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Time analysis of sensory gating using P50 evoked potential in patients with schizophrenia

Relatori
Prof. Enrico GRISAN
Prof. Paolo GARGIULO (Reykjavik University)

Studentessa
Elena PEGOLO

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Elena Pegolo

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Abstract

The present thesis is the result of my work completed during the Erasmus+ experience at Reykjavik University, Iceland. It is part of the ongoing "Icelandic AVH TMS" project which aims to study the effectiveness of repetitive transcranial magnetic stimulation (rTMS) treatment for schizophrenic patients with persistent auditory verbal hallucinations (AVH).

The objective of this thesis is the development of a methodology to be employed in the analysis of the neurophysiological data of the project. This was achieved using the software MATLAB and the application Brainstorm.

Data from 3 healthy subjects and 3 patients before the treatment were studied in order to evaluate the method developed. Preliminary results of one patient before and after the treatment are also reported. The signal we analysed was the P50 evoked potential; in particular, differences in sensory gating between the cohorts were investigated. The novelty of the project is given by the fact that data were acquired using high-density EEG (256 channels). Having this large amount of information, we managed to obtain topological representations of the P50 in different brain regions, for all the individuals.

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List of abbreviations

AM task - Auditory motor task

AVH - Auditory verbal hallucinations

EEG - Electroencephalogram

EP - Evoked Potential

ERP - Event-related potential

FDA - Food and Drug Admininstration

HD EEG - High-density EEG

HS - Healthy subject

MEP - Motor evoked potential

ROI - Region of interest

RU - Reykjavik University

rTMS - Repetitive transcranial magnetic stimulation

TMS - Transcranial magnetic stimulation

Chapter 1

Introduction

1.1 Schizophrenia

Schizophrenia is a chronic and severe mental disorder affecting more than 21 million people worldwide [25, 40]. The word *schizophrenia* means literally "split of the mind" (*schizo*=split, *phrenia*=mind), not to be confused with the split of the personality. Indeed, schizophrenia is characterized by distortions in thinking, perception, emotions, language, sense of self and behaviour.

Three major symptom domains can be observed in schizophrenia:

- **Negative symptoms** are related to the absence or the alteration of a normal process; they may include the loss of interest or emotions that the subject can express
- **Positive symptoms** can be described as psychotic symptoms since they are not exhibited in healthy subjects but are present in schizophrenic patients; they comprehend delusions and hallucinations
- **Cognitive symptoms** are difficult to notice and they are associated with memory, learning and understanding deficits; not all the schizophrenic patients exhibit this type of symptoms.

The symptoms can be very disabling; they usually appear during adolescence or early adulthood. The diagnosis of schizophrenia includes the occurrence of at least two of the symptoms listed before and they have to be ongoing for 6 months; moreover, symptoms must be not related to other conditions (e.g. drug abuse).

The causes of schizophrenia are unknown. There are some studies that prove the correlation between the symptoms and the levels of dopamine in the body: the increase of the neurotransmitter concentration can be related to the causes of the disease, as antipsychotic medications block the dopamine receptors (D2). These

treatments are not always effective, and this implies that there are more factors that contribute to the development of the disorder. Furthermore, research suggests the involvement of the environment as a possible cause. Indeed, the incidence of the illness is higher in patients that have experienced stressful life events and drug abuse. Another incident factor is genetics: the risk increases in people having a first-degree relative (FDR) with schizophrenia [28, 6].

As regards the neurophysiological aspects of the disease, studies about the electroencephalogram of schizophrenic patients were carried out since the 1960s. Some constant anatomical characteristics were found although the heterogeneity of the disease. The first research demonstrated anomalies at the hippocampus level in terms of disorganization, the reduced dimension of the cerebellum (correlated with the negative symptoms) and abnormal grouping of neurons [43].

Another important aspect related to the EEG is the one concerning the auditive and visual evoked potentials. Alterations in amplitude and latency of specific evoked potentials documented problems about the information processing in patients with schizophrenia.

1.2 EEG and High-density EEG

Electroencephalogram (EEG) is the recording of the electrical activity of the brain measured through the scalp of the head, it is a non-invasive, non-destructive and painless technique. Since the brain activity of a pathological person can be distinguished from the one of a healthy subject, EEG is also used as a clinical tool to diagnose brain related diseases and their symptoms.

EEG reflects the activity of communication between neurons using electrical impulses. The postsynaptic potentials of the cortical nerve cells sum in the cortex and extend to the scalp surface where they are recorded as EEG.

As regards the acquisition, EEG measures potential changes between a single electrode and a reference electrode [15]. Electrodes are applied to the scalp with a specific positioning: the usual arrangement adopted is the "International 10-20 system" (International Federation in Electroencephalography and Clinical Neurophysiology) [13]. The numbers 10 and 20 refer to the fact that the distances between adjacent electrodes are either 10% or 20% of the total front-back or right-left distance of the skull (Figure 1.2). Each electrode is labelled with a letter and a number in order to be identified. The letter indicates the position on the scalp: F, T, C, P and O stand for frontal, temporal, central, parietal and occipital respectively (Figure 1.1). The number distinguishes the side of the brain: even numbers represent the right side whereas odd numbers stand for the left hemisphere. A “z” is used to identify the midline (Figure 1.2).

EEG presents some typical and repetitive patterns in time that are categorized

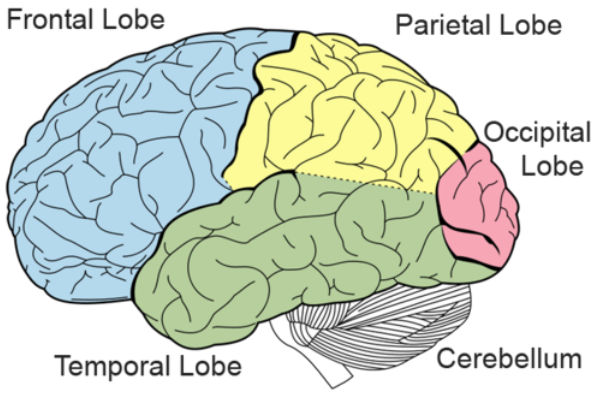

Figure 1.1: Brain lobes

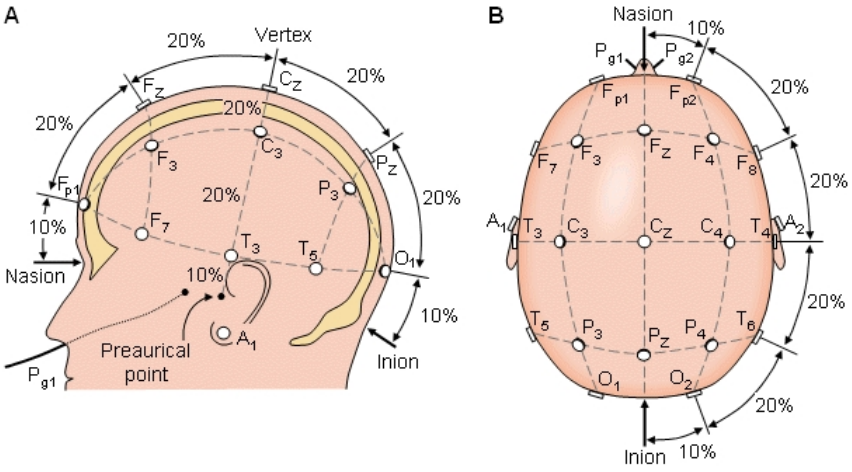

Figure 1.2: International 10-20 system

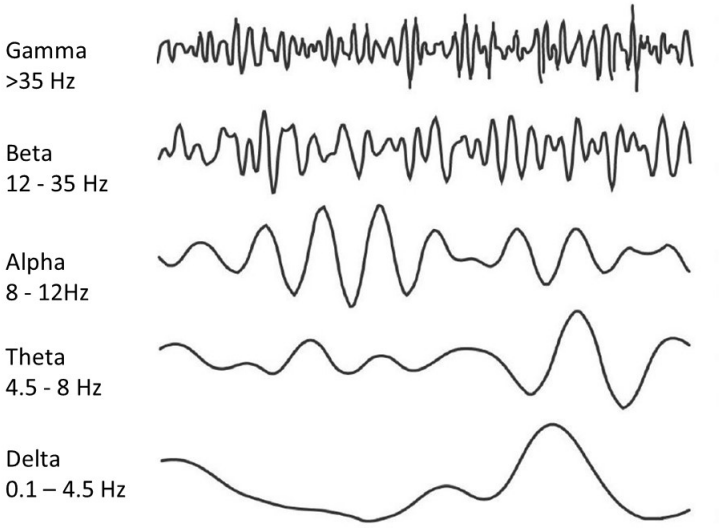

Figure 1.3: Brainwaves classification

with respect to their frequencies (Figure 1.3). These brainwaves are divided into:

- **Delta waves** (0.1-4.5 Hz) represent slow brainwaves found in all stages of sleep, but in particular it is related to deep sleep;
- **Theta waves** (4.5-8 Hz) are associated with subconscious activities such as meditation and dreaming during deep sleep;
- **Alpha waves** (8-12 Hz) are detected during wakeful relaxation with eyes closed;
- **Beta waves** (12-35 Hz) are related to the conscious state and they can be observed when the subject has eyes open and he is involved in a cognitive task;
- **Gamma waves** (> 35 Hz) are fast brainwaves that are correlated to the processing of information.

EEG is the gold standard technique for the recording of brain activity with a high temporal resolution, but it lacks spatial resolution in its standard montages (international 10-20 system) since it is unable to locate the exact source of the signal.

High-density EEG (HD EEG) is a dense array EEG that tries to overcome this deficit. It uses 256 electrodes applied to the scalp and this helps the increase of

the spatial accuracy. However, it does not provide more qualitative information with respect to the standard EEG.

HD EEG set-up consists of a pre-cabled cuff into which the electrodes are embedded, a digital amplifier and a dedicated computer. An example of a HD EEG cap is represented in Figure 1.4. The amount of data recorded is extremely large therefore high storage capacity and post-processing computing are needed.

Thus far, HD EEG is used only for clinical research in various applications for brain investigation [27].

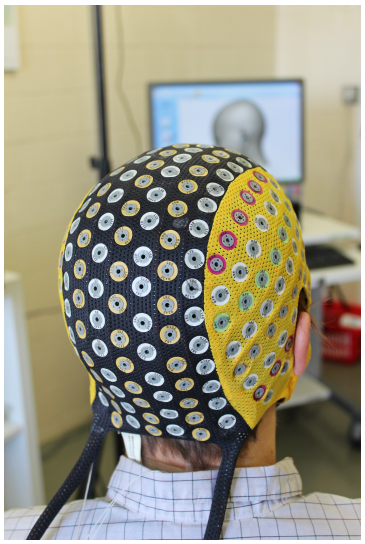

Figure 1.4: 256 electrodes cap

1.3 EEG analysis

The analysis of the EEG signal is the process of significant information extraction from raw data, exploiting mathematical signal analysis methods [36]. These methods can be divided into four categories based on the domain they operate with: time domain, frequency domain, time-frequency, and nonlinear methods [15].

With respect to the type of signal analysed, EEG is classified as a stochastic signal since it is not possible to predict the exact characteristic of the signal in terms of amplitude, duration or morphology and it is a non-stationary process if studied in a long time period.

The analysis of the signal begins with a preprocessing step; being still an active area of research, there is not an universally recognized pipeline. It generally includes artifact recognition and data filtering from the raw data. The former is

one of the most challenging steps in the EEG analysis. During the recording of the signal, artifacts and interference waves are superimposed to the signal of interest. These are undesired electrical potentials recorded by EEG but not generated by the brain that must be detected and then removed to clean the data and to have a more accurate interpretation of the data [32].

There are two types of artifacts: physiological (or internal) and non-physiological (or external) [31]. The former ones are related to the patient whereas the latter ones refer to the technology used in the signal acquisition.

The non-physiological artifacts are related to the environment as well as to the set-up of the experiment. They may be caused by the electrodes like incorrect positioning, electrode popping, loose electrodes or by the electrical noise frequency range emitted by instrumentation [20] (USA: 60Hz artifact, Europe: 50Hz artifact).

On the other hand, physiological artifacts originate from body activities and are due to biopotentials or movements. The main ones are ocular movements and eye blinks, respiration, cardiac activity and muscle potentials of the scalp associated with talking, swallowing and smiling. Given the fact of their origin, this type of artifacts can rarely be avoided. As previously mentioned, since detection and removal of artifacts are very laborious, there is not a unique method to deal with.

