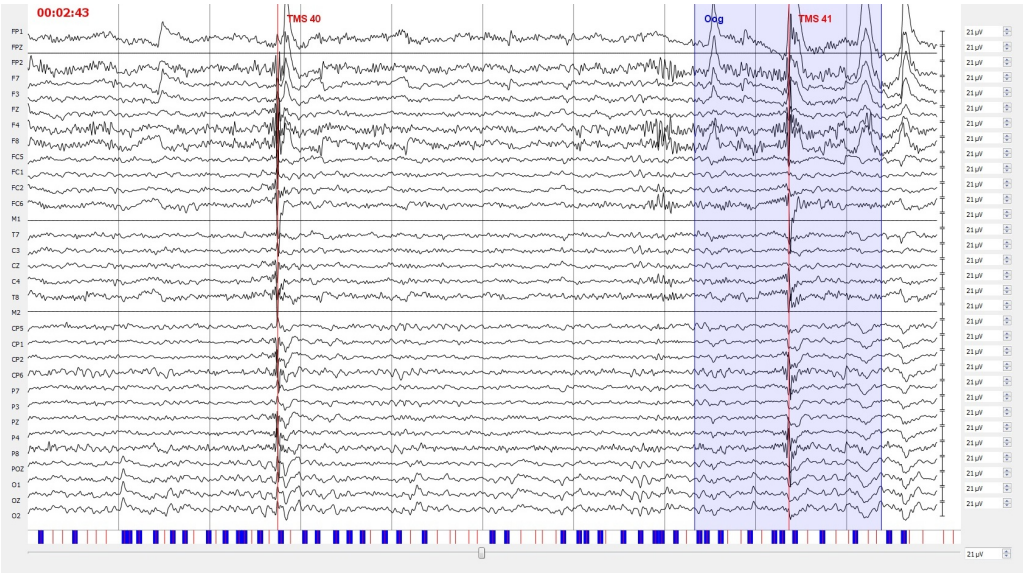


Figure 3.13: Use of the software of NeuroCenter Viewer to identify the trials which contain artifacts and which will be removed from the follow-up analysis for patient 7, stimulation site MCR.

3.3.6 Sensitivity and Specificity

The primary goal of the technique developed is to obtain information regarding differences between patients and healthy controls. This means there will be changes in the likelihood of the presence of a certain condition (e.g. epilepsy). In some cases, it is acceptable to not be 100% certain about the diagnosis; similarly if a particular treatment is available that has certain risks, it is necessary to have a high degree of confidence in the result. In other words, there will be a decision criterion, based on a probabilistic decision variable, to assist in determining how well the method can distinguish between the healthy controls and patients with epilepsy [21,74].

For this, the use of table 3.1 is essential, because for each threshold a new table will be filled. D+ is the presence of the disease, D- is the absence. Test + indicates a positive test outcome, while Test - represents a negative test outcome.

Table 3.1: Table built to establish the sensitivity and specificity of the results determined. D+ is the presence of the disease, D- is the absence. Test + indicates a positive test outcome, while Test - represents a negative test outcome.

	D+	D-
Test +		
Test -		
Total		

The construction of a receiver operating characteristic (ROC) curve, which illustrates the performance of a binary classifier system as the discrimination threshold is varied, will be done by plotting the fraction of true positives out of the positives (sensitivity) *versus* the fraction of false positives out of the negatives (one minus the specificity). The

ROC curve can isolate the effect of the placement of the decision criterion in order to achieve a pure measure of precise intrinsic discrimination.

The sensitivity is also defined as the likelihood of obtaining a positive test outcome, given that the condition is present in the subject. It is determined by P(D+|T+). Specificity, on the other hand, is the likelihood of a negative outcome, knowing that the condition is absent, P(T-|D-). Sensitivity and specificity relate to the quality of the test. Both results do not say how likely it is that a patient will suffer from a particular condition [21]. There is, however, a relation between the test performance and the likelihood of a disease, as stated by the Bayes formula, but that will not be addressed in this study.

This analysis will provide tools to select a possibly optimal model and also to discard suboptimal ones independently to cost context or the distribution of the class. ROC analysis is related in a direct and natural way to perform a cost/benefit evaluation of diagnostic decision making.

4

Results and Discussion

The dynamics found while stimulating the brain are shown in this chapter. There were several attempts to develop a quantification tool, by using the methods described in chapter 3. In the presentation of the results, there is a follow-up discussion on whether the outcomes were expected and if they can be used to explain what occurs after stimulation.

From the six major stimulation areas that are studied in this project, the main focus was given to the motor cortex, both right and left side. This is related to the fact that previous studies have been performed in these locations [3–10, 12, 71] and could thus be used for understanding of the results. Spatial distribution has also been a common approach for some of these studies [4, 8–10]. The foundation for this project is based on the work developed by Valentín *et al.* [12]. We decided to investigate if we could replicate the results obtained in this study of epilepsy.

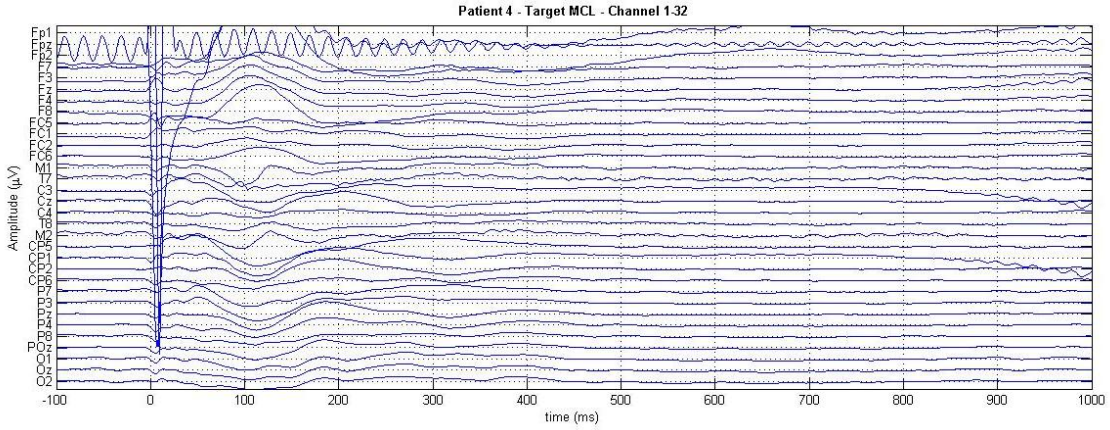
4.1 Results

4.1.1 Amplitude

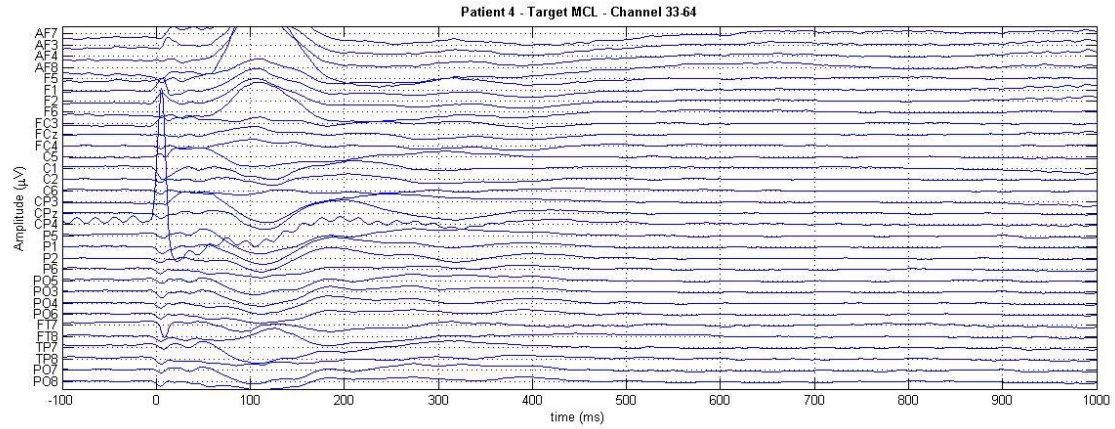
Although it is possible to plot any quantity, such as amplitude or even power, initially only the amplitude was plotted. Amplitude can provide information regarding where the stimulation took place. The use of figures 4.1a and 4.1b enables close monitoring of the EEG in response to the stimulation originating in the TMS pulse. In this case, the data is from 100 ms before the pulse and one second after the pulse.

Looking at the time-series can be somewhat challenging, because it is not always easy to interpret what is present. Due to the existence of so many channels in a clinical EEG, the amount of information is significantly high. Figures 4.1a and 4.1b are an example of how complicated an analysis can be for identifying any distinctive responses throughout the time-series. This was, however, the method chosen by Valentín *et al.* [12] in the analysis of their data.

The use of a topographical plot, showing all 64 electrodes, can provide a clear and accessible way to interpret these results. One of the disadvantages is the loss of



(a) First 32 channels.



(b) Remaining 32 channels (numbers 33 to 64).

Figure 4.1: EEG time-scale results obtained from 64 channels from an epilepsy patient, after baseline removal, interpolation, and filtering. Results from patient number 4 with stimulation site MCL.

time resolution, because in order to decrease the number of images and not create a topographical plot for every millisecond, we define time intervals in which the signal is averaged over said time period. Yet, space resolution is gained because it is possible to clearly identify what occurs in each electrode in certain time frames.

The signals are acquired by samples and not time. Due to this, despite having the intervals defined as 5 ms in figure 4.2, the conversion from samples to time leads to an occasional rounding down, where intervals will only be of 4 ms.

In figure 4.2 it is possible to identify the peaks P30 (29 to 34 ms), N45 (44 to 49 ms), and P55 (54 to 59 ms) in channel Cz. Figure 4.3 has the additional peaks N100 (98 to 146 ms) and P180 (146 to 195 ms). Since the representation is done in intervals of 50 ms, the peaks represent the average of that time frame and therefore it could be more difficult to identify them.

Dipoles can be identified in the various topographical plots. There is a clear separation between negative and positive amplitudes and these amplitudes are located very short distances from each other. In reality, a true quantitative measure cannot be extracted from these results because they demonstrate what is already seen in the time-series figures. The construction of the topographical images is based only on the

