

POLITECNICO DI TORINO

Master of Science Program
in BIOMEDICAL ENGINEERING

Master Degree Thesis

**Spectral analysis of P300 paradigm using High
Density EEG in patients with schizophrenia
undergoing TMS treatment**



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Academic Year 2019/2020

Contents

1	Introduction	1
1.1	Background	1
1.2	EEG	2
1.3	ERP	6
1.3.1	Neurophysiology	6
1.3.2	Studies based on ERPs	7
1.4	Schizophrenia	10
1.4.1	General characteristics	10
1.4.2	AVH	12
1.4.3	Treatments	12
1.4.4	Psychometrics	15
2	Previous studies	17
3	Experimental Set up	21
3.1	Participants	21
3.2	P300 recordings	24
4	Material and Methods	25
4.1	Acquisition	25
4.2	EEG pre-processing	25
4.2.1	A brief introduction to pre-processing	25
4.2.2	Protocol	27
4.3	Data Analysis	31
4.3.1	A brief introduction to PSD and Statistical Analysis	31

4.3.2	Protocol	33
4.3.2.1	Brainstorm: Power spectral density computing	33
4.3.2.2	Matlab: Comparison within cohorts	33
4.3.2.3	Matlab: Comparison between cohorts	36
5	Results	38
5.1	Frequent - rare	38
5.2	Post-TMS - pre-TMS	41
5.3	Healthy - patients comparison	42
5.4	Variability between subjects of the same cohort	43
5.5	Psychometric	45
5.6	Discussion	45
6	Conclusion	47
7	Future Goals	49
	Appendices	56
A	Informed consent	57
A.1	Invitation and introduction form	58
A.2	Information form for participants in the healthy control group	60
B	Protocol	61
B.1	Brainstorm: P300 Protocol	62
B.2	Matlab: Data analysis Protocol	71
C	Supplementary data	74

List of Figures

1.1	EEG acquisition [A]	2
1.2	Example of EEG signals in the various frequency bands	3
1.3	International 10-20 Electrode System seen from (A) left and (B) above the head [B]	4
1.4	Electrodes displacement	5
1.5	Synapse [C]	6
1.6	Pre- and post-synaptic neurons [D]	7
1.7	Event related potential	8
1.8	ERP parameters	9
1.9	Repetitive Transcranial Magnetic Stimulation applied to the head of a subject [E]	14
2.1	ROI creation	18
2.2	P50 suppression ratio and N100-P300 voltage measurements	19
2.3	P50 suppression in three healthy participants and three patients with schizophrenia (baseline)	20
3.1	Data acquisition and processing workflow	23
3.2	Example of P300-peaks for frequent (F) and rare (R) stimuli	24
4.1	ANT Neuro System	26
4.2	Experiment set-up	26
4.3	Band-pass filter	28
4.4	Notch filter	29
4.5	Outliers	30
4.6	Bad time sequence	30

4.7	PSD matrix	34
4.8	Example of plots of the same cohort's PSD values of significant channels (marked with 'x')	35
4.9	Example of plots of PSD subtraction between significant channels (marked with 'x') of different cohorts	37
5.1	PSD Subtraction of significant channels: Frequent - Rare stimuli	39
5.2	PSD values of a single patient	40
5.3	PSD Subtraction of significant channels: post - pre TMS	41
5.4	PSD Subtraction of significant channels: HS cohort - TG cohort	42
5.5	PSD difference post - pre treatment (rare stimulus) of subjects of the same cohort	44

List of Tables

4.1	Epochs creation	31
5.1	Increase (↑), decrease (↓) or constancy (-) of the psychometric of each subject of TG and CG after treatment	45
C.1	Averaged PSD difference - cohort comparison : pre-post, F-R, Healthy-Patients. Unit : $(\mu V^2/Hz) \times 10^{-15}$	75

List of Acronyms

AH Auditory Hallucination

AVH Auditory Verbal Hallucination

CNS Central Nervous System

DASS Depression Anxiety Stress Scales

DFT Discrete Fourier Transform

EEG Electroencephalogram

EPS Extrapyrarnidal Side Effect

ERP Event-Related Potentials

FAD Familiar Alzheimer Disease

HD EEG High-Density Electroencephalogram

PSYRATS Psychotic Symptom Rating Scales

QoLS Quality of Life Scale

RTMS Repetitive Transcranial Magnetic Stimulation

SNR Signal to Noise Ratio

TPC Temporo Parietal Cortex

WSS Wide Sense Stationary

Abstract

This thesis is the result of my contribution to the ongoing "AVH TMS project" at Reykjavik University, Iceland.