After the detection of a specific artifact is performed, the portion of the signal containing the artifact itself is removed. Therefore, it is important to complete this step consciously in order to prevent the deletion of informative segments of the signal.

Once the signal is free from artifacts, the data are filtered in the frequency range of interest and the analysis continues according to the method chosen.

1.4 Evoked potential

Evoked potentials (EPs) are small voltages generated in the brain in response to specific stimuli or events. In some circumstances, it is worth recalling that EPs are also called event-related potentials (ERPs) even though there is no agreement in the academic community [34, 37].

EPs reflect the activity of the brain related to the reception of external sensory stimuli and they are overlapped to the normal spontaneous activity (EEG). Therefore, they are studied independently from the background EEG, but they can be measured simultaneously with the EEG signal i.e. placing electrodes on the scalp. They are considered deterministic signals since they are not spontaneous but, as their name suggests, they are induced. The electrical signal of a single neuron can not be observed on the scalp hence EPs represent the summated activity of large

populations of nervous firing in synchrony while they are processing information [20].

The typical waveforms of EPs are made of a sequence of peaks and troughs and they are time-locked to the stimulus. Therefore, the most important parameters for clinicians are the amplitudes of maxima and minima (sometimes indicated with P and N, respectively) and the latency i.e. the time that elapses between the stimulus and the appearance of the EP, usually indicated with a number (millisecond) following the "N" or the "P" [37]. For instance, P300 is an EP with a positive peak appearing in a latency range of 250-400 ms [37].

The amplitude of the EP is usually very small compared to the EEG one (tens of μV): from less than 1 μV to several μV. Therefore, it is difficult to identify the EP from the background EEG that can be considered noise for research purposes in the EP field. Because of this low amplitude, signal averaging is usually required.

As regards the categorization of EPs they are classified according to the type of stimulus presented. In this way four types of EPs are recognized:

- **Visual EPs** are elicited by a stroboscopic flashing light or a changing pattern on a screen;
- **Auditory EPs** where the stimulus is a click, or a tone presented through headphones;
- **Somatosensory EPs** are induced by an electrical or a tactile stimulation;
- **Cognitive EPs** have different types of stimuli e.g. the identification of a specific letter in a string.

EPs can also be divided into two groups based on their latency. The latency is related to the speed of the stimulus classification and the discrimination of the type of event. The EPs that peak early (latency $<$ 100 ms) depend on the physical parameters of the stimulus and the integrity of the sensory pathway, whereas the late components (latency $>$ 100 ms) represent the cortical responses to the processing of the presented information.

EPs can be used as a diagnostic and clinical non-invasive tool as they measure the function within sensory pathways (optic nerve, brainstem or somatosensory). They are useful for evaluating the brain function as the absence or the delayed latency in the EPs may reveal some sort of abnormalities.

1.5 P50 and sensory gating

P50 is an early component EP since its latency is less than 100 ms. Indeed, P50 is defined as the most positive peak between 30 and 70 ms after the stimulus is

presented [37].

Usually, while studying the P50 wave, a paired-click paradigm is used. It consists of two identical auditory clicks with an interval of 500 ms between them. The first stimulus is called "conditioning stimulus" whereas the second one, "response stimulus". The amplitudes of the two responses are called S1 and S2, respectively [19].

The P50 waveform is analysed as a sensory gating indicator. Sensory gating represents the ability of an individual to discriminate between informative stimuli and repetitive, trivial or extraneous information [10]. In the paired-click paradigm, this can be defined as the amount of attenuation in the neural response to the second stimulus and its value is usually measured as the ratio between S2 and S1: S2/S1 [42] (Example in Figure 1.5).

Sensory gating of a healthy subject has a reduced amplitude S2 with respect to S1 [42].

It is thought that abnormalities in the P50 wave might represent endophenotypes of schizophrenia [3]. In fact, it has been observed that patients with schizophrenia have a deficit in sensory gating as they present a reduced P50 suppression: there is no decreased amplitude in the response to the second stimulus as in healthy subjects [19]. This may demonstrate the compromised ability of the brain to filter out redundant sensory information [37, 10].

At the molecular and chemical level, it is found out that the P50 sensory gating is related to the α-7 nicotinic receptor (CHRNA 7) locus on chromosome 15q [11].

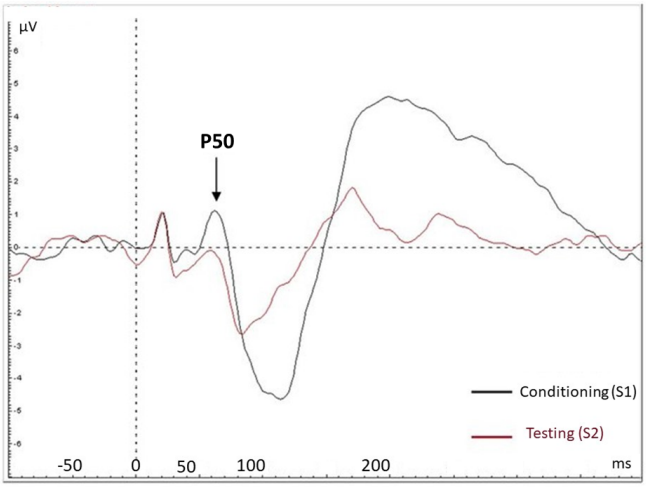

Figure 1.5: Example of P50 with S1 and S2

Chapter 2

AVH TMS Project

This work is part of the ongoing "Icelandic AVH TMS" project [1]. The aim of the project is to study the effectiveness of rTMS (repetitive Transcranial Magnetic Stimulation) treatment for schizophrenic patients with persistent auditory verbal hallucinations (AVH) [1].

2.1 Auditory verbal hallucinations

Auditory verbal hallucinations (AVH) are defined as the experience of hearing voices in the absence of any external stimulus [12]. They are one of the most characteristic symptoms of schizophrenia experienced by 60-80% of people with this disorder. Nevertheless, AVH may occur in other psychiatric diseases or even as isolated events in healthy subjects.

AVH are a form of hallucination that takes the form of other people voices often giving commands or commenting on the subject's actions. It is believed that the content of AVH is related to the environment where the person lives and to their life experiences [5].

AVH are not a homogeneous phenomenon since each person experiences them in a different way. Indeed, they differ in:

- Level of externality
- Number of voices heard (one or more talking voices)
- Intensity/volume of the voice
- Attentional salience

The pathophysiology of AVH is not completely understood and currently, there is still no accordance among the scientific panorama. Several models of their origin have been proposed:

- **Memory-based models**: hallucinations are associated with memories of traumatic events
- **Hypervigilance models**: voices derive from the transformation of ambiguous environmental stimuli
- **Inner-speech models**

The most popular and influential theory is the last one; this model suggests that AVH result from an impairment in the auditory processing. The inner speech is misattributed to an external-to-self source. Inner speech includes verbal thoughts and memories.

At a neurophysiological level, studies in patients with AVH, using fMRI (Functional magnetic resonance imaging), showed hypo activity in the left primary auditory cortex and in related brain regions such as TPJ (Temporoparietal junction) and STG (Superior temporal gyrus).

As regards the treatment of AVH, usually, antipsychotic medications are used. They induce a rapid decrease in hallucinations severity when prescribed to the patient, during one year [33]. If the first-choice drug is not effective within the first 2-4 weeks of treatment, a second medication is chosen. Clozapine is prescribed if the first two drugs do not provide remission; it has superior efficacy compared to the other antipsychotics but it can cause severe side effects. In spite of this, in approximately 25% of patients with schizophrenia, AVH can chronically persist [14].

Nowadays, new treatments are investigated; two examples of these are CBT (Cognitive-Behavioural Therapy) and TMS (Transcranial magnetic stimulation). The former is a therapy that teaches the patient to ignore the voices heard; the aim is to provide the subject a better quality of life. On the other hand, TMS is a non-invasive technique that stimulates the brain and it will be accurately described in the next paragraph. Nevertheless, thus far, both techniques have been studied in patients treated with antipsychotic medications, at the same time [33].

2.2 Transcranial Magnetic Stimulation

TMS is a non-invasive, safe and well-tolerated technique that generates a cortical stimulation in the brain, changing the neural excitability and activity.

The first TMS research were carried out in the 1980s by Anthony Barker and colleagues. TMS is based on the Faraday principle that describes the phenomenon of electromagnetic induction (1831): the electromotive force around a closed path is equal to the negative of the time rate of change of the magnetic flux enclosed by the path, where the closed path is conductive.

In practice, in the TMS application, a brief electrical current of very high intensity (several thousand amperes) flows through a copper wire coil inducing a magnetic field (up to 2T of intensity and it lasts for about 100 μs) perpendicular to that current. The coil is placed over the subject's head in a specific position. This magnetic field pulse can pass through the skin, the scalp and the skull interacting with the brain and produces changes in the current developed in it. This induced current is able to activate brain networks producing action potentials in brain cells [22, 17] (Figure 2.1 represents the TMS functioning).

Neurons are, in fact, electrically conductive material that form circuits inside the brain which, in turn, are part of the so-called brain networks. TMS targets a specific region of the brain and it can change the activity of an entire network; in addition to this, the effects propagate throughout deeper regions. Modulating the frequency of stimulation, the effects can cause either neural firing or inhibition of neurons activation.

Based on the person's cortical excitability, the dose of the TMS treatment is individualized. The amount of electricity delivered is determined by the motor threshold (MT) of the subject. MT is the minimal stimulation needed to produce a movement in the contralateral thumb when the coil is placed over the primary motor cortex; it can be determined visually or more accurately using EMG (electromyography) recordings [9]. Cortical excitability can be affected by sleep, medications, alcohol and drug abuse; all these factors must be considered when choosing TMS dosing. Another aspect regarding the individualization of the treatment is the TMS correct targeting and the positioning of the coil that can be localized either with imaging-based or non-imaging-based techniques.

There are different types of TMS that are classified based on the frequency and the type of magnetic pulse delivered. Single-pulse TMS uses a single magnetic pulse at a given time, whereas rTMS (repetitive TMS) delivers repeated single magnetic pulses of the same intensity. The frequency of the latter type can vary from less than 1 to 20 stimulation per second. High- frequency rTMS uses >1Hz stimulation for short periods (less than 15 minutes), while low-frequency rTMS delivers <1Hz frequency stimulation for longer duration [22, 17, 29].

TMS is used for both diagnosis and treatment.

As regards the diagnosis, it is mainly used for the recording of MEPs (motor evoked potentials) that are twitches generated in the muscle when a single-pulse TMS is applied to the corresponding motor cortex. They are a reliable method to perform functional mapping of muscle representation within the motor cortex [9]. MEPs are also used to study CSP (cortical silent period) which is the suppression of the EMG activity after the production of the MEP in a contracting muscle [17].

Concerning the treatment aspects of this technique, currently, TMS is FDA (Food and Drug Administration) approved for unipolar depression only. Nevertheless, these days lots of research and investigation are carried on for the treatment

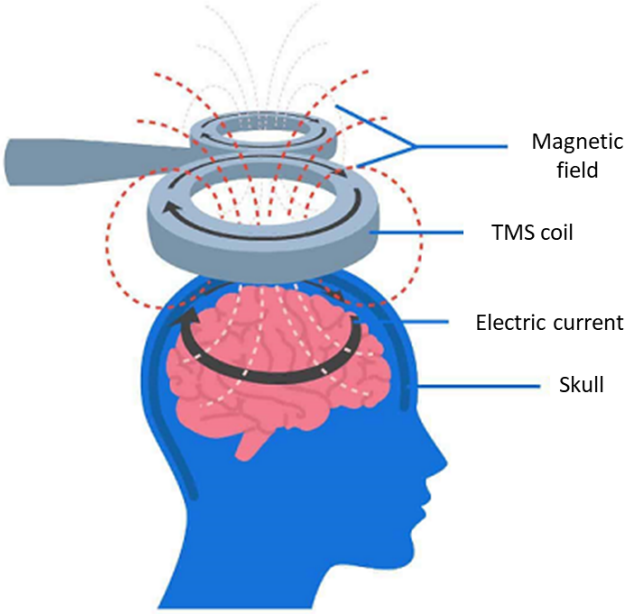

Figure 2.1: TMS functioning

of different diseases both with TMS and rTMS: OCD (obsessive-compulsive disorder), AVH, negative symptoms in schizophrenia, post-traumatic stress disorder, generalized anxiety disorder, autism and bipolar depression.

Being a targeted treatment TMS has few side effects, compared to the systemic treatments. The most common one is headache, especially in the first sessions, but it usually resolves spontaneously. Rarely, severe effects such as syncopes and seizures have been reported [9].