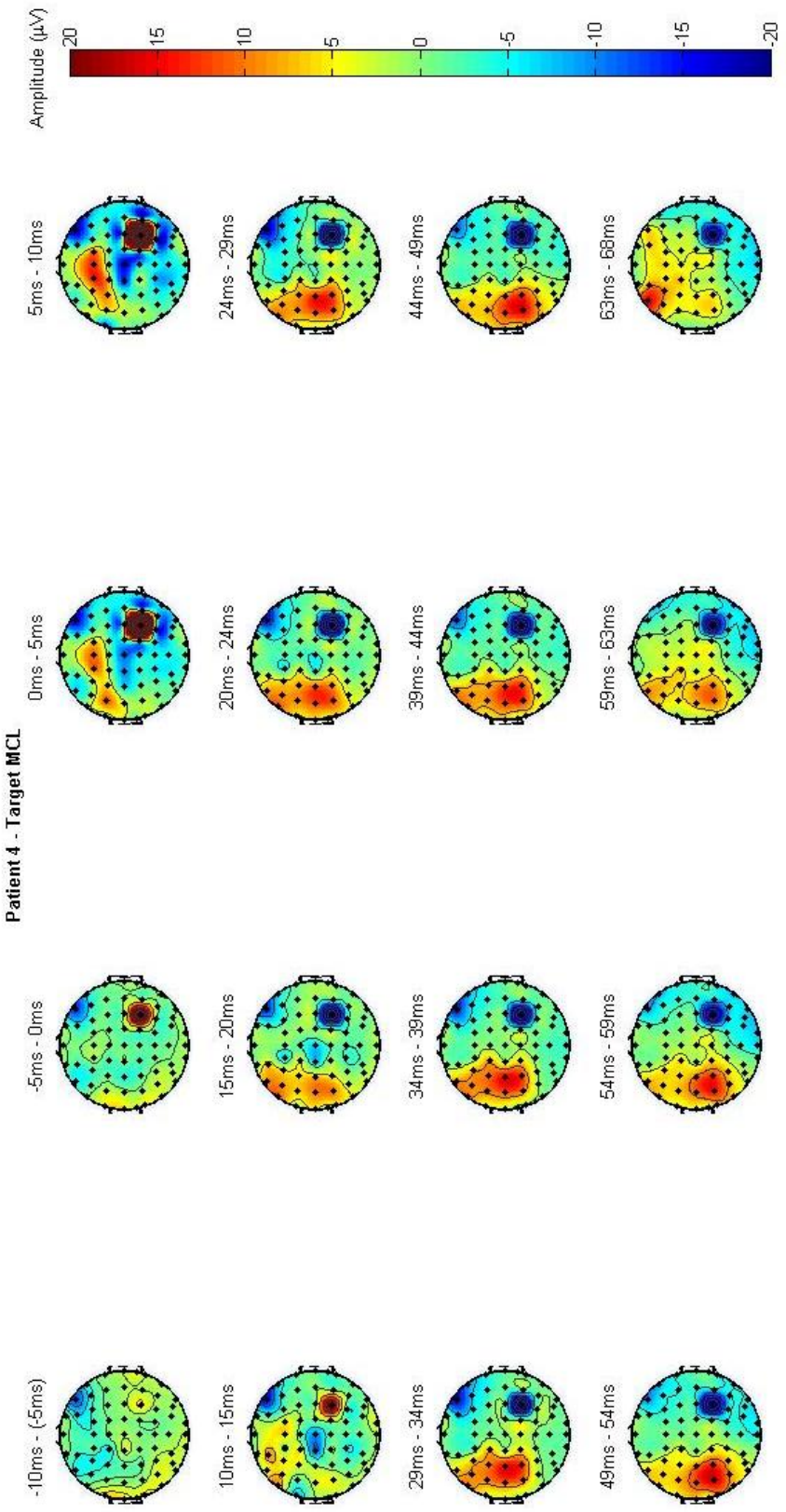


Figure 4.2: Topographical plot showing amplitude with 4 or 5 ms intervals, from -10 to 68 ms. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 2 s. Results from patient number 4 with stimulation site MCL.

Figure 4.3: Topographical plot showing amplitude with 50 ms intervals, from -10 to 928 ms. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 2 s. Results from patient number 4 with stimulation site MCL.

information from the time-series. Both figures 4.2 and 4.3 show which areas/channels of the configuration are activated negatively or positively in certain time intervals and how big that contribution is to the whole response. Localization is an important aspect to determine, as Ilmoniemi *et al.* [6] reported. The response to the TMS stimulation of the motor cortex can, in fact, be observed. These initial results were shown in a poster presentation at the 14th edition of the annual international clinical symposium "Epilepsy, sleep, and neurocognition" [75].

In comparison to figure 4.2, figure 4.3 allows the identification of all of the characteristic peaks found in the time-series figure 2.10 in channel Cz. The time interval has increased to include all the data available, however, the time resolution has diminished. Nevertheless, it is possible to notice a late response in the stimulation site, in this case, on the right side starting at around 600 ms.

After some processing, figure 4.4 is obtained, containing information in the time domain regarding the amplitude of the response in channel Cz as well as how these responses can be seen on a series of topographical plots. This combination enables one to see that channel Cz is representative of the TEP, even if there are some slight time shifts in relation to the results found in literature [51]. The plots enable a much clearer view of what occurs in the response to the TMS. Even though the analysis here is specific to the early response period, where the TEP is located, it gives an idea of how the responses are portrayed in both time and space.

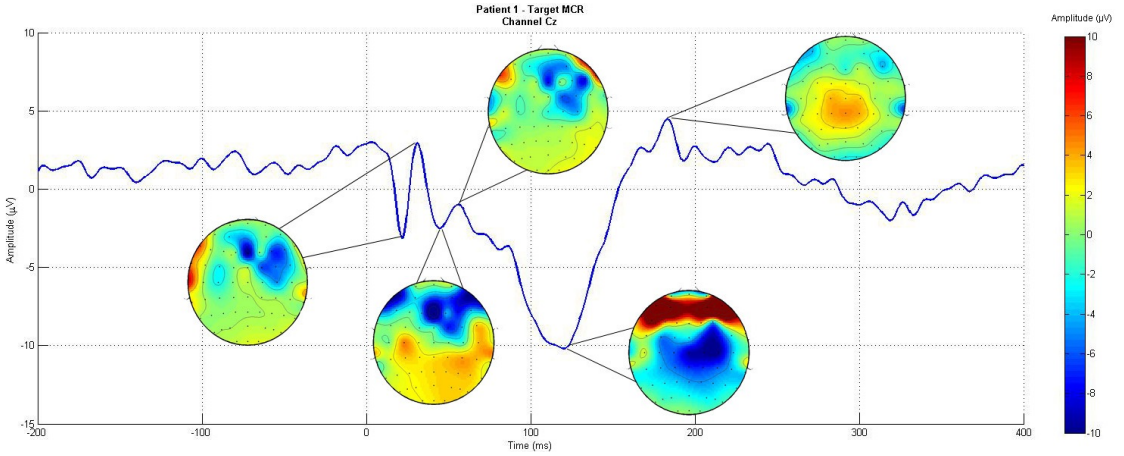


Figure 4.4: Time-series result for channel Cz, from -200 to 400 ms with images from the topographical plots for certain time periods. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 2 s. Results from patient number 1 with stimulation site MCR.

The representation of the EEG data time-series in topographical plots enabled the understanding of what was happening each channel. It was not the ideal method for obtaining a quantitative method, however, it assisted in determining what the next step would be. This method was therefore abandoned, so that it would be possible to obtain the best representation of the responses: calculating the power. By pursuing this new method, it will ensure that any averages that are calculated will not result in a null outcome.

4.1.2 Global Mean Field Amplitude

Scalp potential maps and cortical current distribution density are often plotted in order to estimate activation sites [5, 8]. This provides a better understanding of the electric component of the response evoked by TMS and how the currents are mapped. In order to analyze the data acquired based only on the potential difference, calculating the power was the method chosen. Since GMFA is directly related to power measurements, this will be the way to determine the responses for an established time interval.

By observing the results calculated using equation 3.1 for GMFA, with the use of 61 channels we determine if there is a value above the baseline, previously defined, in the late response period, as stated in chapter 3. The construction of the graphs enables a clear visualization, in time, of the power occurrences. This is for an initial assessment of how power is affected throughout the established time frame.

It is possible to see the early responses for all epilepsy patients and healthy subjects, just like it was expected [12], and in some cases, the TMS pulse is clearly visible near zero seconds in the time scale. Around 800 ms after the pulse a difference between the EEG data and the baseline is visible. There are six patients out of eight that show this difference, even if at times it is hard to identify, only patient number 6 and 7 did not have this difference. This is observable in figure 4.5; however, in some cases the difference of the response from the baseline is difficult to identify, even though it is present.

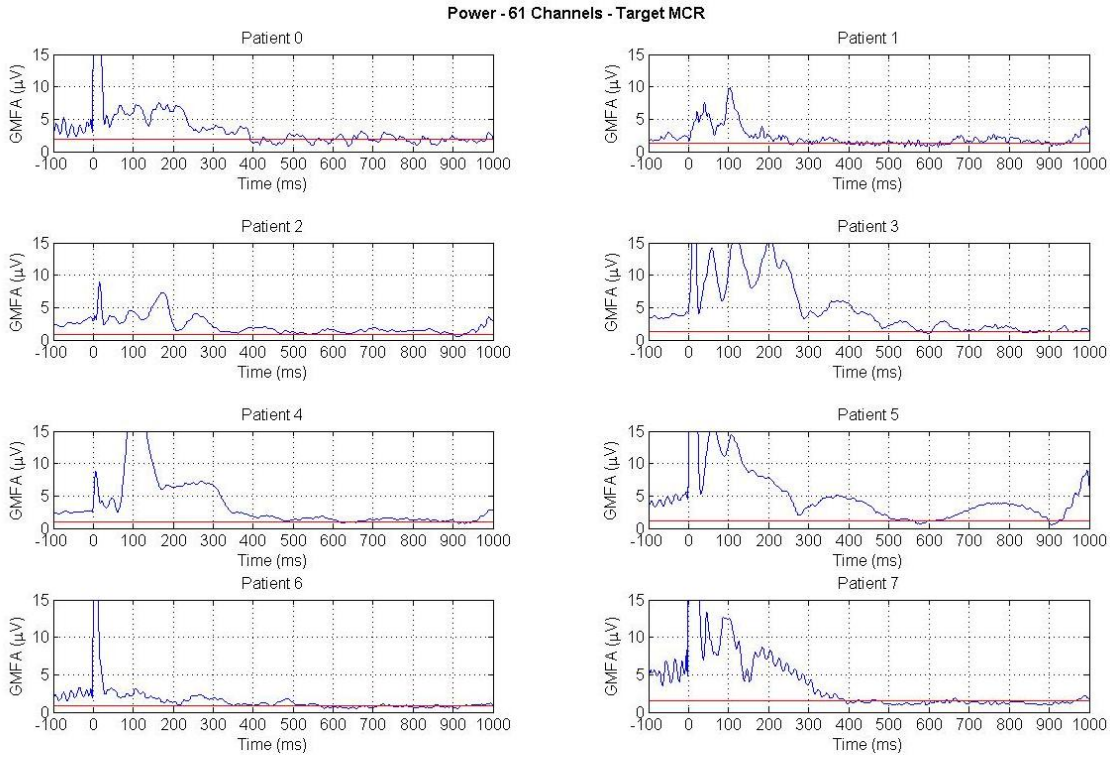


Figure 4.5: Power graphs which quantify the GMFA results obtained for all 61 channels in 8 patients. The baseline from 800 to 200 ms before the pulse is in red and the GMFA is in blue. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 2 s. Results with stimulation site MCR.

In analyzing the data from the healthy subjects only three out of nine show a similar response on the same side - MCR. The scale in the figures has been set to a maximum of 15 µV in order to have some uniformity in the information and to attempt the removal of

the artifacts originated from the TMS pulse, which return a very high power value.

Investigating in more detail the stimulation site, focusing on the nine channels surrounding the place that was stimulated enables a closer look at what happens on a local level. The representation now allows to see the direct influence of the neighboring channels in the stimulation.

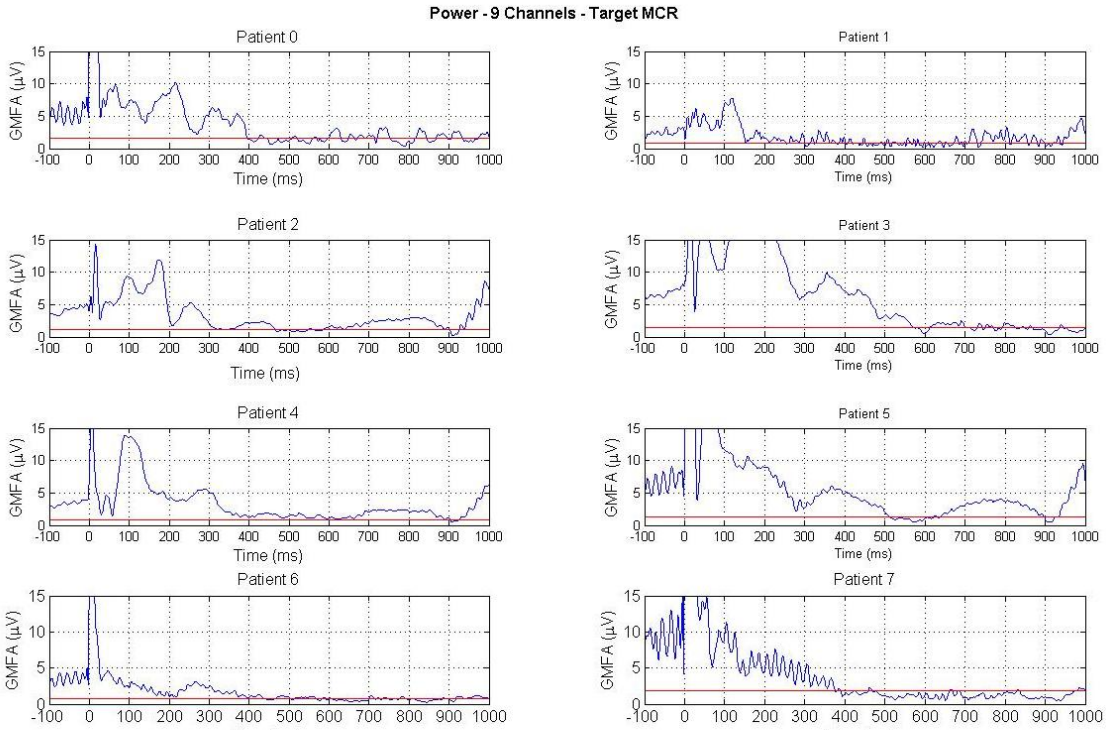


Figure 4.6: Power graphs which quantify the GMFA results obtained for only 9 channels surrounding the stimulation site in 8 patients. The baseline from 800 to 200 ms before the pulse is in red and the GMFA is in blue. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 2 s. Results with stimulation site MCR.