This exploratory work aims to hypothesize a methodological tool and electroencephalogram (EEG) biomarkers for the effect of repetitive Transcranial Magnetic Stimulation (rTMS) on patients with schizophrenia and auditory and verbal hallucinations (AVH). Ten patients diagnosed with schizophrenia and pharmacological persistent AVH were divided into two groups: the first group (TG) received 1Hz frequency rTMS treatment during 10 daily sessions (900 pulses/session) over the left T3-P3 International 10-20 location. The second group (CG) received rTMS treatment over the Cz (vertex) EEG location. Data from the patients were acquired before the rTMS treatment (pre) and 10 days after the treatment (post), and they were compared with the results obtained from a third group of healthy subjects (HS), who did not receive rTMS treatment.

A P300 experiment (oddball auditory paradigm) was done and recorded with a high-density electroencephalogram (HD EEG). The data were analyzed in the frequency domain, focusing on the alpha (8-13 Hz), low beta (13-18 Hz) and high beta (18-30 Hz) frequency bands and the power distribution was computed to show the differences of cortical response between groups.

Despite the limited number of subjects enrolled in our study, this work is a first step towards defining biomarkers assessing the effect of left temporoparietal TMS treatment.

Chapter 1

Introduction

1.1 Background

Schizophrenia is a severe pathology that seriously influences the everyday life of the patient.

Several studies tried to provide a scientific explanation to the cerebral response during the main symptoms of schizophrenia, e.g. hallucinations and delusions. However, the complex neuronal interactions in the brain are very hard to define and today there are only hypotheses about the biological causes of this disorder.

In many cases, patients with schizophrenia do not respond to the treatment with antipsychotic drugs. This is the main reason that brought many researchers to further analyze the pathology in order to find new treatments to cure them.

In 1999, Hoffman and his colleagues started to explore the repetitive transcranial magnetic stimulation (rTMS) as a novel treatment of auditory and verbal hallucinations (AVH) in patients with schizophrenia. It was the turning point that launched the development of a series of new studies and researches on this field that analyze the illness in a new point of view.

This project is an exploratory study aimed at generating hypotheses about how rTMS affects brain function in schizophrenic AVH and to search for biomarkers of this effect using high-density EEG (HD EEG). Response biomarkers are very important from the clinical point of view to create a customized clinical plan. Personalized medicine is indeed decisive to allow doctors and patients to develop targeted treatment and prevention plans.

1.2 EEG

An electroencephalogram (EEG) detects electrical activity of the brain using electrodes placed on the participant scalp (*Figure 1.1*).

The amplitude of the signal varies between 10 and 500 μV , and it can be defined as

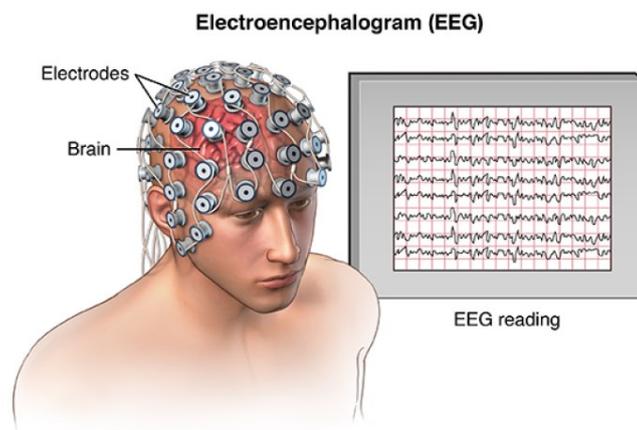


Figure 1.1. EEG acquisition [A].

low ($<30 \mu\text{V}$), medium ($30\text{-}70 \mu\text{V}$) or high ($>70 \mu\text{V}$).

A segment of an EEG signal registered on the scalp can be assimilated as a random process. It is indeed generated by the superimposition of electromagnetic fields produced by post-synaptic potentials of neurons.

It can be divided into 5 different frequency bands:

- *delta* (0.5-4 Hz): related to deep sleep or coma. In waking adults it could represent cerebral injury or severe cerebral pathologies.
- *theta* (4-8 Hz): present in infants, or in the first minutes of sleep in adults. In waking adults it is related to several cerebral diseases.
- *alpha* (8-13 Hz): present during wakeful relaxation with eyes closed.
- *beta* (13-30 Hz): related to the conscious state, when the subject pay attention and he is involved in cognitive tasks.

- *gamma* (>30 Hz): correlated to the processing of information. It is not a spontaneous rhythm, usually evoked by sensory stimulation.