2.3 The Project

The AVH TMS Project [1] is a clinical applied research in psychiatric patients from Iceland. As mentioned before, the study aims to determine the effect of rTMS in schizophrenic patients with persistent AVH.

The complete team of the project consists of:

- Clinicians (TMS laboratory and EEG acquisition): Ovidiu Banea (Clinical neurophysiologist), Eysteinn Ívarsson (Psychologist and neurotechnologist), Aron Dalin Jónasson (Biologist and neurotechnologist)
- Psychologists (Psychometric measurements and EEG acquisition): Viktor Díar Jónasson (Clinical psychologist), Aníta Ó. Georgsdóttir (BCs student

in Psychology at RU)

- Data analysts (Analysis of neurophysiological measurements and EEG acquisition): Sara Marcu (MSc student in Bioengineering at University of Padua) and Elena Pegolo (MSc student in Bioengineering at University of Padua)

Eighteen patients in total were recruited from the psychiatric wards and outpatient clinics of the University Hospital of Iceland.

Concerning the recruitment of patients, the following criteria of inclusion and exclusion were applied in the project to the subjects willing to participate [1]:

INCLUSION CRITERIA:

- Patients that are between 18-55 years old
- Patients with treatment resistant AVH due to schizophrenia or schizoaffective disorder for at least 1 year (*treatment resistance AVH* refers to two pharmacotherapy attempts that used recommended dosage for at least 6-8 weeks)
- Patients that experience AVH at least once per hour

EXCLUSION CRITERIA:

- History of epilepsy
- Daily Cannabis use
- Use of other hard drugs within one month prior to the study or during the study
- Drinking more than three units of alcohol daily
- Use of benzodiazepine daily
- Use of antiepileptic agents
- Meeting any of the exclusion criteria on the rTMS safety screening list
- Left handed (assessed with the *Edinburgh Handedness Inventory*)

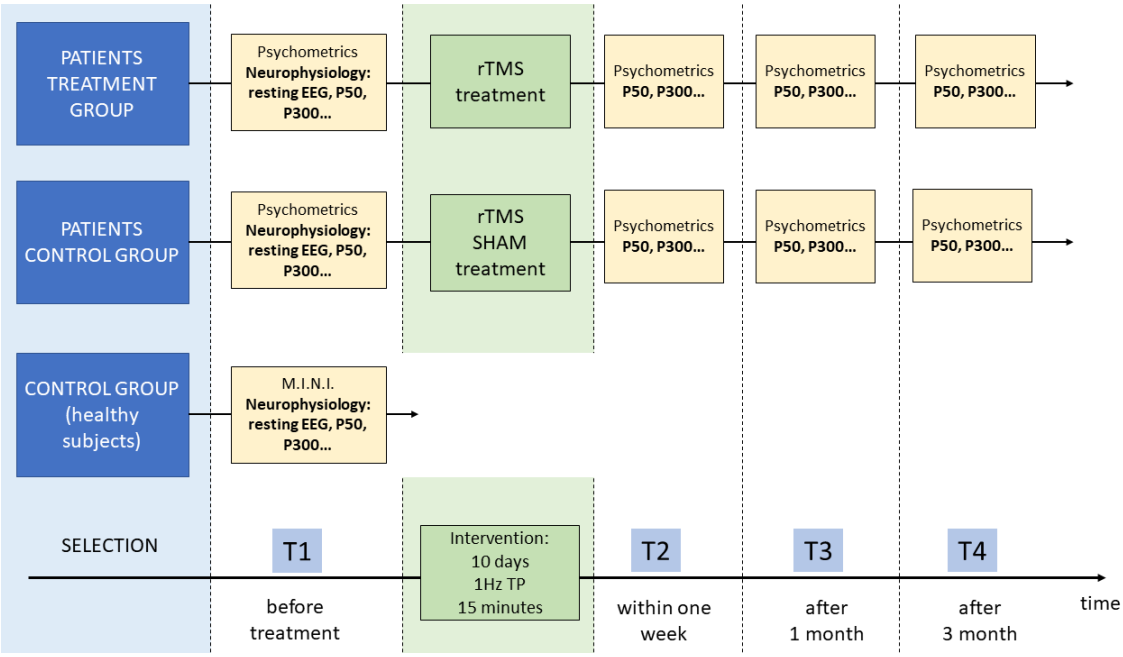

Figure 2.2: Protocol design of the Project

Nine patients underwent the treatment (patient-treatment group) whereas nine of them were sham-treated (patient-control group). The treatment consists of 10 days of rTMS. Furthermore, nine healthy subjects took part in the project (control group).

rTMS treatment is delivered at the National University Hospital of Iceland by a specialist personnel.

Two types of measures were acquired in the research: psychometric scales and neurophysiological measurements; changes in these data were examined before and after the treatment. As regards the psychometric aspects of the research the following scales were used: PSYRATS (Psychotic symptom rating scales) DASS (depression, anxiety and stress scale) and QoL (Quality of Life scale). Whereas, the neurophysiological measurements included: P50, P300, resting EEG and AM task, as described above.

Data were acquired in healthy subjects and compared with the patients' ones before and after the treatment (real or sham): at the beginning of the project (T1), right after the treatment (within one week, T2), one month after the treatment (T3) and three months after (T4).

The scheme of the whole project (time and experiments) can be observed in Figure 2.2.

The experiments were double-blind: neither the participants nor the data analysts were informed about who was receiving real or sham treatment. Only clin-

icians working in the rTMS laboratory knew the patients that were real treated. Consequently, after the analysis of the data, clinicians designated the true classification of the cohorts.

Some neurophysiological hypotheses were formulated, regarding the effects of the treatment in patients with AVH.

In particular, concerning the P50 evoked potential, it is assumed that rTMS treatment is going to improve sensory gating. This is investigated calculating the P50 second response (S2) and the ratio S2/S1; S2, in fact, should be decreased in real-treated patients, compared to healthy subjects and sham-treated patients [1].

Moreover, we believe this is the first study that aims to quantify rTMS effects in schizophrenic patients with persistent AVH through neurophysiological measurements.

A second novelty of the project is given by the fact that data are acquired with high-density EEG. The specific experimental set-up for the EEG acquisition will be described in Chapter 4.1. However, here we would like to specify that, to the best of our knowledge, this is the first study that uses HD EEG to evaluate evoked potentials. In fact, EP acquisitions usually exploit from one (plus a reference channel) up to a maximum of 64 electrodes [21]. Having this large amount of data, topological studies of the P50 can be performed in a non-invasive way. Multiple sites of P50 suppression can be evaluated and studied in the different cohorts.

Chapter 3

Aim of the work

The present thesis aims to develop a methodology to be employed in the analysis of the "Icelandic AVH TMS" Project's neurophysiological data in order to evaluate the effects of the treatment in schizophrenic patients. Preliminary results obtained with this method are presented. In this work, only P50 data are analysed; comparison between schizophrenic patients before the treatment (T1) and healthy subjects are performed with the purpose of demonstrating differences in sensory gating. Moreover, a case of one patient before (T1) and after the treatment (T2) is analysed. All the data are analysed in the time domain. The novelty of the project is given by the fact that data are acquired with HD EEG, as mentioned before. Therefore, a further goal is the topological representation of the P50 wave; in this way, differences or analogies between subjects and cohorts are highlighted.

The experimental set-up and the data analysis will be presented (Materials and methods: Chapter 4) focusing on the preprocessing and the analysis of the data. The implementation of these steps is achieved offline with the toolbox Brainstorm [38] and with MATLAB [41]. Brainstorm is a collaborative, open-source application dedicated to the analysis of brain recordings like EEG, developed with MATLAB [38]. Consequently, the results will be described and discussed in the following sections (Results and Discussion: Chapter 5 and Conclusion: Chapter 6).

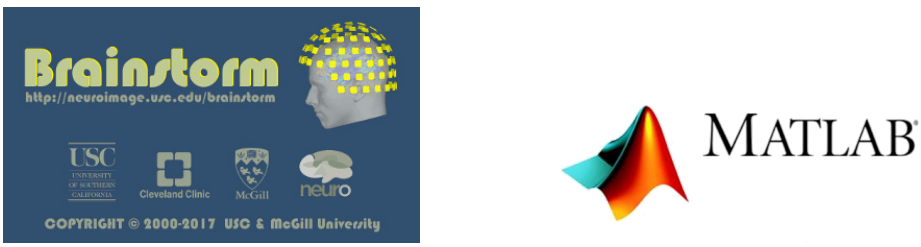

Figure 3.1: Brainstorm and MATLAB logos

Chapter 4

Materials and methods

The measurements were carried out in the Icelandic Center for Neurophysiology Laboratory located at Reykjavik University.

In this thesis the data of 3 healthy subjects and 3 schizophrenic patients are analysed.

During the data acquisition session, five measurements were performed per each patient/healthy subject: P50, P300, Auditory motor (AM) task right, Auditory motor (AM) task left, resting EEG. This work will focus on the P50 data acquisition only.

4.1 Experimental set-up

The continuous EEG data were recorded, at a sampling rate of 1024 Hz, in real time while the experiments were performed. The participants were seated in front of a screen and watched a silent movie. Even if subjects were aware that some clicks would be presented through the headphones, they were not supposed to focus on them or other noises; for this reason, a silent movie was displayed [20].

The P50 protocol used in the EEG acquisition was arranged in the following way: a paired-click paradigm was presented through headphones with a click sound made by a pure tone (*frequency*: 1500 Hz, *duration*: 6 ms). The two clicks were presented 500 ms apart. The interstimulus interval lasted 10 seconds; 150 paired stimuli formed the length of the experiment with a total duration of 25 minutes. However, the length of the whole session was greater (approximately 2 hours) because the preparation for the measurement and the time needed to perform the other experiments must be considered.

Each subject had a P50 file that recorded all the 25 minutes of acquisition.

The signals were acquired using the *eego* software and the 256 channel cap ***wave****guard original*, both provided by ANT-Neuro [2]. In Figure 4.1 an example

Figure 4.1: The ANT-Neuro [2] System

Table 4.1: Amplifier specifications

AMPLIFIER SPECIFICATIONS	
Dimensions	160x205x22 mm
Weight	<500 gr
Referential input noise	< 1.0 V rms
Referential input signal noise	150-1000 mV pp
Input Impedance	> 1 GΩ
Resolution	24 bit
Trigger input	8 bit
Common-mode rejection ratio	>100 dB

of ANT-Neuro system is presented.

The caps are made up of a lightweight, flexible, and sintered Ag/AgCl material. After the cap was placed on the subject head, the cap connectors were plugged into the amplifiers. Four amplifiers were used, and their specification can be observed in Table 4.1. A dedicate syringe with a blunt needle was used to apply a conductive gel (*OneStep-Cleargel*) to induce connectivity and reduce the impedance between the scalp and the electrodes [2]. The syringe was filled with gel and a small amount of it was injected through the holes at the top of each electrode; to remove air bubbles this procedure was done with a circular hand movement. Each electrode should be completely filled. In order to avoid too much drift and noise the impedance was checked through the software: channels showing values below 10 kΩ were accepted.

A trigger box, created in the RU laboratories, was used to detect the two stimuli. A trigger marked “011” appeared when the first stimulus was presented (conditioning stimulus), while a trigger marked “012” appeared with the second stimulus (response stimulus).

The paradigm and the two triggers used are presented in Figure 4.2.

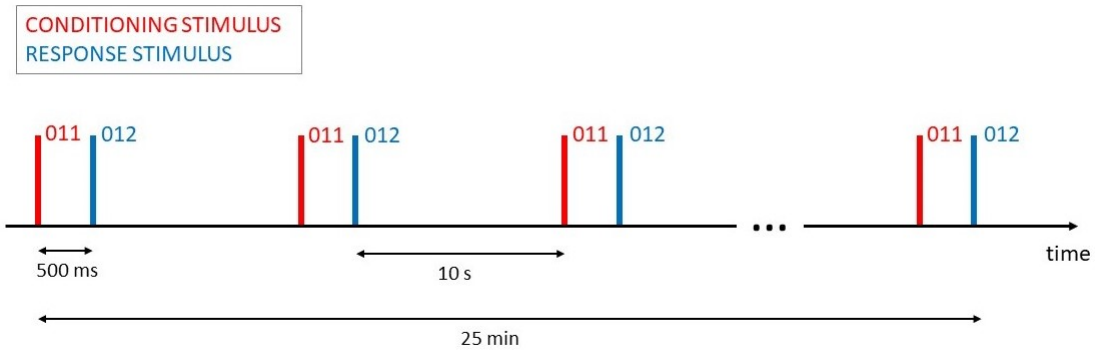

Figure 4.2: P50 paradigm

In Figure 4.3 the entire experimental set-up for the acquisition of the P50 of one subject is presented.