A summary of the previous results is presented in table 4.1. An example of how this change influences the outcomes for both stimulation sites is that for the epilepsy patients there is an increase in the number of patients that show some response around 800 ms.

This method does not necessarily give clear outcomes and interpretation. This is because a number of healthy subjects and patients show somewhat of a response near 800 ms and it is sometimes not very distinguishable from the baseline. Some of the less distinguishable peaks were still accounted for as responses in the late period. This led to a decision of not pursuing this method of analysis of the stimulation site for the healthy subjects.

The time resolution is maintained; however, it no longer becomes possible to distinguish between electrodes. The space resolution deteriorates because of the mathematical sum done for all electrodes. The purpose of this project is to attempt a quantification of the differences in epilepsy patients and healthy subjects, which means that this procedure is not suitable for this purpose because even though it was possible to determine when the differences occur, it would not be possible to know where - *which* channel caused such differences. For detecting and monitoring the neural circuits, localization is extremely important.

Table 4.1: Overview of the presence of late responses in epilepsy patients and healthy controls, using GMFA to calculate the power in 61 channels. Late responses are considered to exist around 800 ms. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 2 s. Results from stimulation site MCR.

	Epilepsy patients	**Healthy controls**
61 channels MCR	6/8	3/9
9 channels MCR	7/8	-
61 channels MCL	6/8	2/9
9 channels MCL	7/8	-

These results can be considered not very reliable, due to the non strict way of defining a response. In some cases the response was not above the defined average baseline, even if a peak was visible. This means that the baseline has power with higher or similar values to the late response period data. Other situations (see patient number 0 in figure 4.6) showed a high variability in a small time window. This variability could be due to artifacts present even after signal treatment and processing.

In previous studies using GMFA as a basis for calculations, there is a steep increase of GMFA correlated to increasing intensities in motor cortex TMS [9]. Since we only stimulated at one intensity for each subject and each target, it is not possible to confirm this information. However, we can state that in patients with higher stimulation intensity, the GMFA values were not necessarily higher. A difference is visible between patient number 5 and 6 in figure 4.6, both had stimulation intensity of 100%. While patient number 5 has a clear response above the baseline around 800 ms, number 6 does not have this type of response.

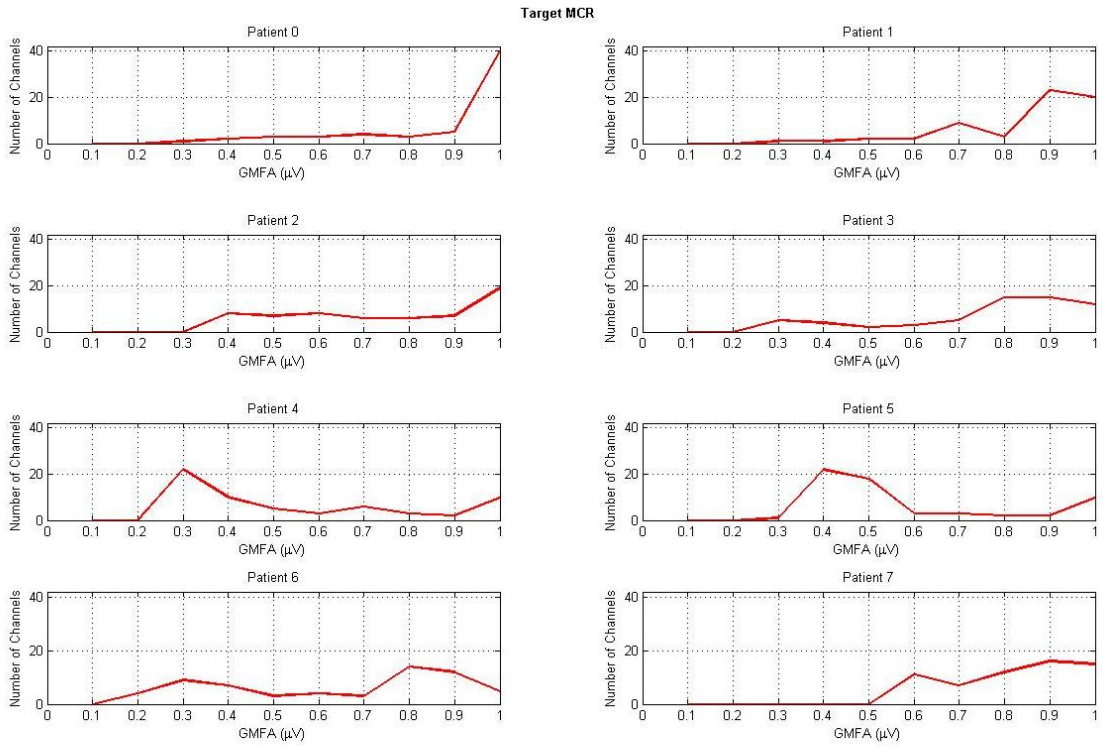


Figure 4.7: Number of channels in each interval of normalized power. Time interval is from 0 to 1 second after the TMS pulse. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 2 s. Sample of 8 patients with stimulation site MCR.

If there is a response identified, it is important to understand how large, in terms of area, it is. For this, we determined how many channels show a high power value. The power values obtained through GMFA for every electrode, are added from zero to one second after the TMS pulse, in what can be interpreted as a histogram. Results for each subject and target are normalized, with respect to the highest amplitude. To facilitate analysis, the outcomes are grouped in ten 0.1 intervals. The particularity of these graphs means that, for example, the plotting of the energies between 0.3 to 0.4 is done at 0.4. The outcome of this data treatment can be seen in figures 4.8 and 4.7.

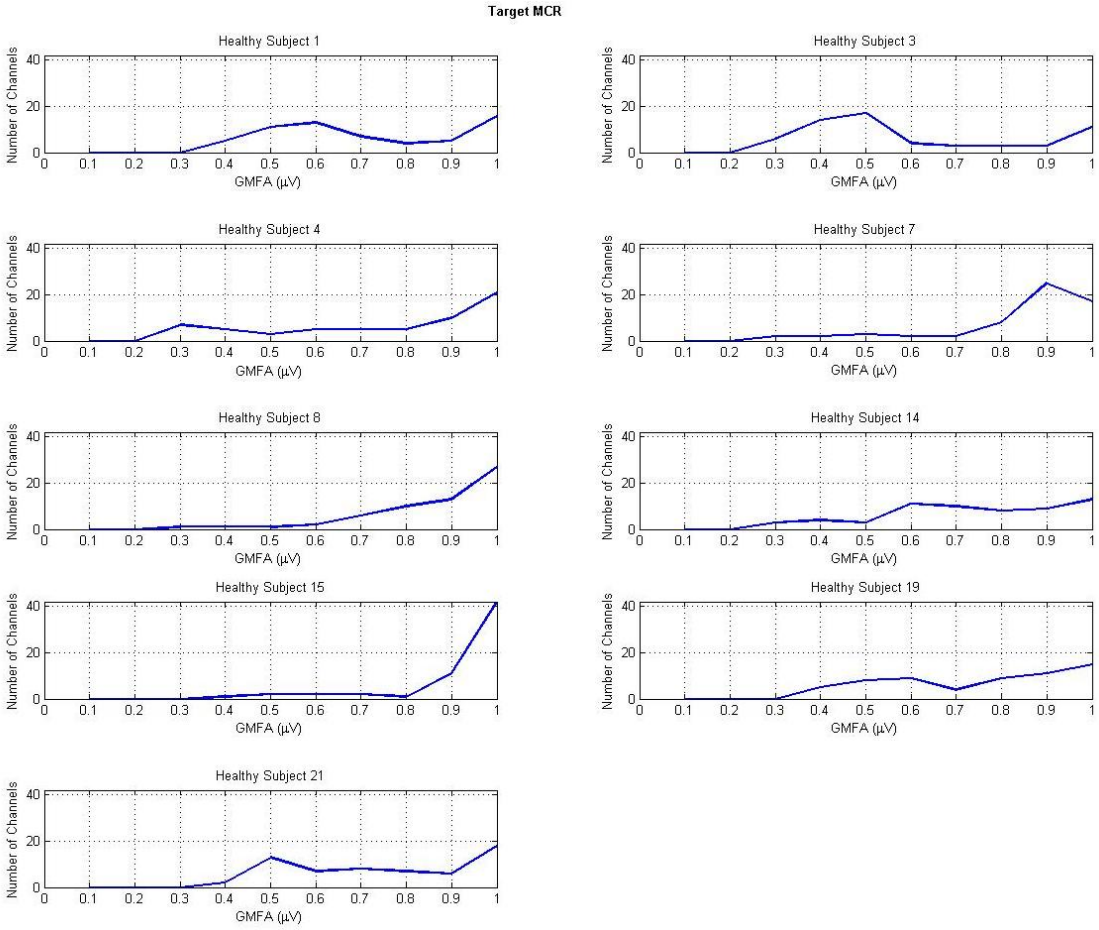


Figure 4.8: Number of channels in each interval of normalized power. Time interval is from zero to one second after the TMS pulse. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 2 s. Sample of 9 healthy subjects with stimulation site MCR.

After analysis of the figures, a trend is visible in the results. Only one of the healthy subjects shows a decrease in the interval 0.9 to 1.0, as seen in figure 4.8. The remaining healthy controls have the last value of the histogram as the highest peak. This means that there are more channels with a higher energy.

Half of the patients, see figure 4.7, show a decrease in the same interval (0.9 to 1.0). The highest peak is located at lower energies. Thus, it is logical to reach the statement that more channels in the healthy controls have higher energies. This would go against the predicted outcome of expecting higher energies in patients. Our expectation would arise from the evoked response from the TMS, which by measuring the excitability should be higher in epilepsy patients. However, there may still be artifacts which have not yet been

removed from the signal. This possibility will be addressed later on.

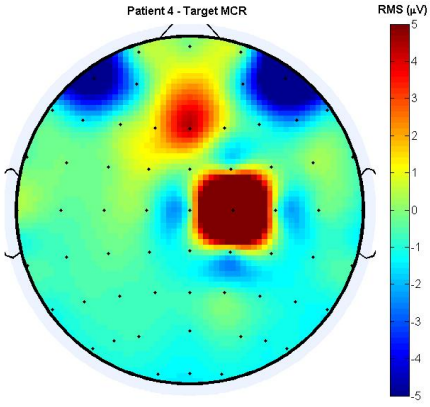
The combination of these methods does not enable a determination of where the response is located because the spatial resolution is lost. Nonetheless, a different type of information regarding the size of the area that is influenced by the pulse, is provided, even if the channels are not all neighbors.

4.1.3 Root Mean Squared

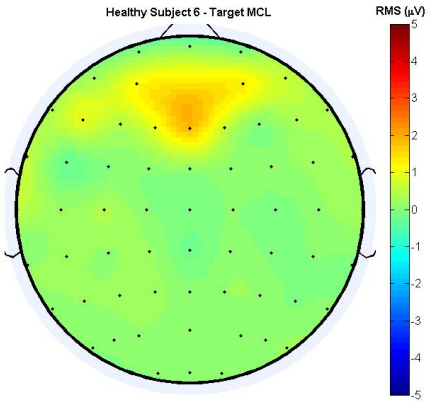
Through logical reasoning from the theoretical concepts explained for the basis of epilepsy, it is to be expected that there will be higher power differences in patients due to the higher excitability of epilepsy and the fact that TMS is used to provoke that excitability. However, the distinguishing feature could also be in the form of a greater number of electrodes being stimulated, even if the power difference is not higher.