The first four are showed in *Figure 1.2*.

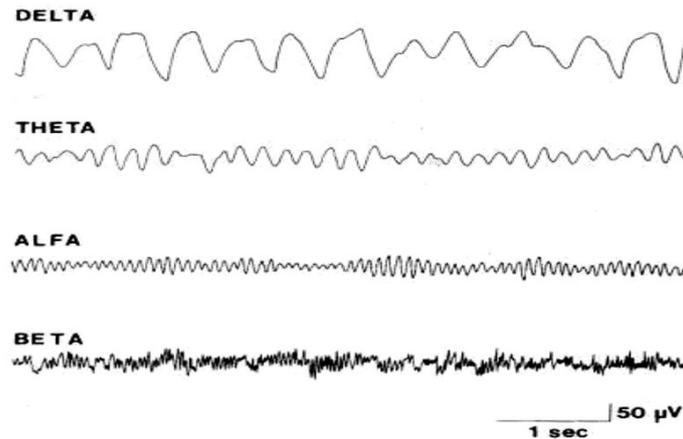


Figure 1.2. Example of EEG signals in the various frequency bands.

A typical EEG system uses electrodes, an amplifier, an analog filter, an analog-to-digital converter (A/D) and a computer with EEG acquisition software.

Electrodes are metal disks or cups, they act as filters to not distort the EEG signals that have to measure. They should record very slow changes in potentials (generally the amplitude of the signal is less than 100 μV) [1].

Electrodes are positioned following a standard placement map, usually the *international 10-20 electrode system*, as shown in *Figure 1.3*. This system aims to standardize the outcome of the study and it is based on the relationship between the location of an electrode and the underlying area of the brain. The numbers 10 or 20 are referred to the percentage (10% or 20%) of the distance between electrodes positioned in some ideal places related to two main lines:

- from *nasion* to *inion* points: antero-posterior midline;
- from *left preauricular point* to *right preauricular point*: from one ear of the subject to the other.

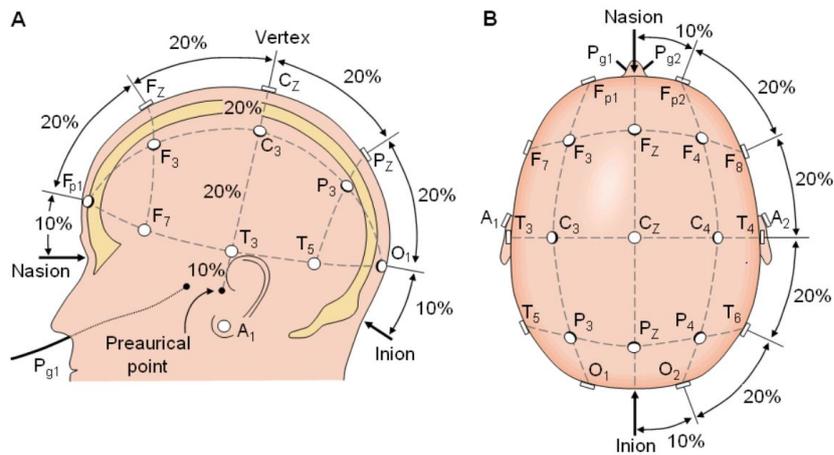


Figure 1.3. International 10-20 Electrode System seen from (A) left and (B) above the head [B].

The exact locations of the electrodes can be determined relative to some fiducial points and each one is labeled with a letter and a number to be identified. The letter represents the part of the head: frontal (F), temporal (T), central (C), parietal (P) and occipital (O). Numbers are used to indicate the side of the brain, even numbers for the right hemisphere and odd numbers for the left one.

The electrodes are usually attached to an elastic cap and can be placed with a conductive paste or gel to reduce the artifacts.

Analog filtering cuts the very high frequencies and the very low frequencies, and it is usually performed at the same time as the amplification.

A/D converter converts the signal from analog to digital form so that it can be recorded and processed by the computer. Optimal sampling would use a separated A/D converter for every channel so that all channels could be sampled simultaneously.

A typical EEG system for gathering ERP data uses a differential amplifier, which requires an active electrode (A), a reference electrode (R), and a ground electrode (G). The *active electrode* is placed at sites over which activation is expected. The *reference electrode* could be placed in the scalp or computed off-line, but the most important thing is that the reference should not affect the characteristics of the ERP component of interest. The *ground electrode* is placed at some convenient location on the participant's head or body. An example of placement of these electrodes is shown in Figure 1.4.