Figure 4.3: Example of acquisition

4.2 Data analysis

4.2.1 Preprocessing

The complete protocol for the preprocessing is described in Appendix A.1: “Brainstorm – P50 Preprocessing”. The method included 4 steps:

- **Epoching of the trials**
- **Filtering**
- **Removal of bad channels**
- **Interpolation of bad channels**

The data were imported in Brainstorm in order to be preprocessed using the following procedure. The protocol "P50" was created and a new folder per each subject was generated. Each subject had two sets of trials related to the two stimuli (011 and 012).

4.2.1.1 Epoching of the trials

The first step was the epoching of the EEG. This step is a specific phase performed in the EEG analysis while studying the evoked potentials. It consists in creating specific time-windows from the signal. These are called “epochs” and they are time-locked to the event of interest.

The data acquired during the P50 experiment (25 min approximately) were imported in Brainstorm. They were divided into epochs according to the events 011 and 012 (S1 and S2 respectively). The application automatically recognized the times associated to the stimuli. The time-window used was [-100 ms, 400 ms] with respect to the stimulus, with a total length of 500 ms per each segment [10]. In each epoch, the time associated with the stimulus is 0 s. The pre-stimulus interval is 100 ms long whereas the post-stimulus one is 400 ms. At the end of the epoching step, 115/120 epochs were generated per each event.

While importing the data, the baseline value of each channel was removed in order to eliminate the possible DC offset in the signal. The time range chosen as baseline was the pre-stimulus period [-100 ms, -2ms]. For each epoch, Brainstorm computed the average of each channel over the baseline and subtracted it from the channel at every time instant (full epoch interval).

4.2.1.2 Filtering

The data were then filtered in order to eliminate high-frequency noise and to improve the signal-to-noise ratio.

Two different filters were applied. The first one was a band-pass filter from 0.1 Hz to 80 Hz, covering all the frequencies in the EEG brainwaves [21]. In Table 4.2 the properties of the bandpass filter are reported. The second one was a Notch filter at 50 Hz, used to eliminate the 50 Hz interference. Its properties can be observed in Table 4.3.

All the epochs of both the 011 and the 012 sets were filtered.

Table 4.2: Band-pass filter properties

BAND-PASS FILTER PROPERTIES	
Band-pass	0.1-80 Hz
Low transition	0-0.1 Hz
High transition	80-80.5 Hz
Stopband attenuation	60 dB
Passband ripple	0.1%
Filter type	Kaiser, linear phase FIR filter
Filter order	37124
Sampling frequency	1024 Hz

Table 4.3: Notch filter properties

NOTCH FILTER PROPERTIES	
Frequency to remove	50 Hz
3-dB Notch bandwidth	1 Hz
Filter type	Second-order IIR Notch filter
Filter order	2
Sampling frequency	1024 Hz

4.2.1.3 Removal of bad channels

After the filtering of the signal, the removal of bad channels was performed.

As mentioned in Chapter 1.3, artifacts in the signal can appear when electrodes are misplaced, loose or have high impedances. It is important to remove the channels showing a poor signal quality in order to avoid the propagation of them in the following steps.

Since it is one of the most important steps of the preprocessing phase, the removal of them was performed visually, trial by trial.

Epochs showing outliers or channels over +/-80 μV were marked as “bad channels” and were removed from the selected epoch. An example of a bad channel can be seen in Figure 4.4: it is highlighted in red.

If the outlier was present just for a short period (< 0.2 ms), it was considered in the following steps and not marked as “bad channel”.

When a bad channel appeared in most of the epochs, it was rejected from the whole set. It was marked as "bad channel" and eliminated from all the trials.

If more than 10% of the channels showed bad behaviours, the whole trial was rejected, and it was discarded from the analysis (Example of a bad trial: Figure 4.5).

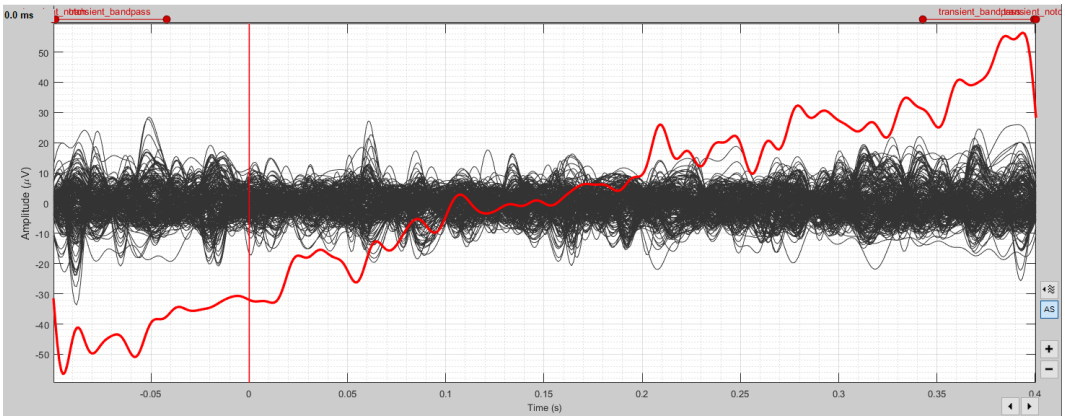

Figure 4.4: Example of bad channel highlighted in red. The vertical red line at 0s represents the stimulus

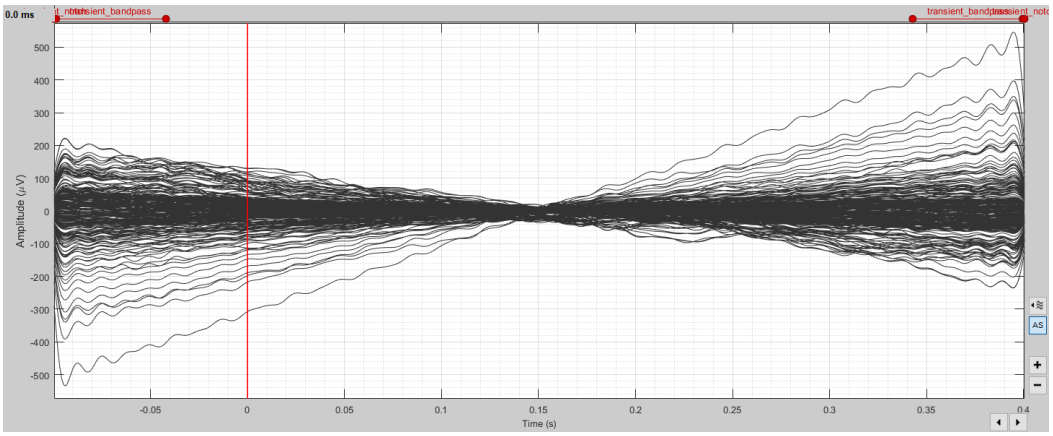

Figure 4.5: Example of bad trial. The vertical red line at 0s represents the stimulus

4.2.1.4 Interpolation of bad channels

The last step concerns the interpolation of bad channels. The missing data that had been removed in the previous point, must be filled with the information given by the data from the good channels.

There are different ways of interpolation: replacement of the bad channels by the plain average of all neighbours, by a weighted average of all neighbours, by an interpolation based on a surface Laplacian, or by spherical spline interpolating [8, 26].

The method used in this work was the weighted average of the neighbours with a maximal distance between them of 5 cm (default of Brainstorm).

Figure 4.6 represents an example of one trial at the end of the preprocessing phase.

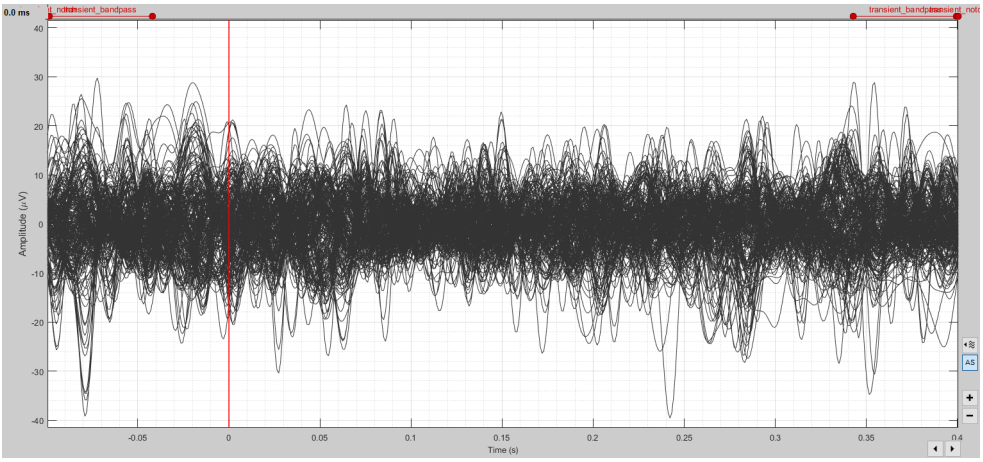

Figure 4.6: Example of one trial after the preprocessing step. The vertical red line at 0s represents the stimulus

4.2.2 Analysis

In the analysis step, the average of the data was performed. The first part of the analysis was completed in Brainstorm whereas the second one in MATLAB. The complete protocol is described in Appendix A.2: "Brainstorm - P50 Averaging".

All the trials marked as good were averaged using the pipeline editor in Brainstorm. The setup used was the arithmetic mean applied to all the trial group (grand average).

Then the data were re-referenced. This procedure can be done offline, since it is a linear transformation of the data. The montage used as the new reference was the bimastoid [21, 16] (average mastoids) created in Brainstorm. The channels of reference were “L19L” and “R19R” which, in fact, represent the mastoids (localization of the L19L electrode in Figure 4.7).

The continuation of the analysis was carried out in MATLAB. Each subject had, at this point, two files regarding the average of the 011 trials and the 012 trials, both re-referenced to the bimastoid. These files were created as .mat files in a specific folder of Brainstorm; they were imported in MATLAB as structure arrays (*struct*).

The fields of the struct that were used in the analysis are:

- **matrix F**: (254 channels x 513 samples) collects all the averaged data re-referenced to the mastoids. There are 254 channels since two of the 256 electrodes were used as reference
- **vector Time**: (1 x 513 samples) represents the time samples

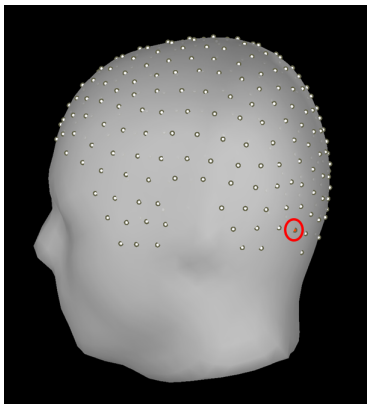

Figure 4.7: Localization of the L19L electrode

The analysis of the data of one subject will be now explained. Afterwards, the code for the organization of the cohorts will be illustrated .

4.2.2.1 Time analysis: single subject

In order to locate spatially the P50 wave on the scalp, the electrodes were grouped together in seven different ROIs.

A similar procedure to the one used by Hall and colleagues [10] was employed. They divided the channels into 13 ROIs each one containing from 3 up to 6 electrodes.

In this analysis, the localization of the ROIs on the scalp was chosen in the same way. However, less ROIs were created and the same number of channels per each ROI was selected. After consulting with the neurophysiologists, the following arrangement was decided: 7 different ROIs each one containing 15 electrodes.