Based on these results, some of the advantages of calculating power and plotting topographical data are joined in one method. The development of this process was not initially planned, but was reached after the first outcomes. This provides a illustrative measure of power, given by equation 3.2 due to the fact that RMS is directly related to power measurements. The use of RMS comes into play because the results that are needed to obtain need to be per channel, and GMFA does not offer that outcome. After subtracting the baseline results from the late response period as defined in figure 3.11, the plots were constructed.

The examples in figure 4.9 show a patient and a healthy subject with their corresponding late period responses. By carefully interpreting the plots provided by figures 4.9a and 4.9b, a clear difference is present and distinguishable. The plot of patient number 4 shows a response on the right side, where the stimulation was performed. The power difference value is clearly above 5 µV. In healthy subject number 6, no response near the stimulation site is visible when using the same scale.



(a) Patient number 4 for stimulation site MCR. A response is found at channel C2, which is near the stimulation site.



(b) Healthy subject number 6 for stimulation site MCL. No response is visible near the stimulation site.

Figure 4.9: Topographical plot showing the absolute power difference (post-stimulus result subtracted by baseline) calculated using RMS. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 2 s.

The definition of late TMS-EEG responses differs from Valentín *et al.* [12], that considered late responses seen with a variable latency greater than 100 ms and less than 1 s after TMS stimulus. In our definition the interval starts at 400 ms up to 950 ms. In the same way, delayed responses could be seen as clearly different from eye blinking or eye movement artifacts.

Careful analysis of the figures obtained through the developed script was performed. All patients showed a late response when results are combined for MCL and MCR stimulation, with five out of eight showing this late response in both TMS targets. In the healthy subjects, only one out of eighteen showed a late response for both targets, however, nine of those eighteen showed a response for MCR or MCL stimulation.

For these outcomes, it is important to establish that when observing the resulting topographical plots, only the absolute differences that were greater than |2| µV were considered as a response. This decision was established after rigorous analysis of the data and where the most significant difference between both study groups was present. There was a clear separation of the subjects with responses from those that were defined as not having a response, which means that the latter group had values much smaller than |2| µV.

A summary of these results can be found in table 4.2. The calculations also involve determining if the absolute difference in power from the baseline to the late responses is significant or not. This is done through the use of the t-test defined in chapter 3 by equation 3.3.

All of the results that represent a response were determined to be statistically significant, with $p < 0.01$. These initial results were submitted as an abstract and accepted for a poster presentation which will take place at the 66th Annual Meeting of the American Epilepsy Society in November 2012 [76].

Table 4.2: Overview of the presence of late responses in epilepsy patients and healthy controls. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 2 s. For $p < 0.01$ and RMS values > |2| µV.

	Epilepsy patients	**Healthy controls**
Late response MCL	7/8	5/18
Late response MCR	6/8	5/18
Late response MCL or MCR	8/8	9/18
Late response MCL and MCR	5/8	1/18

In this situation, it has been possible to maintain the spatial resolution and some of the temporal resolution because the time frame has been defined to observe only the late response period (i.e. by averaging the values for the time interval). For every subject there is a distinct baseline, due to the fact that every individual's background EEG signal is slightly different, it was decided not to use relative power difference, but the absolute value. In this manner, even if the difference is from 100 to 110 µV or from 20 to 30 µV, the topographical map will always show an increase of ten microvolts.

4.1.4 Modification of Initial Definitions

After a closer analysis to these results, we explored variations in the initially defined parameters in order to confirm that the results are in fact present in any circumstance and originate from the response given to the TMS pulse. This means that the epochs were altered to four seconds, as was discussed in chapter 3.

In figures 4.10 and 4.11 it is possible to see that when the epochs, over which the filter is applied, are smaller, the filters create artifacts at the endpoints. These artifacts

will interfere with the time periods defined in figure 3.11. This can cause the signal to create a curve going up or down at either or both of the endpoints. This does not occur for all signals we analyzed, which justifies our initial approach of two second epochs. Consistency is necessary and therefore this will be applied to the EGG signals of all individuals.

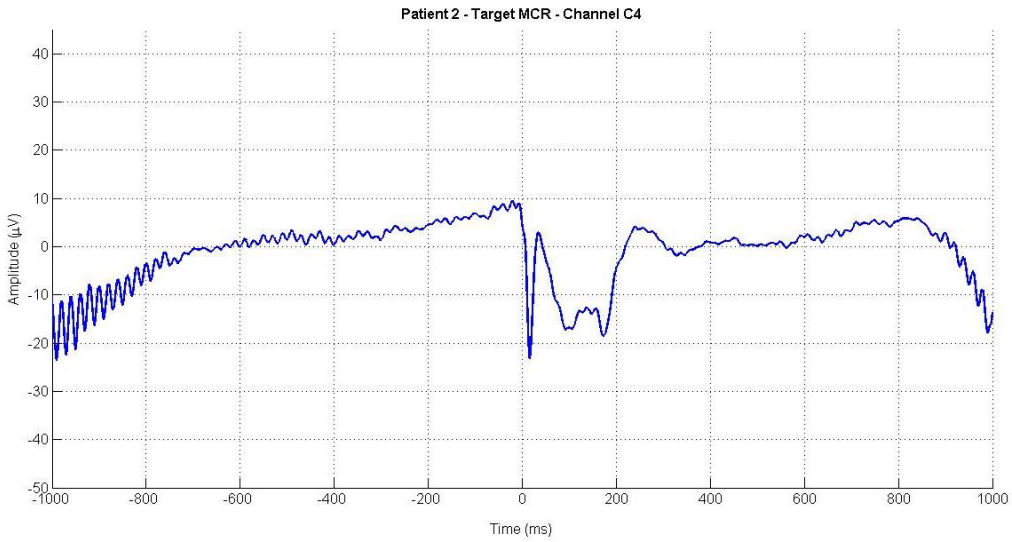


Figure 4.10: Average signal consisting of epochs of two seconds, at channel C4 for patient number 2 with stimulation site MCR. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz.

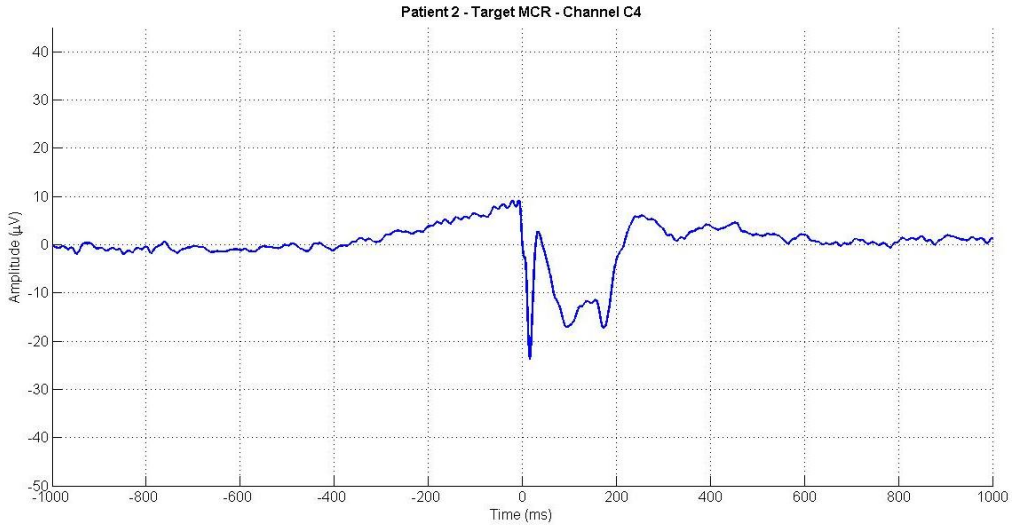
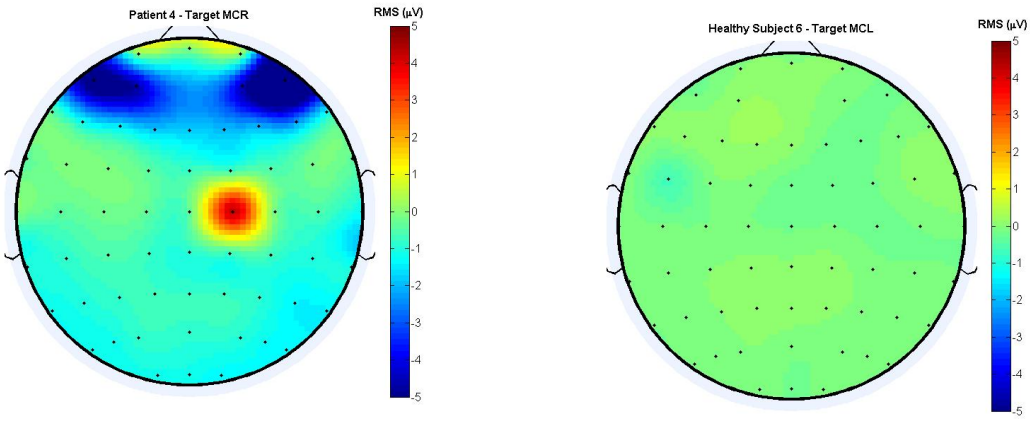


Figure 4.11: Average signal consisting of epochs of four seconds, at channel C4 for patient number 2 with stimulation site MCR. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz.

In figure 4.11, the epoch was increased one second in both directions, which means that the artifacts are still present at the endpoints but do not influence the part of the signal before the TMS pulse that is the baseline and the late response period. Results of power can once again be seen in examples of both patients and healthy controls in figures 4.12a and 4.12b.

The response displayed by patient number 4 with stimulation site MCR is still present even through it has decreased in size and (see figure 4.9a), what could be considered as artifacts in the frontal channels, have also reduced. This means that the difference from the late response to the baseline is smaller. No change has occurred in healthy control number 6 except the decrease in power in a few of the frontal channels (see figure 4.9b). The scale of the colormap has been kept the same for better comparison.



(a) Patient number 4 with stimulation site MCR. The response displayed by patient number 4 is still present at channel C2, even through it has decreased in size.

(b) Healthy subject 6 with stimulation site MCL. No response in visible near the stimulation site.

Figure 4.12: Topographical plot showing the absolute power difference (post-stimulus result subtracted by baseline) calculated using RMS. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 4 s.

In analyzing the topographical plots for all individuals, the outcomes were those that can be found in table 4.3. For this method there was the inclusion of more patients, after acquisition of their EEG signal. An important note, however, is that due to a malfunction, data for patient number 8 with stimulation site MCR is not available. This leads to a number of total signals for MCR as nine, while for MCL it is ten. When considering either target the total number is ten and for both targets it is nine.

Only three out of nine patients showed this late response in both TMS targets, which is a decrease from previous results. In the healthy subjects, the number of responses has increased from the previous analysis. In the combination of both targets there were five out of eighteen controls showing a response and seven showed a response for either target. All of these results were determined to be statistically significant, with $p < 0.01$ and a response was defined as a power difference higher than $|1|$ μV. This change was made because overall the differences between the baseline and the late response period decreased significantly.

The first alteration - increasing the epoch interval - leads to what can be seen as a cleaner signal. Differences between patients and controls have a less defined line separating them.

One of the objectives of this project is to quantify the differences between these two groups, but the fact is that they may not exist and the power differences may be due muscle and filter artifacts, and even eyeblinks that were not previously removed. For each of the patients and healthy controls, the use of the NeuroCenter Viewer software enables the determination of which channels and trials should be excluded from the

Table 4.3: Overview of the presence of late responses in epilepsy patients and healthy controls. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 4 s. For p <0.01 and RMS values >|1| µV.

	Epilepsy patients	**Healthy controls**
Late response MCL	6/10	7/18
Late response MCR	4/9	5/18
Late response MCL or MCR	7/10	7/18
Late response MCL and MCR	3/9	5/18

subsequent analysis, and these can be seen in appendix C.