The names of the ROIs and the specific channels per each ROI are here reported:

1. **LEFT ANTERIOR (LA)** L1E-L5E, L1D-L5D, L1C-L5C88, 89, 90, 91, 92, 79, 80, 81, 82, 83, 71, 72, 73, 74, 75;

2. **LEFT POSTERIOR (LP)** L5F-L8F, L6E-L10E, L6D-L9D, L6B-L7B 102, 103, 104, 105, 93, 94, 95, 96, 97, 84, 85, 86, 87, 69, 70;

3. **MEDIAL ANTERIOR (MA)** L3L-L7L, Z3Z-Z7Z, R3R-R7R 42, 43 44, 45, 46, 120 121, 122 123, 124, 169, 170, 171, 172, 173;

4. **MEDIAL CENTRAL (MC)** L8L-L12L, Z8Z-Z12Z, R8R-R12R 47, 48, 49, 50, 51, 125, 126, 127, 245, 246, 174, 175, 176, 177, 178;

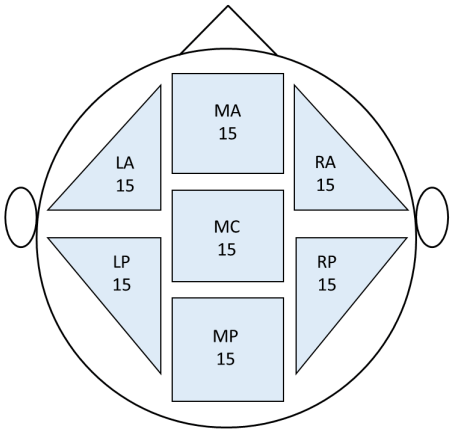

Figure 4.8: Localization of the ROIs on the scalp

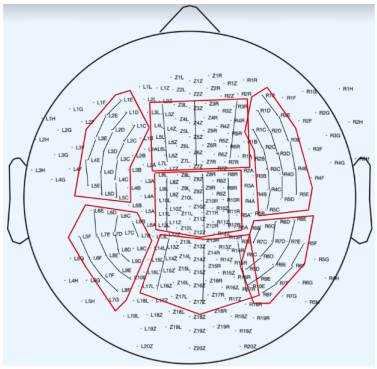

Figure 4.9: Electrodes used in the ROIs

5. **MEDIAL POSTERIOR (MP)** L13L-L17L, Z13Z-Z17Z, R13R-R17R 52, 53, 54, 55, 56, 247, 248, 249, 250, 251, 179, 180, 181, 182, 183;

6. **RIGHT ANTERIOR (RA)** R1E-R5E, R1D-R5D, R1C-R5C 215, 216, 217, 218, 219, 206, 207, 208, 209, 210, 198, 199, 200, 201, 202;

7. **RIGHT POSTERIOR (RP)** R5F-R8F, R6E-R10E, R6D-R9D, R6B-R7B 229, 230, 231, 232, 220, 221, 222, 223, 224, 211, 212, 213, 214, 196, 197.

The disposition of the ROIs on the scalp and the electrodes selected per each ROI are shown in Figures 4.8 and 4.9.

The specific choice of the electrodes was made in accordance with the clinicians team. Channels were selected in order to have a symmetrical division of the scalp with respect to the midline. Moreover, channels on the perimeter were discarded in order to avoid the ones that could lead to muscle and eye blinks artifacts.

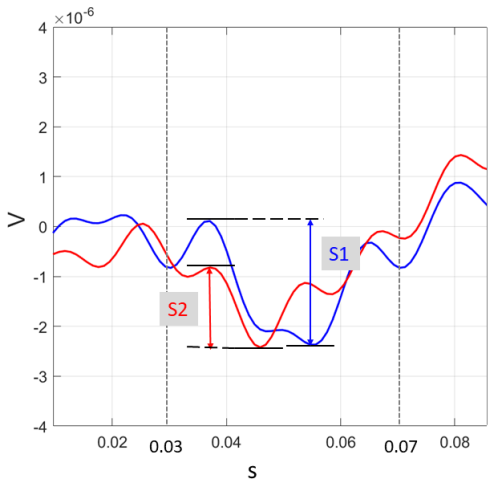

Figure 4.10: P50 calculation

A custom-made code per each subject was created to complete the analysis in order to obtain the topological representation of the P50 EP and to compare it between the different ROIs.

The data of both S1 and S2 signals were averaged with respect to the channels following the scheme described above.

Per each ROI the P50 signal was calculated according to its definition; the peak-to-peak amplitude was measured from the preceding positive peak to the negative trough in the 30-70 ms range from the stimulus onset for both the stimuli. An example of the calculation is reported in Figure 4.10.

Maxima and minima values of the two signals were determined in this time interval and reported in an Excel file. The amplitude of S1 and S2 was calculated as the absolute value of the difference between maximum and minimum. S2/S1 was also calculated in order to evaluate sensory gating.

The same procedure was used for both healthy subjects and patients, before (T1) and after (T2) the treatment.

The obtained results are discussed in the following chapter (Chapter 5).

4.2.2.2 Time analysis: cohorts

The last part of the analysis aimed to organize the data in 3D matrices in order to perform analysis on the cohorts.

The dimension of the matrices was 254x513xN, where 254 was the number of channels, 513 was the number of samples per each channel, and N represented the number of subjects in the cohort. A matrix was created for each cohort and event (Example of 3D matrix in Figure 4.11).

These structures can be filled in with the preprocessed data every time there are new ones. In this way further analysis can be performed by subject and cohorts.

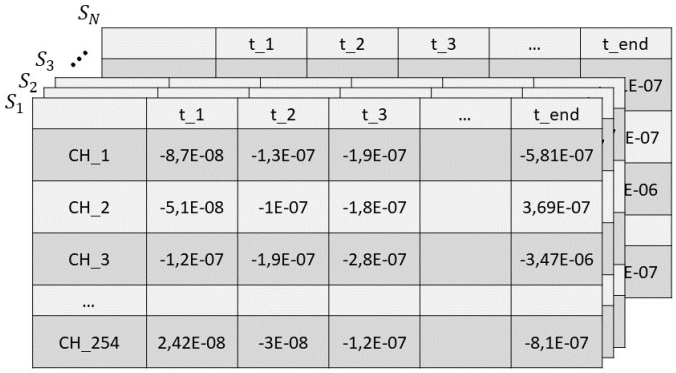

Figure 4.11: Example of a 3D matrix for one cohort

Chapter 5

Results and discussion

P50 evoked potential data measured with HD-EEG from 6 subjects (3 patients and 3 healthy subjects) were analysed in order to highlight differences of sensory gating in the two cohorts. Analogies in the ROIs were found through comparisons in the topological representation of the P50 wave. Some preliminary results about a single patient before and after the treatment are also presented.

The subjects were identified by codes: P1, P2 and P3 refer to the patients whereas C1, C2, and C3 refer to the HS.

T1 and T2 represent the timing of the analysis: T1 is the pre-treatment and T2 is the post treatment within one week. T3 and T4 are not analysed in this work.

As regards the topological representation, the following codes are used to identify the different ROIs:

1. **LA**: LEFT ANTERIOR
2. **LP**: LEFT POSTERIOR
3. **MA**: MEDIAL ANTERIOR
4. **MC**: MEDIAL CENTRAL
5. **MP**: MEDIAL POSTERIOR
6. **RA**: RIGHT ANTERIOR
7. **RP**: RIGHT POSTERIOR

Qualitative results will be presented in sections *Healthy subjects* and *Patients*; quantitative results and comparison between them will be reported in the last two sections (*Comparison between healthy subjects and patients* and *Analysis of one patient before and after the treatment*)

5.1 Healthy subjects

One healthy subject (C3) is taken as a reference in the presentation of the results. Graphs of the other HS are inserted in the appendix (A.3).

In Figure 5.1 the topological representation of the P50 is presented. The interval [0.03 s 0.07 s] is highlighted with a box, S1 is the blue signal, S2 the red one. The two signals are overlapped in the same time window in order to highlight differences in sensory gating. In this representation, time 0 s represents both stimuli.

Sensory gating (S2<S1), in fact, is clearly visible in some specific ROIs such as LA, RA, LP, RP: the signal is circled in these regions. As expected, the P50 wave is more visible in the temporal area (LA, RA, LP, RP) since the P50 response is generated in this area (primary auditory motor cortex). In the other ROIs, P50 EP is still recognizable but the sensory gating is not as evident as the lateral areas.

Generally describing all the HS, P50 response is generated in the temporal area, as already mentioned, but it is thought that also the midfrontal region (MA) could be a relevant area, being a candidate for an early gating [16]. In this region, in fact, there are areas of attention and sensory inhibition. For all the 3 healthy subjects MC and MP were the ROIs where the P50 wave was more difficult to identify, in accordance to the literature about P50 topography.

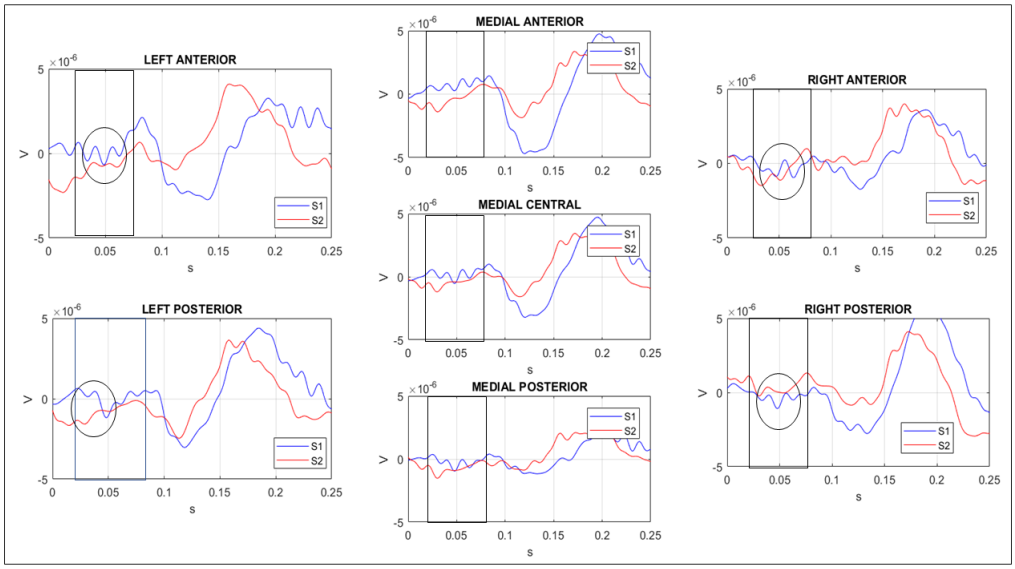

Figure 5.1: Topological representation of the P50 wave in subject C3

5.2 Patients (T1)

Subject P2 is considered as a reference in the discussion of the data regarding patients analysed before the treatment (T1); results regarding P2 will be accurately described. The images of the other patients are then presented.

In Figure 5.2 it is portrayed the topological representation of P50: S1 is visible in all the ROIs, whereas S2 is not always identified. In the MA region S1 is greater than S2 (good sensory gating) but in all the other ROIs S2 is more significant or even not well recognized (MC).

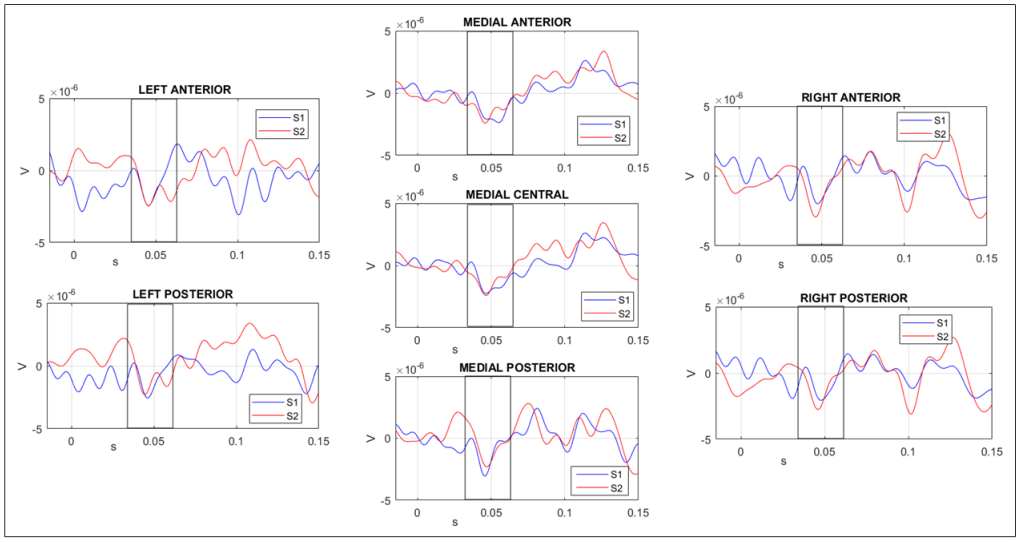

Figure 5.2: Topological representation of the P50 wave in subject P2

The grand average of all the ROIs (with the correspondent standard deviation) was calculated in order to have an overview of the P50 wave through the whole scalp. In Figure 5.3 the calculation of S2 and S1 can be observed. They are visible but sensory gating is not present, having even a greater value for S2 with respect to S1.

Regarding the analysis of all the patients, P1 and P3 showed a behaviour similar to the one of P2: S1 was well-identified in mostly all the ROIs, whereas S2 was not so easy to highlight. Therefore, sensory gating was not well evaluated in all the regions. Nevertheless, maximum and minimum values of S1 and S2 in the [0.03 s, 0.07 s] were reported and they were used to calculate S2/S1 in order to quantify the sensory gating per each ROI (Section 5.3 *Comparison between healthy subjects and patients*).

The topological images of patients P1 and P3 are presented below (Figures 5.4 and 5.5).

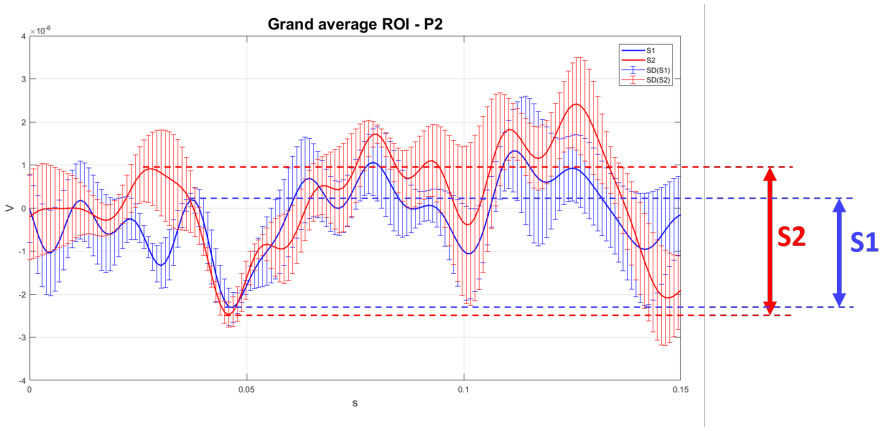

Figure 5.3: Grand average of all the ROIs in patient P2

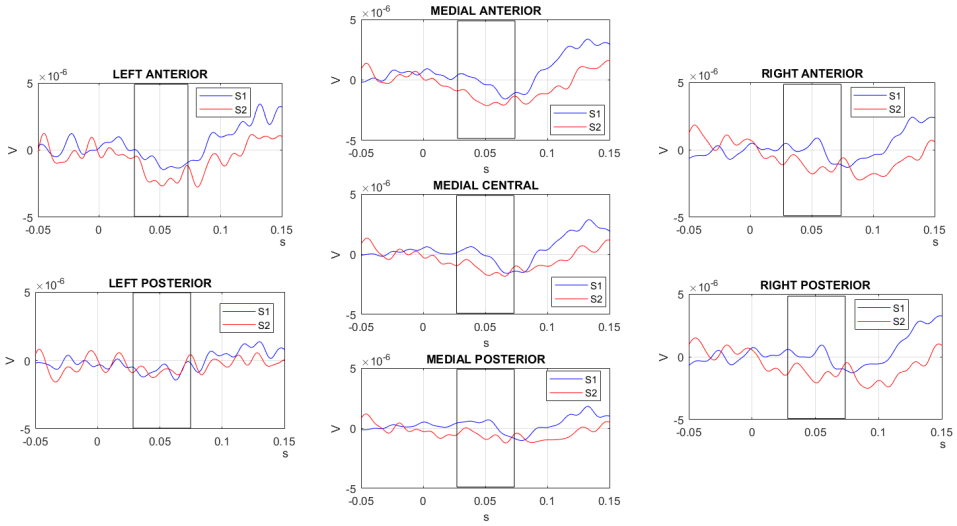

Figure 5.4: Topological representation of the P50 wave in subject P1

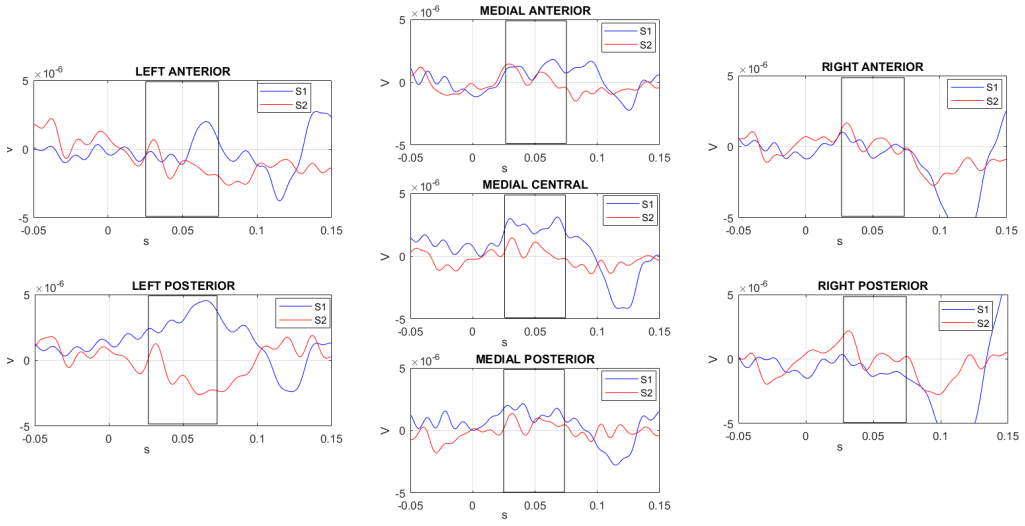

Figure 5.5: Topological representation of the P50 wave in subject P3

5.3 Comparison between healthy subjects and patients (T1)

In this section comparison between healthy subjects and patients is performed.

Sensory gating is evaluated in a quantitative way. S2/S1 is calculated as a measure of sensory gating. This quantity has typical dimensionless values between 0 and 1. Values close to 1 or greater than 1 indicate poor sensory gating.

Maximum and minimum values of S1 and S2, are reported in an Excel file; their amplitude are calculated as the absolute difference of these values. S2/S1 is computed and the mean of the obtained values per each subject and per each ROI is calculated.

In Table 5.1 the values of S2/S1 for the healthy subjects are reported. It can be noticed that the lowest values are in the LA and LP regions (0.4790 and 0.5321); these values are reliable having a good SD: they represent good sensory gating in these areas. On the other hand, the ROIs on the right part have values higher than 1 (1.1780 and 1.0871). Also MP has a value really close to 1: 0.9986.

As regard the patients, Table 5.2 describes the values of S2/S1 for the 7 ROIs averaged per patients with the respective SD, in the same way as the healthy subjects. In this case, all the values, except one, are higher than 1. However, the only value left is close to 1 (0.9106 for ROI MA). Therefore, patients show deficit in sensory gating in all the analysed ROIs. This result is in accordance with literature. This topological representation highlights the deficit in all the analysed ROIs.

Table 5.1: S2/S1 in healthy subjects with the correspondent mean and SD per each ROI

HEALTHY SUBJECTS (S2/S1)					
	C1	C2	C3	MEAN	SD
LA	0.4729	0.5395	0.4247	0.4790	0.0577
LP	0.6631	0.5794	0.3539	0.5321	0.1599
MA	0.4297	1.5223	0.6801	0.8774	0.5724
MC	0.4633	1.0218	0.8693	0.7848	0.2887
MP	0.9172	0.7496	1.3290	0.9986	0.2982
RA	1.4297	0.7745	1.3298	1.1780	0.3530
RP	0.7475	0.5308	1.9831	1.0871	0.7835

Table 5.2: S2/S1 in patients with the correspondent mean and SD per each ROI

PATIENTS (S2/S1)					
	P1	P2	P3	MEAN	SD
LA	1.4929	0.8148	0.9088	1.0722	0.3674
LP	0.8770	1.2990	1.5839	1.2533	0.3556
MA	0.6324	0.9398	1.1597	0.9106	0.2649
MC	0.6350	1.1435	1.2687	1.0157	0.3356
MP	0.8893	1.2553	1.1512	1.0986	0.1886
RA	0.7309	1.2106	1.2554	1.0656	0.2908
RP	0.8225	1.0615	2.0218	1.3019	0.6348

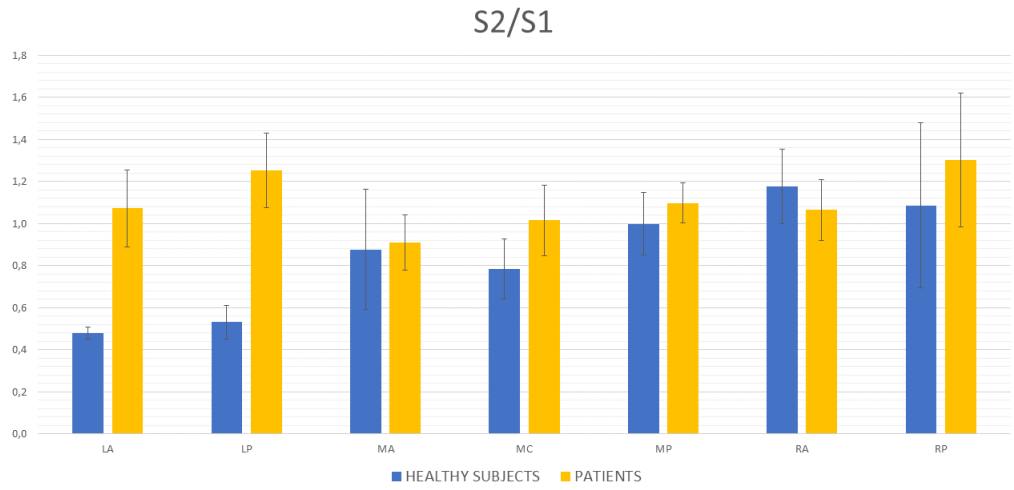

Figure 5.6: Comparison between healthy subjects and patients using S2/S1 in the different ROIs

The data just described are then reported in a bar chart (Figure 5.6) in order to compare the two cohorts. In almost all the ROIs, data from healthy subjects are less than the ones from patients.

Only in RA the value of S2/S1 is greater for healthy subjects.

RP has non reliable values in both healthy subjects and patients having high values for the correspondent SD (0.7835 and 0.6348 respectively).

In general, sensory gating ratios vary considerably among individuals, also in the same group. Overall, the results are satisfactory since the values of the healthy subjects are below 1 for almost all the ROIs and they differ from the patients substantially, as expected from literature.

Moreover, it can be underlined that LA and LP are the two ROIs in which there's more difference between the two cohorts. Recalling that patients with AVH show hypo activity in the left primary auditory cortex (Chapter 2.1), the present results are consistent with the theory. Patients show higher values of S2/S1 (poor sensory gating) with respect to the healthy subjects' ones in LA and LP that, in fact, represent the area of the left primary auditory cortex.

5.4 Analysis of one patient before (T1) and after the treatment (T2)

In this section, a comparison between data before and after the treatment of subject P2 is presented. As already mentioned, being a double-blind study, at the time of the analysis it was not known if the patient had been real treated or not. This was revealed after obtaining the results only.

As regards the topological representation of the P50, Figure 5.9 shows the data of P2 after the treatment. S1 is clearly visible in some regions such as LA, LP and MA and S2 can be identified considering the same latency as S1. Using the same method, S2 can be highlighted also in the other ROIs although S2 data are slightly noisier than S1 ones. In general, S1 is easier to identify with respect to S2.

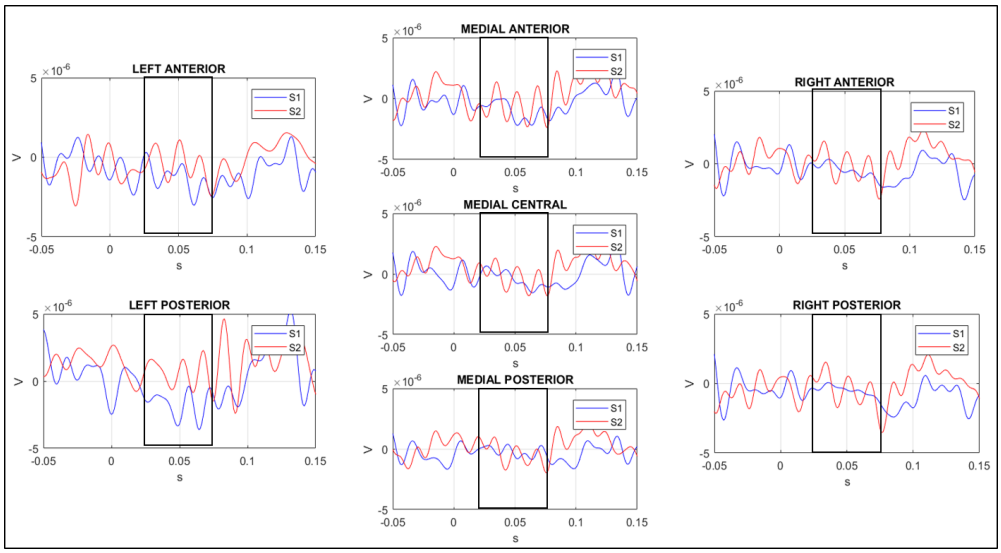

Figure 5.7: Topological representation of the P50 wave in subject P2 at T2 (post treatment)

The grand average of all the ROIs is then computed (Figure 5.8) and compared with the one of the pretreatment (Figure 5.3). Similar observations to the ones made for the topological representation can be done in this case. S1 is clearly visible, whereas S2 is calculated taking into account the same latency of S1.