In analysis of the topographical maps in figure 4.13 some significant changes are visible in the responses that were stimulated by the pulse. For patient number 4, in figure 4.13a, the response earlier identified in channel C4 has disappeared (see figure 4.12a), even with the use of a smaller scale. The data no longer contains certain trials and channels, which means that a channel that used to show a response, such as channel C4, was highly contaminated with eyeblinks. This statement derives from the fact that this channel was not removed from the data. The topographical map for healthy subject number 6 still has no response, see figures 4.9b and 4.12b for comparison.

Other images have been included in these results so that it is possible to see that in some patients there are responses even after *cleaning* the signal, small or large - but nonetheless present. This is visible in figures 4.13c and 4.13e. The response can be positive or negative, relatively to the baseline. In the same manner, there are healthy controls that now have the presence of a response, such as number 12 with stimulation site MCL, see figure 4.13d.

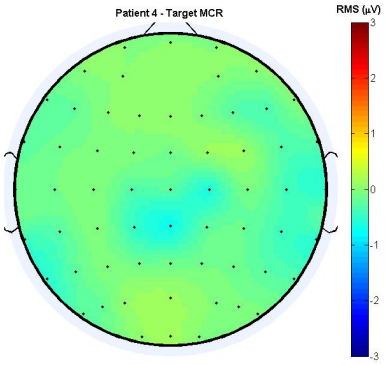
In the construction of table 4.4 we evaluated the numbers obtained in the calculation of the average power difference for the late response period and not just by analysis of the topographical plot. This is essential for confirmation of the responses evoked by TMS in a quantifiable manner and for the construction of a threshold to attempt the separation between healthy controls and epilepsy patients.

If the p-value is increased to 0.05 there is a difference for the stimulation site MCR represented as a increase in the number of healthy subjects identified as having a late response. A majority of the power values, either in patients and healthy controls, are situated around |1| µV, which makes it more difficult to establish it as the threshold. However, a decision was necessary and to evaluate the effect of the choice, we studied the sensitivity and specificity in subsection 4.1.5.

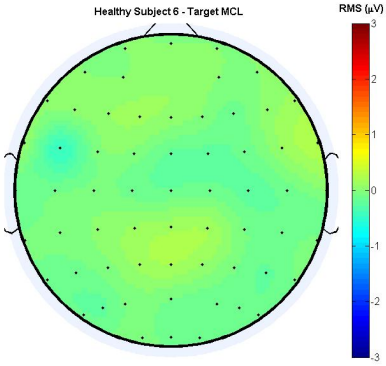
Table 4.4: Overview of the presence of late responses in epilepsy patients and healthy controls. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 4 s. After removed trials and channels. For p <0.01 and RMS values >|1| µV.

	Epilepsy patients	**Healthy controls**
Late response MCL	4/10	9/18
Late response MCR	4/9	7/18
Late response MCL or MCR	5/10	10/18
Late response MCL and MCR	3/9	6/18

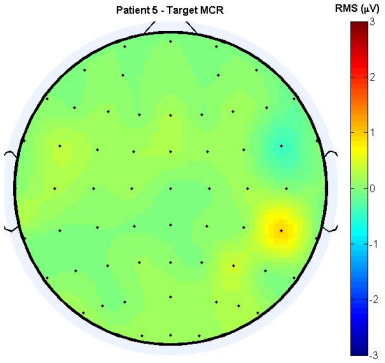
In the cases where responses are located on the other hemisphere or on another location of the scalp, i.e. differences in reactivity of different cortical areas, the reasons could be due to remote effects of TMS. There have been studies which explore the effects of TMS with functional MRI, showing that TMS activates not only the site of stimulation but also distant brain areas [9].



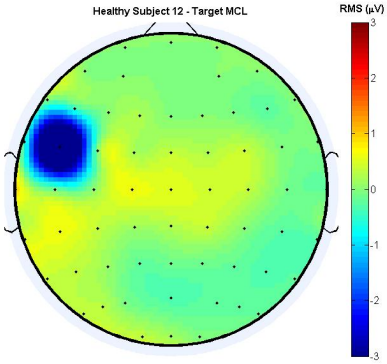
(a) Patient number 4 with stimulation site MCR. The response that was previously visible near the stimulation site has disappeared.



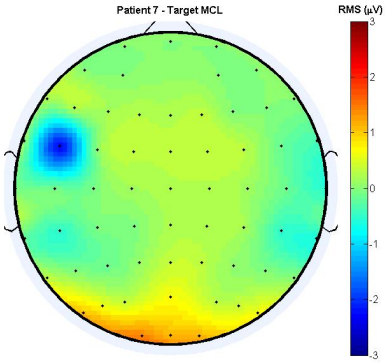
(b) Healthy subject number 6 with stimulation site MCL. No response in visible near the stimulation site.



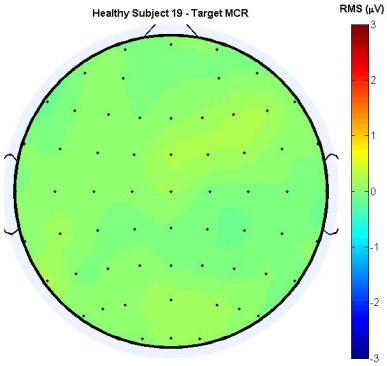
(c) Patient number 5 with stimulation site MCR. A response is visible near the stimulation site, at channel CP6.



(d) Healthy subject number 12 with stimulation site MCL. A response is visible near the stimulation site, at channel FC5.



(e) Patient number 7 with stimulation site MCL. A response is visible near the stimulation site, at channel FC5.



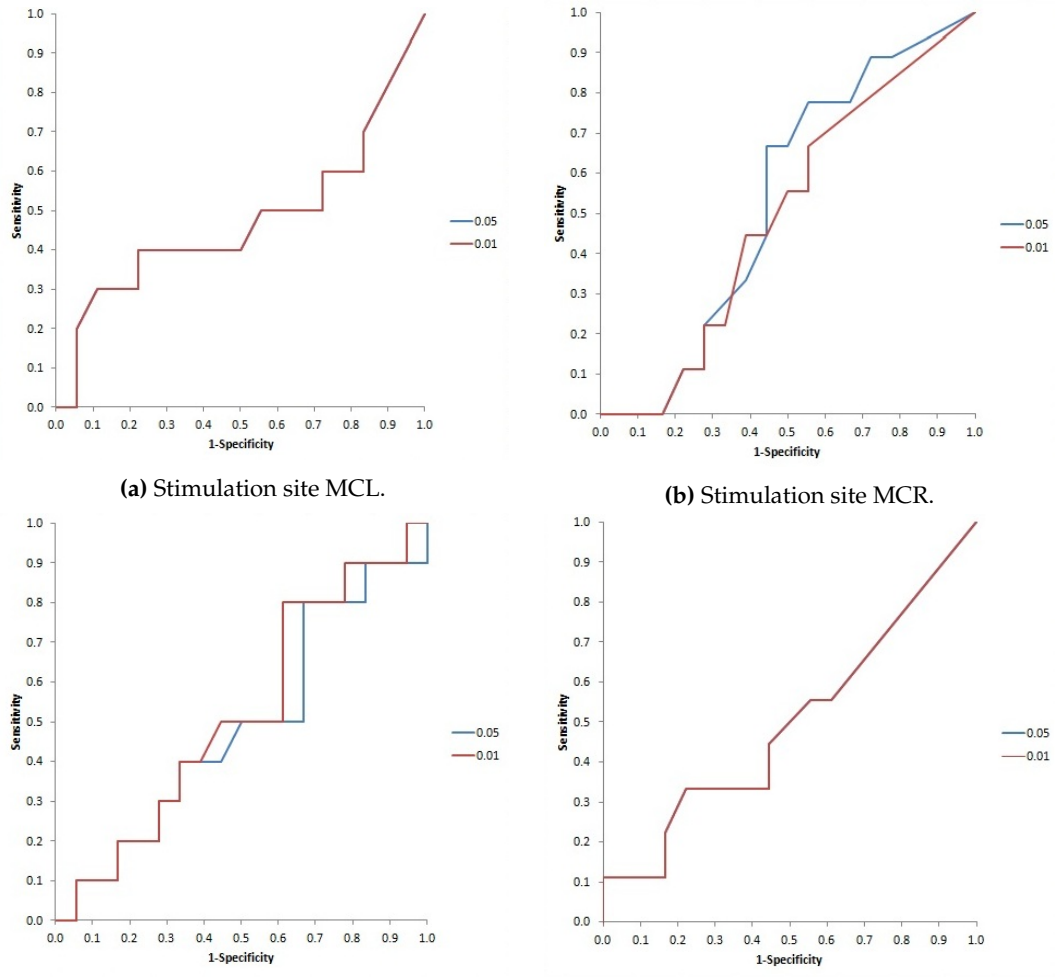
(f) Healthy subject number 19 with stimulation site MCR. No response in visible near the stimulation site.

Figure 4.13: Topographical plot showing the absolute power difference (post-stimulus result subtracted by baseline) calculated using RMS. Several examples of both stimulation sites. Filter definitions are band-pass 1 to 80 Hz and band-stop 49 to 51 Hz, with epochs of 4 s.

4.1.5 Specificity and Sensitivity

The definition of what is a response was subject of great struggle throughout this project. For the latest collection of results we decided to specify several thresholds of response in order to better understand how this definition influences the percentage of the results obtained. This means that for several values of calculated power, there will be a determination of the fraction of individuals that fall into the category of having a response. Starting at the highest value obtained, which for the healthy subjects it is 6 µV and for the patients it is 4.9 µV, there will be a decrease in the RMS value in steps of $|0.1|$ µV. This will enable the construction of ROC curves.

The stimulation area is defined as nine electrodes for each of the hemispheres (see figure 3.10). After determining the response values for every channel and the corresponding significance values, several thresholds which could separate the evaluation of the individuals that can be classified as patients and as healthy subjects, are defined. With two established significance levels, 0.01 and 0.05, only responses below those values will be assessed.



(a) Stimulation site MCL.

(b) Stimulation site MCR.

(c) Stimulation site MCR or MCL.

(d) Stimulation site MCR and MCL.

Figure 4.14: ROC curves constructed with the sensitivity and specificity results obtained from the several thresholds for determining a response and thus separate patients from healthy controls. The blue line represents a p-value of 0.05 and the red line represents a p-value of 0.01. When only one line is visible, the results are the same for both p-values.

The expectation is that there are no artifacts left after the removal of the eyeblinks and channels through the use of NeuroCenter Viewer and also by increasing the epoch interval. This, therefore, means that any response evoked on the opposite hemisphere of stimulation is evoked by the pulse and is not due to anything else that was noticeable in this analysis.

The ideal curve shows a high sensitivity when the specificity is high, or in other words, 1-specificity is low; and remains high when specificity decreases. Careful analysis of these ROC curves leads to stating that this method does not have enough quality to distinguish between patients and healthy controls. The sensitivity should be higher right at the first threshold analysis, which means more epilepsy patients should have an identifiable response above 1 µV. In the same manner, less healthy controls should have such a high response.

By observing figure 4.14a, when the stimulation site is MCL, there is no distinguishing feature separating the curve when the significance level decreases. For MCR (see figure 4.14b), one modification alters the ROC curve. It is expected that for a higher significance value (0.05), the likelihood of obtaining a test outcome given that the condition is present, increases.

4.2 Discussion

Patients included in this study had either generalized or focal epilepsy, there was no specific target group so that all the data acquired could be analyzed. When more EEG data is gathered, then there could be a separation of individuals based on epilepsy type. The comparison to Valentín *et al.* [12] is done, however, it is relevant to keep in mind that in their study only patients with focal epilepsy were included.

It is important to note that the development of the methods to be used in this project occurred as new data was becoming available. For this reason, not all subjects were evaluated for every quantification technique. This is true, for example, for patients number 8 and 11. Due to a recording malfunction there is no *.cnt* data file for patient number 8 at stimulation site MCR.

For both groups of subjects, the stimulation intensity was set to 110% of motor threshold (MT). This intensity value could go up to 100% of 1.5 T, which is the maximum allowed by the stimulator. In the study by Valentín *et al.*, if the MT exceeded 55% of the maximal stimulator output, then the procedure was abandoned for that particular individual. The intensity used for each stimulation was also 100% of the subject's resting MT. This difference may have lead to TMS output saturation and consequent heating, while Valentín *et al.* avoided such situations. However, there is no registered case of these events, so we assumed it was safe to keep the stimulation levels as they were.

Due to the differences in head size and the location of the motor cortex, there may be some variability of the stimulated area between the subjects [9]. This will be seen by the fact that the evoked response by TMS is not found in the same electrodes/channels for all individuals.

In our protocol there was an average of 75 single pulses performed at the different scalp positions. This differs from Valentín *et al.*, that used only 15 single TMS pulses. Our choice was made to ensure that enough trials were averaged, to improve the signal-to-noise ratio, on the signals that are analyzed. While we stimulated only in six specific locations: motor cortex, temporal lobe, and Brodmann's Area 19 - both hemispheres, Valentín *et al.* opted for a whole scalp analysis which is why there were successive series carried out, from left to right and from front to back. We aimed to study

specific sites in the brain, while the existing experiment did a general analysis of the whole scalp. This will most likely account for the differences existing between both sets of results.

A way to deal with the artifact problem is to exclude the channels that are strongly affected by said artifact. Yet, there is a problem with this solution because these channels are usually the ones closest to the stimulation site, thus they are usually the most informative about the early stages of response [5]. It becomes clear that some of the responses that disappeared after our modifications to the initial definitions were only present before due to these *bad* channels. However, because this was not visible in the initial analysis of the time-series, it was only in later stages of this project that we decided to investigate with the use of NeuroCenter Viewer.

By removing the trials that had a presence of eyeblinks, it becomes clear that a significant amount of data is removed from the signals acquired. This is visible in the four tables presented in appendix C. The removal of so many trials leads to a decrease in the signal-to-noise ratio. The influence due to eyeblinks originates in the frontal channels (see figure 4.12a) and can sometimes be seen in the back channels as well in the time series. However, because these channels are not taken into consideration in the study of the power around the stimulation site, we can assume that they will not *contaminate* the data. What occurs in the final results is that some of the responses that were present, for example in patient number 4 with stimulation site MCR (see figures 4.12a and 4.13a), disappear because of the signal-to-noise ratio, when in fact it should be counted as a response.

As Valentín *et al.* [12] stated in his study, the identified delayed TMS-EEG responses consisted of spikes or sharp waves which would sometimes resemble the patient's epileptiform discharges. These responses were seen with a variable latency, but were greater than 100 ms and less than 1 s after the TMS pulse. Taking into consideration the TEP, that identifies a positive peak at around 180 or 200 ms, it was our opinion that any response found in this time period would be considered an early response. With this approach, it was expected that the results obtained would be different than those that Valentín *et al.* displayed.

The approach of this project was based on obtaining a quantifiable measure of a response, in the late period of a stimulation. With this measure, an attempt would be made to distinguish between patients with epilepsy and healthy controls. The important word in this work is definitely *quantifiable* because in Valentín *et al.*, the delayed TMS-EEG responses seen in 11 out of the 15 patients and 0 out of the 15 healthy subjects were all obtained through observation of the time-series. This is the method currently used when looking for epileptiform discharges in the routine EEG, however, due to reasons discussed in chapter 2, a novel approach is necessary to remove subjectivity.

The ROC curve analysis is only performed for the nine electrodes around the stimulation site, as defined in figure 3.10, and there is no consideration of what is happening on the other side of the brain. In other words, if MCR is stimulated then only the right side of the brain is addressed. This is because it would be expected that if the stimulation is performed on that side then the response would originate there. By observing all of the topographical plots from both groups, in some situations it is clear that a response is also present in the other hemisphere and also in the mid-line electrodes. However, we made a choice, and in this case, only the highest value in the nine electrodes surrounding the stimulation side were used for analysis of responses. Any other approach is also valid.

It is possible to obtain a quantification measure of the TMS-EEG response through the use of power analysis. However, the calculation of the ROC curves discards this model as an optimum way of distinguishing between both groups. For diagnostic decision making it would be required to explore other options, including slight variations to the last established method.

These results may be influenced by many circumstances, namely the presence of AEDs and what can be determined as a response. AEDs regulate hyperexcitability, which means that since all the patients were taking them, and some have already been seizure free for some time, the excitability has reduced and there is no longer a difference between these epilepsy patients and the healthy controls. Why this occurs and further development of these suggestions are discussed in chapter 5.

5

Conclusions

A fundamental issue in neuroscience is understanding how large neuronal circuits work together in the brain, and what mechanisms underlie in this cooperation. That is the basis for all sensory, cognitive, and motor activities. The combination of magnetic stimulation with EEG recordings allows for the exploration of the brain's connectivity. By stimulating a particular area, the goal is to explore how the activity propagates to other areas, in this case, later in the response. This stimulation will excite the brain and the study of this excitation was the aim of this project.

Correct diagnosis directly after a first seizure would mean great health benefit, minimizing the risk of epilepsy-related accidents because AEDs could be prescribed immediately and also leading to the diminished use of unnecessary AEDs in patients who do not have epilepsy. Time and resources would be saved, thus implying great benefits in clinical practice.

During the development of this project, there were a number of contributions which enabled a better understanding of how excitation propagates in the brain, in particular, in the late response period after a stimulation. The analyses were built on foundations established from a project with a wider scope. Since a quantification technique for the response was obtained, in figure 5.1 there is an enumeration of the steps which led to the outcome. There are some ideas that can be developed in other studies related to the use of TMS-EEG in epilepsy.

Through the several methods developed and the logical steps that lead to the obtaining of the results, whether related to amplitude or power, in time or in space, there is a somewhat comprehensive study of the late responses in individuals who undergo TMS-EEG. Even though there are other features to be explored, the first logical step was to assess the amplitude in relation to the time-series and from there determine how power differs when compared to the time prior to the TMS pulse. Perhaps it would be beneficial to evaluate some of the initial methods with the new definitions. This would assist in confirming the results that were obtained, especially because the calculation of the power difference and its representation in topographical maps does not determine a threshold where it is possible to separate patient responses from healthy controls.

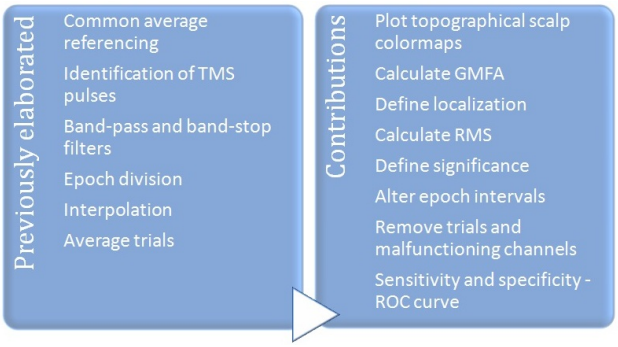


Figure 5.1: Previously elaborated steps to this project and the contributions in signal processing that this thesis made to obtain a quantification of the TMS-EEG response in epilepsy.

Considering what the final results were in chapter 4, subsection 4.1.4, no difference can be found between patients and the respective controls. The several established thresholds do not enable a clear separation, which means that in order to classify individuals as patients, some of the healthy subjects will also fall into that category. We were able to confirm this by studying the sensitivity and specificity. The ROC curve shows that the method developed leads to a poor classification of subjects. However, a true quantitative measure was obtained for the late response period, where the power calculations represent which channels remain activated 400 ms after the TMS pulse.

The results obtained are definitely influenced by the trials that were removed. This number varies between two and 59, which considering the majority of cases where the number of pulses is a total of 75, causes a significant decrease in the trials being subject averaging and analysis. Eyeblinks are an involuntary reaction which cannot be controlled because the stimulation evokes this response from patients as well as healthy subjects. Increasing the number of trials could increase the probability of the accepted number of trials. This would ensure a signal averaging with more data, and thus more representative of the response given by the individual.

Since the calculations were performed for late responses, as established in chapter 4, it could still be possible to determine these differences in the early responses. Ideally the modification to the initial definitions would have been made early on in the project, however, this was only explored after the first promising results arose and in a need to confirm what was obtained.

An important aspect that caused some struggle during the development of this project was the answer to the question *when is something a response?* Although it is clear that all patients and healthy subjects show some form of early response (up to about 400 ms), the purpose was to determine if there was something that would distinguish patients and healthy controls in the later responses. As such the definition was established as the following:

- Significant power change;
- Above a certain amplitude;
- Localized.

The definition of late and early responses could also be modified. In this project there was one definition, but this study could further be improved by exploring other alternatives of definitions, especially one that is more related to the definition made by Valentín *et al.* [12].

The patients involved in this study were all under medication, and this medication was not the same. Due to this, results may not show the difference between epilepsy patients and healthy controls. The fact is that, if the difference does in fact exist, it might not be visible because IEDs are not present. Some studies show [3] that there is a decrease in cortical excitability when AED use is present. The AEDs are thought to target voltage-gated channels, neurotransmitter receptors or both. They do this by modulating their cellular target in a way that is sufficient to change the pattern of the pre-existing cortical hyperexcitability found in epilepsy. These alterations to the excitability have the purpose of reducing or eliminating seizures which in turn could reduce or remove responses related to the TMS pulse.

The different types of epilepsy, generalized or focal epilepsy can also influence the type of response that is given. In this study both types of epilepsy patients are present, which means that, in case there are differences in the responses such as a partial epilepsy patient on the right side, shows only a response when stimulated at the motor cortex right. In generalized epilepsy patients responses, one would expect responses in both sides of the brain. The study by Valentín *et al.* [12] included only patients with focal epilepsy.

While Valentín *et al.* [12] determined there to be late responses (delayed or repetitive) simply by observing the time-series of signal, here there was an attempt to get a better visualization technique and to obtain a measurable quantity. These late responses, defined as clearly different from the background EEG (increased amplitude at particular frequencies) have not been able to be reproduced.

5.1 Future Work

In a first attempt to establish a method to quantify the TMS-EEG response, there is little information on which to begin with. Considering this, there is a lot that can still be done in the analysis of these responses. Yet, the approach which is taken can result in different outcomes, however, if the response is there it is most likely that it will still be there under any circumstances.

In many situations, information is not clearly seen in the time-domain but can be seen in the frequency-domain. A wavelet is a short mathematical function that represents a wavelike oscillation with a amplitude that starts at zero, increases, and then returns to zero. This function can be scaled and translated. It is possible to combine wavelets (using a technique called convolution) with unknown signals, in order to gather information about those unknown signals [77, 78]. They take any signal and express it in terms of scaled and translated wavelets, by cutting up data into different frequency components.

Wavelet analysis is becoming a more commonly used tool for the evaluation of localized variations of power within a time series. The decomposition of a time series into time-frequency space enables the determination of both the dominant modes of variability and how these vary in time [79]. An advantage, in comparison to the Fourier transform (FT), is in analyzing physical situations where the signal contains discontinuities and sharp spikes, because wavelet analysis requires substantially fewer wavelet basis functions that sine-cosine functions in order to obtain a comparable approximation.

There was a slight approach to the wavelets but the pursuit was discouraged. This was due to time constraints, and also the need to investigate the reproducibility and validity of the absolute power difference obtained through the topographical plots.

An important aspect of scalp topography is the rhythms, such as delta (δ), theta (θ),

alpha (α), beta (β), and gamma (γ). By looking at the different frequency bands, as seen in Table 2.1, it might be possible to identify the contribution of each to the response. These should enable a better comprehension of what occurs at each frequency and if the difference between epilepsy patients and healthy controls is perhaps situated. Therefore, further investigation of the frequency bands would be beneficial in order to determine if there is a specific frequency that has a bigger or smaller response to the stimulation.

A further work suggestion also includes a pilot study with the inclusion of individuals who have has their first or second seizure and it is suspected that they have epilepsy. This means the diagnosis is not yet definite, however, it will ensure that no AEDs have been administered and thus the analysis will be performed on the EEG signal of a patient with epilepsy with no reduction in excitability. With this it will be easier to understand how the hyperexcitability due to epilepsy originates a response. A follow-up examination one or two years later would monitor the differences if these are present in the first measure.