As regards quantitative results of this analysis, Table 5.3 reports the values of S2/S1 in the different regions at time T1 and T2. A bar chart is used to represents graphically these values in order to highlight the differences.

It can be observed that all the values of S2/S1 are higher than 1, except for some values still really close to 1, in both conditions.

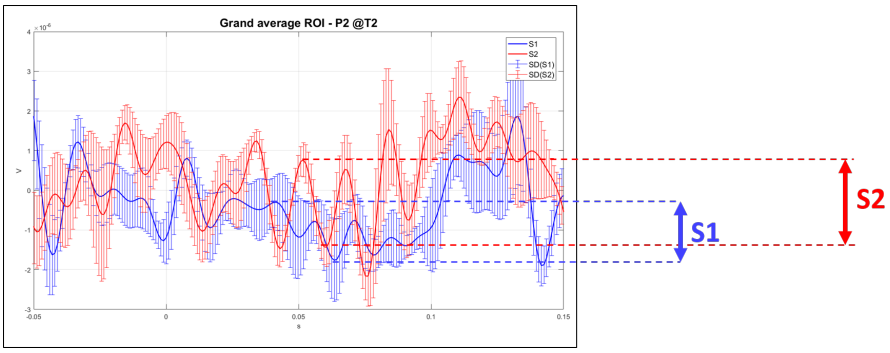

Figure 5.8: Grand average of all the ROIs in patient P2 at time T2

Table 5.3: Values of S2/S1 in the different ROIs before and after the treatment

	S2/S1	
	T1	T2
LA	0.8148	0.9184
LP	1.2990	0.9782
MA	0.9398	1.4899
MC	1.1435	1.2566
MP	1.2553	1.3511
RA	1.2106	1.6298
RP	1.0615	2.2557

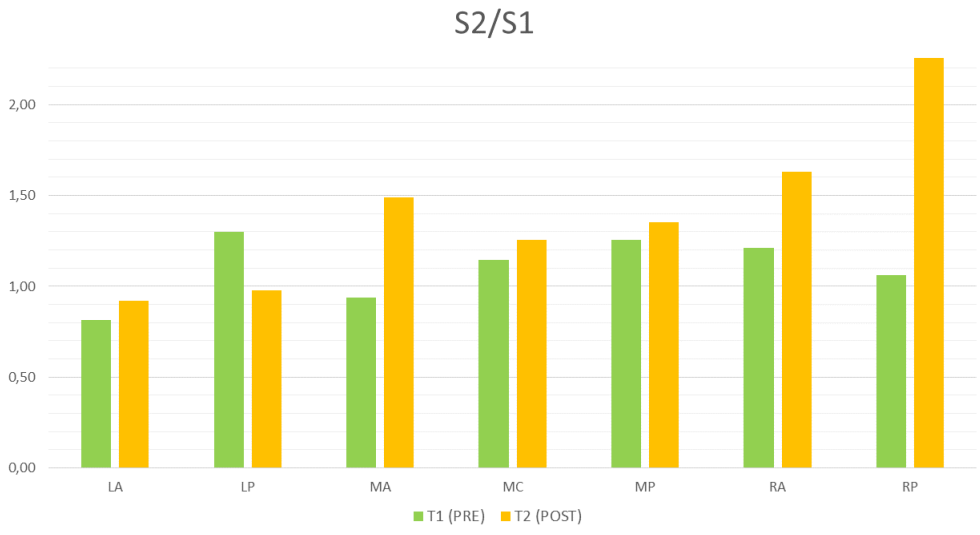

Figure 5.9: Comparison between T1 and T2 using S2/S1 in the different ROIs

RP in T2 is an outlier compared to the other values. Its value is 2.2557 and it is more significant than the others and also it is not expected. Thus, it is not considered in the following statements.

Examining all the results, data regarding T2 are not improved, with respect to T1. Values are still really high and comparable with the T1 condition. If the patient had been real treated it would have been expected a decreasing in the values but this happens in only one region (LP) and the values of the post-treatment is still really high: 0.9782.

This indicates a compromised sensory gating not only in the pretreatment condition (as already reported in the previous section) but also in the post treatment data analysis.

It can be assumed that patient P2 underwent sham rTMS.

After the inspection of all the data before and after the treatment and after obtaining all the results, this hypothesis was confirmed by the clinicians team.

This is a preliminary result since only one patient was analysed in the comparison before and after the treatment.

In addition to this, it has been assumed an improvement after the treatment but this is still not proved for the P50 evoked potential. A further analysis comparing real and sham treatment is necessary in order to validate or contradict this hypothesis.

Chapter 6

Conclusion

The present work aimed to develop a method to analyse data from the "Icelandic AVH TMS" Project. P50 evoked potential recorded with high-density EEG was studied in order to test this method.

Concerning the comparison between healthy subjects and patients before the treatment, the results we found in this preliminary phase are promising. Only 3 individuals per cohort were studied but the data we have obtained are in accordance with literature.

As regard the study of a single patient before and after the treatment, the results we discovered are in agreement with the hypothesis of the Project. However, further analysis is indispensable to assess this result.

More patients and healthy subjects data have to be analysed in order to perform statistical tests and validate these results.

In conclusion, the method we developed is efficient for the data tested until now. The further goal is the analysis of all the available data in order to evaluate the effects of the rTMS treatment in schizophrenic patients.

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Appendix A

Appendix

A.1 Brainstorm - P50 Preprocessing

BRAINSTORM - P50 preprocessing

Elena Pegolo, @elena.pegolo@gmai.com, April 2019

Download and install Brainstorm software (step by step using the link below):

https://neuroimage.usc.edu/brainstorm/Introduction

Open MATLAB

» type brainstorm in command window to open

File » New protocol

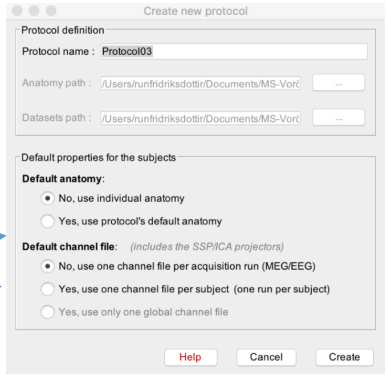
» Name your protocol.

Default anatomy

» Yes, use protocol's default anatomy

Default channel file

» No, use one channel file per aq. run



Go the the functional data window

Right click on the protocol

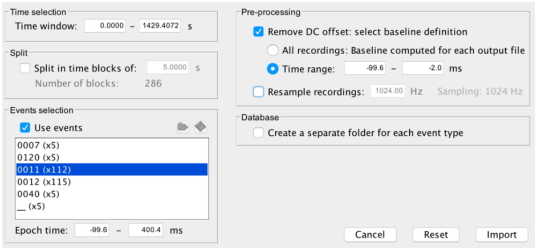
» New subject » Name the Subject

Right click on the Subject

» Import MEG/EEG

» open the *.cnt file (ANT EEPROBE format)

Choose the time window of the file imported



When importing events, choose events and epoch time

In AVH TMS project:

- P50: 0011 is the first event and 0012 is the second event
- Import 0011 and 0012 events seperatly.Start by 0011.
- For P50 choose epoch time [-100ms 400ms]

Remove DC Offset: Check this option, select Time range: [-100, -2] ms.

For each epoch, it will: Compute the average of each channel over the baseline (pre-stimulus interval: [-100,-2] ms) and subtract it from the channel at every time instant (full epoch interval: [-100,400] ms).

Remove DC offset: select baseline definition » Time range: [-100 ms -2ms]

Click Import (if you get a warning about replacing a channel file – click no). Import again and now choose event 0012.

Core Registration

Right click on the Subject folder

» import channel file (Warning appears, click yes to overwrite)

» choose the channel location file (standard_waveguard256_duke.elc) in ANT ASA *.cnt format

» Warning appears, click yes.

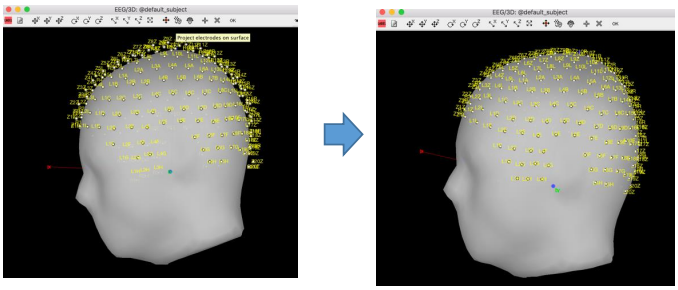
check if the electrodes location is coregistered for all the studies of the subject, otherwise copy paste the correct intra-subject file into all the studies

Right click on ANT Xensor under the Common Files folder

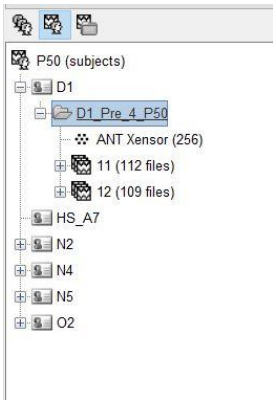
» MRI registration

» Edit

» Project electrodes on surface » Refine registration » OK



The Subject window should look similar to this:



Preprocessing

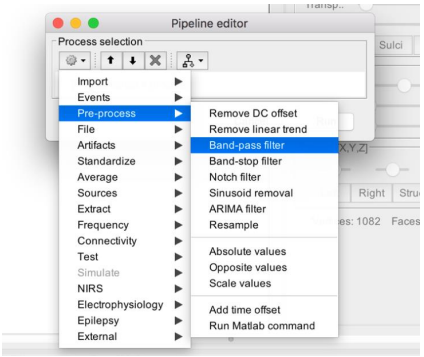
Select the subject folder and drag it to the processing panel below. (Filtering 0.1-80 Hz)

Select

» run » add process » preprocess » band-pass filter (0.1 Hz – 80 Hz) » run

Check ‘overwrite input files‘ if you want to overwrite the files

A new file folder ‘| band ‘ is created for each sub folder.



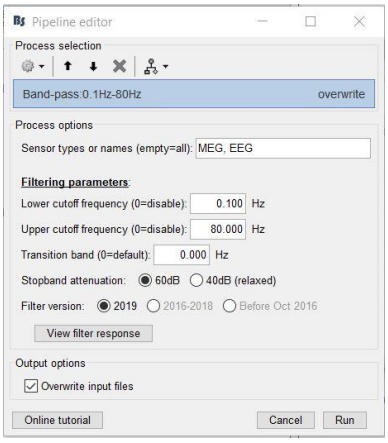
Now select the ‘| band ‘ folder for both events and drag them to the processing panel below (Notch filter)

Select

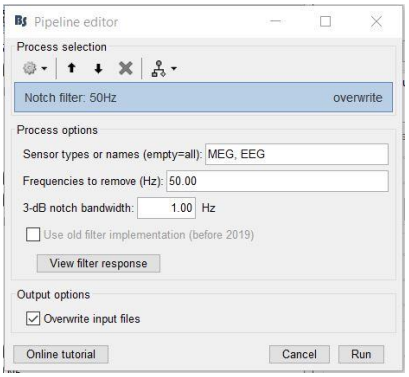
» run » add process » preprocess » notch filter » run

Check ‘overwrite input files‘ if you want to overwrite the files

A new file folder ‘| band | notch ‘ is created for each sub folder.