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Appendix

Adapted questionnaire from Van Strien [69] used to confirm handedness. It is necessary to obtain a minimum score of 9 for healthy subjects.

Vragenlijst handvoorkeur

Met de onderstaande vragenlijst kunt u bepalen hoe uitgesproken links- of rechtshandig u bent. De lijst bestaat uit één vraag over de hand waarmee u bij voorkeur schrijft en tien vragen met betrekking tot uw voorkeurshand voor andere handelingen. Geef voor elke vraag aan met welke hand u betreffende handeling gewoonlijk uitvoert.

Schrijfhand

Omcirkel met welke hand u schrijft:

links rechts op school gedwongen rechts te schrijven

Handvoorkeur

Hieronder staat een aantal activiteiten die u met uw linker of rechterhand kunt uitvoeren. Omcirkel welke kant u gewoonlijk gebruikt voor elk van deze activiteiten. Indien u hent antwoord niet meteen weet, voer dan de betreffende handeling in gedachten uit. Heeft u geen duidelijke voorkeur, omcirkel in dat geval 'beide'.

1. Met welke hand tekent u?	linker	rechter	beide
2. Welke hand gebruikt u om met een tandenborstel te poetsen?	linker	rechter	beide
3. In welke hand houdt u een flesopener vast?	linker	rechter	beide

4. Met welke hand gooit u een bal ver weg?	linker	rechter	beide
5. In welke hand heeft u een hamer vast als u ermee op een spijker moet slaan?	linker	rechter	beide
6. Met welke hand houdt u een (tennis-)racket vast?	linker	rechter	beide
7. Welke hand gebruikt u om met een mes een touw door te snijden?	linker	rechter	beide
8. Welke hand gebruikt u om met een lepel te roeren?	linker	rechter	beide
9. Welke hand gebruikt u om met een gummetje iets uit te vlakken?	linker	rechter	beide
10. Met welke hand strijkt u een lucifer aan?	linker	rechter	beide

Scoring

Om de totaalscore op de tien items te bepalen, geeft u het antwoord 'linker' de score -1, 'beide' de score 0 en 'rechter' de score +1. De score kan variëren van -10 voor extreme linkshandigheid tot +10 voor extreme rechsthandigheid. De schrijfhandvoorkeur wordt niet in de totaalscore betrokken. De overgrote meerderheid van de rechtsshrijvenden zal in de range van +8 tot +10 vallen.

Bron

Van Strien, J.W. (1992). Classificatie van links- en rechtshandige proefpersonen. Nederlands Tijdschrift voor de Psychologie. 47. 88-92.

Original questionnaire version by Van Strien [69] regarding handedness.

Handedness questionnaire

With this questionnaire you can measure the extent of your left- or right-handedness. This questionnaire contains one question about the hand you preferentially use for writing and ten questions about your hand preference for other activities.
Writing hand

Circle which hand you use for writing:

left right forced to use the right hand in school

Hand preference

Below, a number of activities are listed that you can perform with either your left or right hand. Indicate which hand you usually use for each of these activities. If you do not immediately know the answer, imagine performing the activity. Only if you have no clear preference, circle 'both'

1. Which hand do you use to draw?	left	right	both
2. Which hand do you use to brush your teeth?	left	right	both
3. Which hand do you use to hold a bottle opener?	left	right	both

4. Which hand do you use to throw a ball far away?	left	right	both
5. Which hand do you use to hammer a nail?	left	right	both
6. Which hand do you use to hold a (tennis) racket?	left	right	both
7. Which hand do you use to hold a knife when cutting a rope?	left	right	both
8. Which hand do you use to stir with a spoon?	left	right	both
9. Which hand do you use to hold an eraser when rubbing out something?	left	right	both
10. Which hand do you use to hold a match while striking it?	left	right	both

Scoring

Left = -1, right = +1, both = 0. The score varies from -10 for extreme left-handedness to + 10 for extreme right-handedness.

Reference

Van Strien, J.W. (1992). Classificatie van links- en rechtshandige proefpersonen. [*Classification of left- and right-handed research participants*]. Nederlands Tijdschrift voor de Psychologie. 47. 88-92.

B

Appendix

Below are the questions asked to the participants, both patients and healthy controls, in order to determine if they should be included or excluded in this study. Individuals with cardiac pacemakers, depth or subdural intracranial electrodes, other electronic implants such as vagus nerve stimulators or implanted metal structures in the brain were excluded [70].

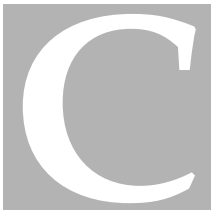
Vragenlijsten voor TMS proefpersonen

1. Heeft u epilepsie of heeft u ooit een aanval of insult gehad?	Ja / Nee
2. Heeft u ooit een flauwte of syncope gehad? Zo ja, beschrijf de omstandigheden (achterzijde formulier).	Ja / Nee
3. Heeft u ooit ernstig letsel (gevolgd door bewustzijnsverlies) aan het hoofd gehad?	Ja / Nee
4. Bent u in verwachting, of is er een kans dat u dit misschien bent?	Ja / Nee
5. Heeft u iets van metaal in het hoofd (behalve titanium)? (Bijvoorbeeld splinters, fragmenten, clips, enz.)	Ja / Nee
6. Heeft u een cochleair implantaat?	Ja / Nee
7. Heeft u een geïmplanteerde neuro-stimulator? (bijv. DBS, epiduraal/subduraal, VNS)	Ja / Nee
8. Heeft u een pacemaker of draden in het hart, of metaal ergens anders in het lichaam?	Ja / Nee
9. Heeft u een infuussysteem voor medicijnen?	Ja / Nee
10. Gebruikt u medicijnen? (Graag opschrijven op achterzijde formulier)	Ja / Nee
11. Heeft u ooit een operatie aan uw ruggenmerg ondergaan?	Ja / Nee
12. Heeft u drains in uw ruggenmerg of ventrikels?	Ja / Nee
13. Heeft u ooit eerder TMS pulsen ondergaan?	Ja / Nee
14. Heeft u ooit een MRI scan gehad?	Ja / Nee

Translation of the TMS experiment questionnaire performed on all individuals, patients and healthy controls.

Questionnaire for TMS subjects

1. Do you have epilepsy or have you ever had a convulsion or a seizure?	Yes / No
2. Have your ever had a fainting spell or syncope? If yes, please describe the circumstances (back form).	Yes / No
3. Have your ever had severe (i.e., followed by loss of consciousness) head trauma?	Yes / No
4. Do you have any hearing problems or ringing in you ears?	Yes / No
5. Do you have metal in the brain/skull (except titanium)? (e.g., splinters, fragments, clips, etc.)	Yes / No
6. Do you have cochlear implants?	Yes / No
7. Do you have an implanted neurostimulator? (e.g., DBS, epidural/subdural, VNS)	Yes / No
8. Do you have a cardiac pacemaker or intracardiac lines or metal somewhere else in your body?	Yes / No
9. Do you have a medication infusion device?	Yes / No
10. Are you taking any medication? (Please write on the back of the form)	Yes / No
11. Did you ever have a surgical procedure performed on your spinal cord?	Yes / No
12. Do you have spinal or ventricular derivations?	Yes / No
13. Have you ever undergone TMS pulses?	Yes / No
14. Have you ever had an MRI?	Yes / No



Appendix

After the alterations performed on the definitions of filters and interval of epochs, it was decided that each signal should be closely analyzed. Using NeuroCenter Viewer, the TMS pulses were identified and if any artifacts are found in the two second epochs surrounding (one second before and one second after) the pulse that specific trial should be excluded. Any channel that is malfunctioning or with continuous artifact, it is also removed from the posterior analysis.

In this section it is possible to find information regarding all the channels and trials removed from the data and that were thus not included in the MATLAB offline analysis.

Patient Data MCL

patient no.	total trials	no. removed trials	removed trials	no. accepted trials	accepted trials	removed channels	removed channel no.	accepted channel no.
0	50	2	17 24	48	1:16 18:23 25:50	FC1	10	4:9 11:32 37:64
1	75	24	1 6 8 9 11 13 16 20 29 42 45 47 48 53 57:59 65 66 68 69 72 74 75	51	2:5 7 10 12 14 15 17:19 21:28 30:41 43 44 46 49:52 54:56 60:64 67 70 71 73	P3 CP3 F6 FC3 F1 C5 F4 F8 FC5	7:9 25 38 40 41 44 48	4:6 10:24 26:32 37 39 42 43 45:57 49:64
2	78	32	2 4 5 9 17 21 23 27 28 32 35 40 41 46 48 50 51 53 56:60 62 63 66 68 71:73 75 78	46	1 3 6:8 10:16 18:20 22 24:26 29:31 33 34 36:39 42:45 47 49 52 54 55 61 64 65 67 69 70 74 76 77	-	-	4:32 37:64
3	73	38	1 3 6 7 9 11 13:18 20 22 24 26:28 33 34 37:39 42:49 51 53 56:58 62 73	35	2 4 5 8 10 12 19 21 23 25 29:32 35 36 40 41 50 52 54 55 59:61 63:72	P3 CP3	25 41	4:24 26:32 37:40 42:64
4	75	59	1:3 5:7 9:16 18:21 27:34 36 39:42 44:53 55:60 64:75	16	4 8 17 22:26 35 37 38 43 54 61:63	-	-	4:32 37:64
5	75	34	1 2 4:6 13 14 21 22 24 26 28 29 33 35 37 38 40 43:45 47:52 55 59 61 67 71 73 74	41	3 7:12 15:20 23 25 27 30:32 34 36 39 41 42 46 53 54 56:58 60 62:66 68:70 72 75	T7 T8 P3 F5 F1 F2 F6 C2 C6 CP3 P5 P2 P6 PO5 PO3 FT7 FT8 TP7 TP8	14 18 25 37:40 46:48 51 53:56 59:62	4:13 15:17 19:24 26:32 41:45 49 50 52 57 58 63 64
6	74	15	2 19 22:25 27 36 42 50 52 63 67:69	59	1 3:18 20 21 26 28:35 37:41 43:49 51:62 64:66 70:74	FT7 T7	14 59	4:13 15:32 37:58 60:64
7	72	34	2 8 9 12 14 15 17 21:23 27 29 33:40 43 46:49 51:53 57:59 65 68 72	38	1 3:7 10 11 13 16 18:20 24:26 28 30:32 41 42 44 45 50 54:56 60:64 66 67 69:71	F4 F8 F6	7 8 40	4:6 9:32 37:39 41:64
8	75	53	1:10 12:19 23 24 30 32 33 35:37 39 41 43 45:47 52:56 58 60:74	22	11 20:22 25:29 31 34 38 40 42 44 48:51 57 59 75	F5 T7 FC5	9 14 37	4:8 10:13 15:32 38:64
11	62	22	1 3 4 7 13 15 17 19 22 24 36 40 41 43 44 52 56:58 60:62	40	2 5 6 8:12 14 16 18 20 21 23 25:35 37:39 42 45:51 53:55 59	-	-	4:32 37:64

Patient Data MCR

patient no.	total trials	no. removed trials	removed trials	no. accepted trials	accepted trials	removed channels	removed channel no.	accepted channel no.
0	32	4	29:32	28	1:28	-	-	4:32 37:64
1	75	9	6 16 21 43 49 57 58 63 64	66	1:5 7:15 17:20 22:42 44:48 50:56 59:62 65:75	F8 FC5 F4 F5 F6 C5 FC3 FT7 FT8 TP7 FC6 T8	7:9 12 18 37 40 41 44 59:61	4:6 10 11 13:17 19:32 38 39 42 43 45:58 62:64
2	75	26	1 8 10 11 18 20 23 25 29 36 38 41 48:53 56 58 60 63 68:70 75	49	2:7 9 12:17 19 21 22 24 26:28 30:35 37 39 40 42:47 54 55 57 59 61 62 64:67 71:74	-	-	4:32 37:64
3	75	27	2 6 8 9 13 14 17 19 20 24 25 29 30 33 35 37 43 45 49 53 57 59 62 66 69 73 75	48	1 3:5 7 10:12 15 16 18 21:23 26:28 31 32 34 36 38:42 44 46:48 50:52 54:56 58 60 61 63:65 67 68 70:72 74	-	-	4:32 37:64
4	90	46	1 3 6 7 10 13 14 19 21:30 32:34 38 39 41:46 48 51 53 56:58 65:67 69 71 73 74 76 85 86 89	44	2 4 5 8 9 11 12 15:18 20 31 35:37 40 47 49 50 52 54 55 59:64 68 70 72 75 77:84 87 88	F8	8	4:7 9:32 37:64
5	92	32	1 3 4 7 16 19 22 23 25 26 30:32 45 47 48 50 57 58 62 64:66 68 72 73 76 77 80 82 83 89	60	2 5 6 8:15 17 18 20 21 24 27:29 33:44 46 49 51:56 59:61 63 67 69:71 74 75 78 79 81 84:88	P3 T8 T7, FT7 FT8 PO5 PO3 TP7 TP8 F1 F2 F5 F6 C2 C6 CP3 P5 P6 P7 P8 P2	14 18 24 25 28 37:40 46:48 51 53:56 59:62	4:13 15:17 19:23 26 27 29:32 41:45 49 50 52 57 58 63 64
6	75	15	1:6 8 9 13 15 21 22 30 41 73	60	7 10:12 14 16:20 23:29 31:40 42:72 74 75	FT7 T7	14 59	4:13 15:32 37:58 60:64
7	80	44	1 4 6 8:11 13 14 16 18:20 22 24 26:30 32 33 35 41 42 47:50 52:56 58:60 63 65:67 69 72 75 76	36	2 3 5 7 12 15 17 21 23 25 31 34 36:40 43:46 51 57 61 62 64 68 70 71 73 74	-	-	4:32 37:64
8	-	-	-	-	-	-	-	-
11	75	37	1 3:5 7 8 10:12 14 16 20 22:24 28 32 40:43 45:49 53:55 58 59 61 63 65 66 68 71	38	2 6 9 13 15 17:19 21 25:27 29:31 33:39 44 50:52 56 57 60 62 64 67 69 70 72:75	C4 T8 FC6 F8 FT8 F6	8 12 17 18 40 60	4:7 9:11 13:16 19:32 37:39 41:59 61:64

Healthy Subject Data MCL

healthy sub. no.	total trials	no. removed trials	removed trials	no. accepted trials	accepted trials	removed channels	removed channel no.	accepted channel no.
1	75	54	1 4:18 21:29 31:34 36 40 41 43 44 46 48 49 51 52 54 56 57 59:63 69:75	21	2 3 19 20 30 35 37:39 42 45 47 50 53 55 58 64:68	-	-	4:32 37:64
2	75	47	1 2 4 6:8 11:14 16 18:20 22 23 30 32:35 37:39 44 46:49 51:54 56:59 61:63 66 67 70:72 74 75	28	3 5 9 10 15 17 21 24:29 31 36 40:43 45 50 55 60 64 65 68 69 73	FC2	11	4:10 12:32 37:64
3	75	55	1:5 8:15 19 20 24:27 29 31 33 34 37 38 41:45 47:51 53:56 59:69 71:75	20	6 7 16:18 21:23 28 30 32 35 36 39 40 46 52 57 58 70	FT8 T8	8 60	4:7 9:32 37:59 61:64
4	75	48	4 7 11 12 14 16 17 21 22 24 26 28 30:33 37 38 40:42 44:46 48:51 53:56 58:66 68 70:75	27	1:3 5 6 8:10 13 15 18:20 23 25 27 29 34:36 39 43 47 52 57 67 69	-	-	4:32 37:64
5	73	16	27 36 37 42 44 45 50 55 56 60 61 65 67:69 73	57	1:26 28:35 38:41 43 46:49 51:54 57:59 62:64 66 70:72	F8 F6	8 40	4:7 9:32 37:39 41:64
6	75	15	3 6 8 9 21 31 32 60 61 67 68 72:75	60	1 2 4 5 7 10:20 22:30 33:59 62:66 69:71	-	-	4:32 37:64
7	75	39	2:4 10:12 14 16 17 19 20 22 25 26 33 39 41 43:50 52:55 57 58 60 64:67 70 73 74	36	1 5:9 13 15 18 21 23 24 27:32 34:38 40 42 51 56 59 61:63 68 69 71 72 75	-	-	4:32 37:64
8	75	15	7 14 16 30 36:38 41 45 54 56 58 60 67 73	60	1:6 8:13 15 17:29 31:35 39 40 42:44 46:53 55 57 59 61:66 68:72 74 75	F8 F7 FC5 F6	4 8 9 40	5:7 10:32 37:39 41:64
10	79	14	1 5 17 22 33 35 39 42 47 60 66:68 71	65	2:4 6:16 18:21 23:32 34 36:38 40 41 43:46 48:59 61:65 69 70 72:79	C6 TP7 FT8 TP8 P7 T8 P8 FC6	12 18 24 28 47 60:62	4:11 13:17 19:23 25:27 29:32 37:46 48:59 63 64
12	75	40	1:4 6 9:11 16 19:21 23:25 27:30 35 40 42 43 45 46 51 59:63 65:73	35	5 7 8 12:15 17 18 22 26 31:34 36:39 41 44 47:50 52:58 64 74 75	F8 F7	4 8	5:7 9:32 37:64
13	75	11	2 3 6 15 41 46 48 54 60:62	64	1 4 5 7:14 16:40 42:45 47 49:53 55:59 63:75	CP6 F7	4 23	5:22 24:32 37:64
14	75	10	6 9 14 30 36 38 48 52 57 71	65	1:5 7 8 10:13 15:29 31:35 37 39:47 49:51 53:56 58:70 72:75	FC1 C1 FT7 T7	10 14 45 59	4:9 11:13 15:32 37:44 46:58 60:64
15	75	48	12 13 17:19 21:26 31:52 54:58 61 68:75	27	1:11 14:16 20 27:30 53 59 60 62:67	-	-	4:32 37:64
16	75	66	1 2 4:18 20:44 46:48 50 52:55 57:61 63 66:75	9	3 19 45 49 51 56 62 64 65	FC5 T7 FT7 T8 F8	8 9 14 18 59	4:7 10:13 15:17 19:32 37:58 60:64
17	75	5	5 19 55 62 63	70	1:4 6:18 20:54 56:61 64:75	-	-	4:32 37:64
18	74	5	26 37 47 50 52	69	1:25 27:36 38:46 48 49 51 53:74	-	-	4:32 37:64
19	75	18	19 32 34:36 47 51:53 58 62 67 68 71:75	57	1:18 20:31 33 37:46 48:50 54:57 59:61 63:66 69 70	P8 T7 T8 F5 FT7 FT8 F6 F8 F7	4 8 14 18 28 37 40 59 60	5:7 9:13 15:17 19:27 29:32 37:39 41:58 61:64
21	75	34	6 12 15 17 18 23 25 27 32 34:36 39 40 42 43 46 48 49 53 56:58 60 63:66 68:71 73 74	41	1:5 7:11 13 14 16 19:22 24 26 28:31 33 37 38 41 44 45 47 50:52 54 55 59 61 62 67 72 75	-	-	4:32 37:64

Healthy Subject Data MCR

healthy sub. no.	total trials	no. removed trials	removed trials	no. accepted trials	accepted trials	removed channels	removed channel no.	accepted channel no.
1	75	49	2 4 5 11 13 14 16:19 21:28 31:33 36:38 40:45 47 48 50 53 55 56 59 60 62:66 68:70 72 74 75	26	1 3 6:10 12 15 20 29 30 34 35 39 46 49 51 52 54 57 58 61 67 71 73	-	-	4:32 37:64
2	74	44	1:7 9:14 17 19 22 23 26:29 31 32 34 35 37:39 45:47 49 51 53 58:61 65 68:70 72 74	30	8 15 16 18 20 21 24 25 30 33 36 40:44 48 50 52 54:57 62:64 66 67 71 73	FC2	11	4:10 12:32 37:64
3	75	55	1:4 6:10 15:18 20:24 26 29 31:35 38:47 49:53 55:58 60 64:69 71:74	20	5 11:14 19 25 27 28 30 36 37 48 54 59 61:63 70 75	FT8 T8 T7 FT7	14 18 59 60	4:13 15:17 19:32 37:58 61:64
4	75	49	4:7 9 11 14 15 21 22 24:28 31:33 35:38 40:43 45:48 50 52:54 57 60:65 67:72 74 75	26	1:3 8 10 12 13 16:20 23 29 30 34 39 44 49 51 55 56 58 59 66 73	-	-	4:32 37:64
5	75	11	2:4 7 13 16 18 40 47 53 54 62 64 70 72 74	64	1 5 6 8:12 14 15 17 19:39 41:46 48:52 55:61 63 65:69 71 73 75	-	-	4:32 37:64
6	75	35	6 34 38 39 41 43 45 47 59 67 74	40	1:5 7:33 35:37 40 42 44 46 48:58 60:66 68:73 75	-	-	4:32 37:64
7	75	39	1 2 4 6 8:10 12 13 15:17 24 26 31:33 41 47:53 57:59 61 63:68 70:72 74	36	3 5 7 11 14 18:23 25 27:30 34:40 42:46 54:56 60 62 69 73 75	-	-	4:32 37:64
8	76	6	24 30 39 46 63 68	70	1:23 25:29 31:38 40:45 47:62 64:67 69:76	F6 F8 F7	4 8 40	5:7 9:32 37:39 41:64
10	75	12	20 23 28 31 35 39 49 57 64 67 69 71	63	1:19 21 22 24:27 29 30 32:34 36:38 40:48 50:56 58:63 65 66 68 70 72:75	TP7 FT8 FC6 T8 C6	12 18 47 60 61	4:11 13:17 19:32 37:46 48:59 62:64
12	75	47	1:7 9 11 13 14 16 19 23 25 27:29 31:41 43 46 48 50 55 58:67 71:73	28	8 10 12 15 17 18 20:22 24 26 30 42 44 45 47 49 51:54 56 57 68:70 74 75	F8 F7 F6	4 8 40	5:7 9:32 37:39 41:64
13	75	21	13 27 37 40 45 47 51:58 62 63 65 67 70 72 73	54	1:12 14:26 28:36 38 39 41:44 46 48:50 59:61 64 66 68 69 71 74 75	C4 FC4	17 43	4:16 18:32 37:42 44:64
14	74	17	7 10 14 17 20 27 30 41 45 47 50 56 59 68 69 72 74	57	1:6 8 9 11:13 15 16 18 19 21:26 28 29 31:40 42:44 46 48 49 51:55 57 58 60:67 70 71 73	-	-	4:32 37:64
15	75	58	2 3 7:16 18:21 23:30 32 33 35 38 41:44 46:49 53:62 64:75	17	1 4:6 17 22 31 34 36 37 39 40 45 50:52 63	-	-	4:32 37:64
16	75	66	1:10 12:27 30:43 45 48:50 53:56 58:75	9	11 28 29 44 46 47 51 52 57	FT8 F8 T8 F7 T7 FC6 FT7	4 8 12 14 18 59 60	5:7 9:11 13 15:17 19:32 37:58 61:64
17	75	6	18 38 42 59 73 74	69	1:17 19:37 39:41 43:58 60:72 75	-	-	4:32 37:64
18	75	19	2 3 10 27 29 34 36:38 53:56 62 68 70 71 73 74	56	1 4:9 11:26 28 30:33 35 39:52 57:61 63:67 69 72 75	-	-	4:32 37:64
19	75	32	3 9 14 15 17 18 20 22 27 30:34 37:39 41:44 46 48:55 71 73	43	1 2 4:8 10:13 16 19 21 23:26 28 29 35 36 40 45 47 56:70 72 74 75	P8 T7 FC5 FT7 PO8 PO7 TP8 TP7 P5 F5	9 14 37 38 51 59 61:64	4:8 10:13 15:32 39:50 52:58 60
21	75	48	1:5 7:11 13 15:17 20 21 23 27 30:35 37 38 42 43 45 46 48:52 54:59 62 65 68:70 74 75	27	6 12 14 18 19 22 24:26 28 29 36 39:41 44 47 53 60 61 63 64 66 67 71:73	-	-	4:32 37:64