Band-pass filter (0.1-80Hz)



Notch filter (50Hz)

Reference

Create a new reference: BIMASTOID

» Double click on the first epoch in events which have been filtered » click the Record tab » click on Avg Ref and select ‘Edit montages… ‘

» Click on new montage » new re-referencing montage (linked ref)

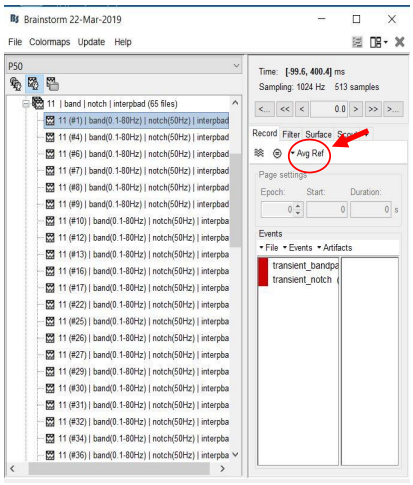
Click OK on the warning message

» New montage name: ‘Bimastoid‘

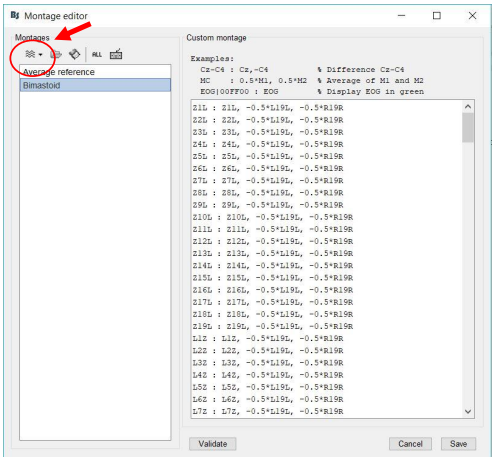
» Select the first reference channel from the list (R19R) » OK

» Select the second reference channel from the list (L19L) » OK

» Save



In the Record tab » select ‘bimastoid reference‘ or ‚average reference‘ (depending on which is appropriate).



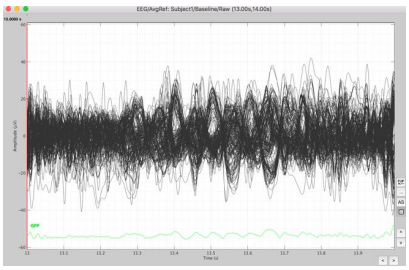
New montage

Remove bad channels

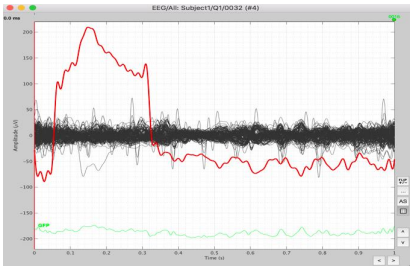
Visually check all epochs to detect weird channels.

» Double click on the first epoch in the events that have been filtered » click on the bad channel (becomes red) » right click » channels » mark as bad (or press delete button on keyboard)

The channel will be interpolated only for the selected epoch. Up to 10% of the channels can be interpolated for each epoch, otherwise reject the whole epoch.



A good epoch



A channel that should be interpolated

We remove outliers or channels over +/-80µV, if the outlier is just for a short period (<0.2 ms) keep it.

If the whole epoch is bad (>10% of the channels)

» right click on the epoch » file » delete

This must be done for all epochs. If in doubt, rather keep the epoch than delete too much.

Interpolate bad channels

Select the folder with the filtered and visual detected data and drag it to the processing panel below

Select

» run » add process » standardize » interpolate bad electrodes (keep all default) » run

A new file folder '| band |notch | interpbad' is created for each sub folder.

Brainstorm saves the file automatically, when you reopen Brainstorm you will arrive to the same step of the preprocessing.

Averaging and process

After the preprocessing the data are averaged and processed in Matlab. The data can be saved on .mat format and opened seperatly from Brainstorm - see Protocol *BRAINSTORM_P50Averaging* to continue the analysis.

A.2 Brainstorm - P50 Averaging

BRAINSTORM - P50 averaging

Elena Pegolo, elena.pegolo@gmail.com, June 2019

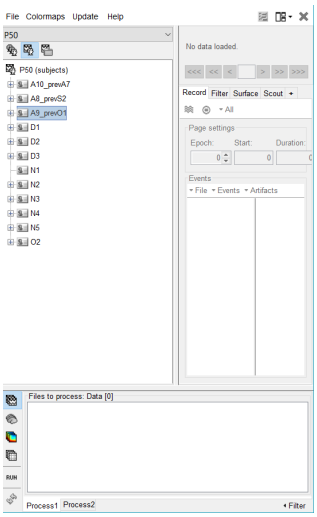
ATTACHED FILES: D2.mat, codice_roi.mat, structures.mat

This is a protocol that describes the process of analysis of the data. It has to be used after "BRAINSTORM – P50 preprocessing" protocol which describes the preprocesing of the P50 data in Brainstorm.

Open MATLAB

» type brainstorm in the command window to open brainstorm

Open the protocol where the P50 preprocessing is.

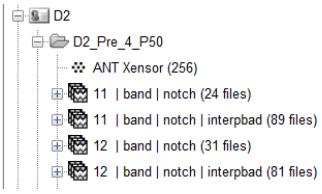


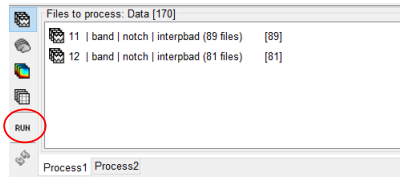
Average of the data

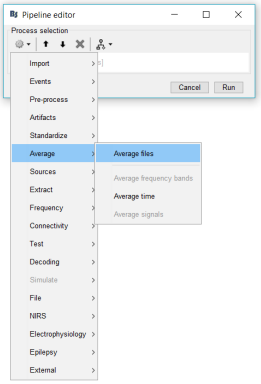
Open the subject you have to work with.

Drag the two folders 11 and 12 '| band |notch | interpbad' in the process box.

» RUN » Average » Average files » Run







Two new files are created: 'Avg: 11 | band |notch | interpbad' and 'Avg: 12 | band |notch | interpbad'

Bimastoid reference

In the section 'Reference' of "BRAINSTORM – P50 preprocessing" it is explained only how to change the visualization of the data. In this section, we apply the reference in our data.

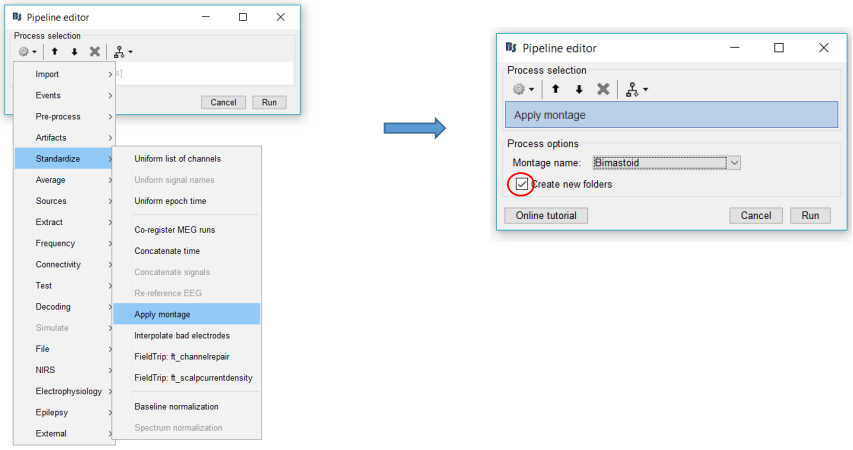
Drag the new files just created 'Avg: 11 | band |notch | interpbad' and 'Avg: 12 | band |notch | interpbad' in the process box.

» RUN » Standardize » Apply montage

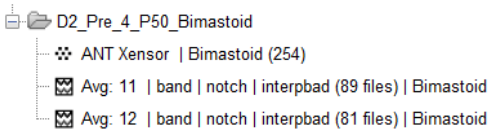
» Select the montage name 'Bimastoid' (created in the "BRAINSTORM – P50 preprocessing" protocol)

» Check 'Create new folders'

» Run



A new folder is created with the two new files inside



Processing of the data in Matlab

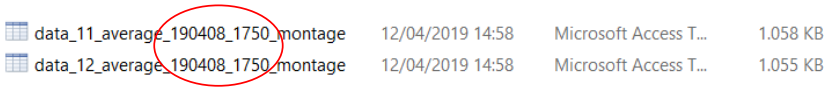
Each subject has a code file named *codeofthesubject.mat*. In this file, there is the code to visualize and average the data. The attached example file *D2.mat* has the comments to understand how to work with the data.

The file *codice_roi.mat* is used to create the variable *roi*, used in the code of each subject.

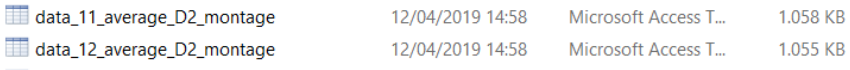
The file *structures.mat* puts together the data of each subject to average and to compare the different cohorts (pre-treatment patients and healthy subjects).

NOTE: How to save the data: from Brainstorm to Matlab

Each file in Brainstorm is saved in the folder *brainstorm_db*. In this case, the P50 data averaged and referenced to the bimastoid are saved in the folder: *brainstorm_db\P50\data\D2\D2_Pre_4_P50_Bimastoid*. The files we're interested in are .mat files and they are named with the date and time in which they're created.



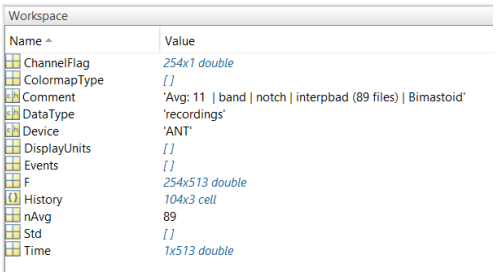
The files can be copied and pasted in the folder of Matlab where we're working in and renamed with the code of the patient.



These Matlab variables can be loaded in the workspace of Matlab. Each file is a struct.

» Matrix *F* contains the signal of each channel averaged for the different trials;

» Vector *Time* contains the time samples;



A.3 Healthy subjects: C1 and C2

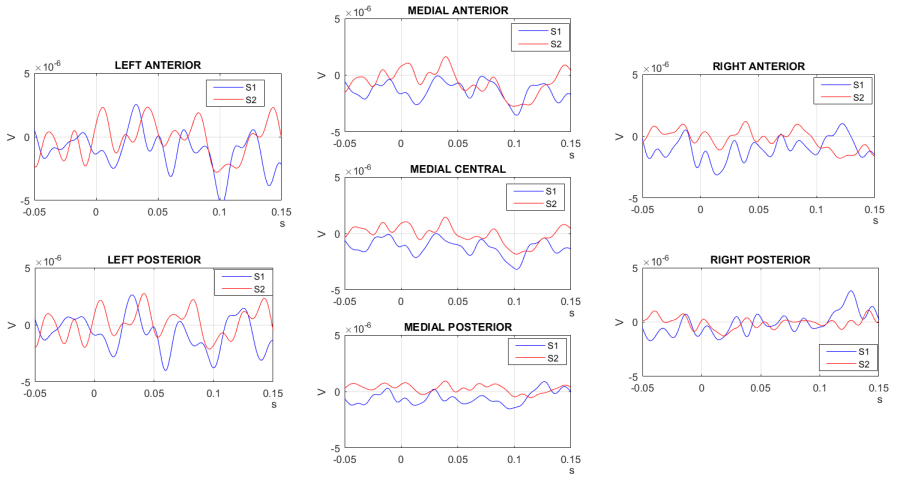


Figure A.1: Topological representation of the P50 wave in subject C1

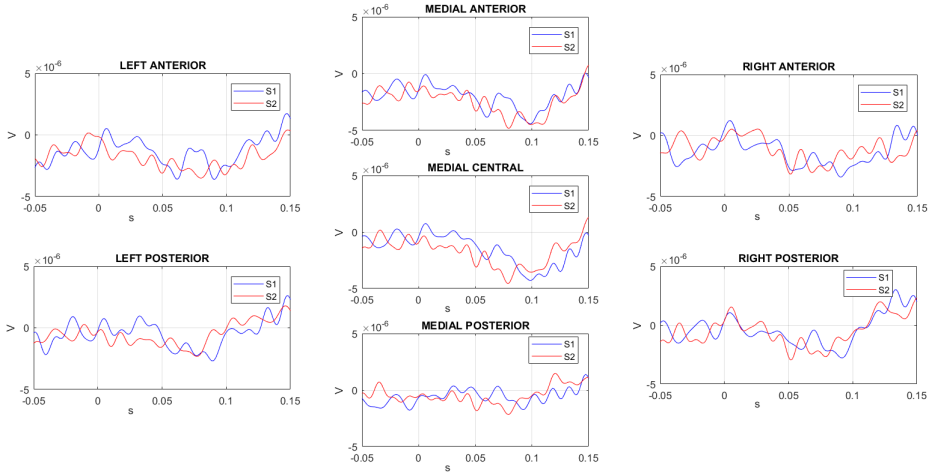


Figure A.2: Topological representation of the P50 wave in subject C2