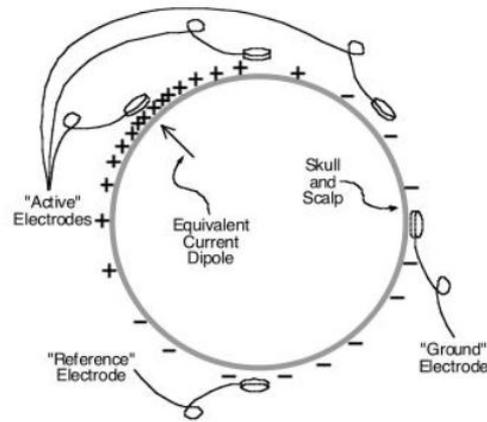


Figure 1.4. Electrodes displacement.

It is called *differential amplifier* because it computes the differences between the signals acquired from two pairs of electrodes, i.e. the difference between the voltage measured between A and G and the voltage measured between R and G. This means that the ground should be eliminated in the output result: $(A-G)-(R-G) = A-R$, to subtract the noise.

In his book, Cohen [2] showed three main reasons why EEG is the perfect tool for studying neurological processes.

First of all, it can capture fast, dynamic, and temporally sequenced events. It is so well suited for cognitive processes which occur within tens to hundreds of millisecond.

The second reason is that EEG direct measures the neural activity of the brain. The changes of voltage in EEG reflects the neural oscillations in the cerebral cortex.

Third, the EEG signal is multidimensional. The data are usually studied as two-dimensional (voltage changes over time and space, where space is measured through different electrodes), but they comprise four-dimensions: time, space, frequency, power, and phase. The last two are discrete elements of a dimension because they provide largely independent information: the power represents the strength of frequency-band-specific activity, while the phase is the timing of the activity ([2]).

In this project, a 256-channels 'high-density' EEG (HD EEG) is used. It attempts to overcome the spatial limitations of a standard electroencephalogram increasing the

number of electrodes [3].

To study the physiological response to TMS treatment, EEG measures generally include event-related potentials (ERPs) [4].

1.3 ERP

Event-related potentials (ERPs) are very useful to analyze the differences between brain processes of different groups of subjects [2]. In particular, for this project, comparing ERPs across the groups is the fastest and easiest approach to find biomarkers that highlight the neural response of patients with schizophrenia.

1.3.1 Neurophysiology

ERPs are direct measures of neural activity. They are scalp-recorded activities generated by a specific neural process, which in turn produces a certain polarity, latency, and scalp distribution.

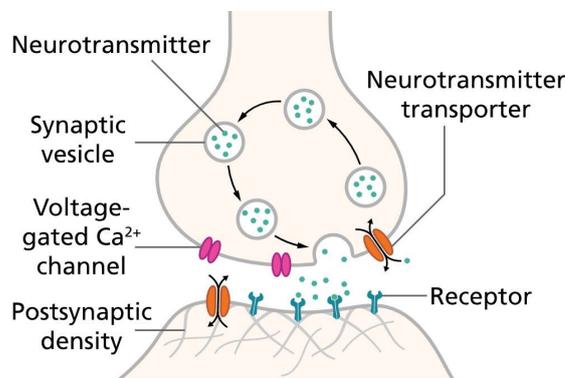


Figure 1.5. Synapse [C].

They are sensory components obtained through the stimulation of a sense, such as view or hearing: in this study, the subjects were exposed to acoustic stimuli, i.e. clicks through headphones. To better understand the process, it is important to know that neurons are the cell units of the nervous tissue and communicate with each other through the transmission of action potentials (electrical impulses). The signal moves through neurons by synapses, in which the electrical stimulus induces the opening of some ion channels of the pre-synaptic neuron and the entrance of positive ions as shown in Figure 1.5. These particles allow neurotransmitters (which are inside vesicles) to leave the cell and be captured by specific sensors of the post-synaptic neuron (Figure 1.6) which transmit the information.

The central nervous system receives and processes the information from the sensory organs to make decisions and to give precise instructions to parts of the body (such as muscles and glands). CNS takes part in learning and memory activities, in thoughts, emotions, language and other complex functions and the brain is the central control organ of the body and coordinates these activities.

ERPs are therefore waveforms of the EEG in specific points of time derived by acoustic stimuli (*Figure 1.7*). They represent the sum of postsynaptic potentials of large groups of neurons (*pyramidal neurons*) that become active in synchrony, propagated through the brain. Auditory ERPs are therefore of interest in psychiatric and neurological studies because they can be used to determine if the disorder impacts sensory input and the subsequent cognitive processing.

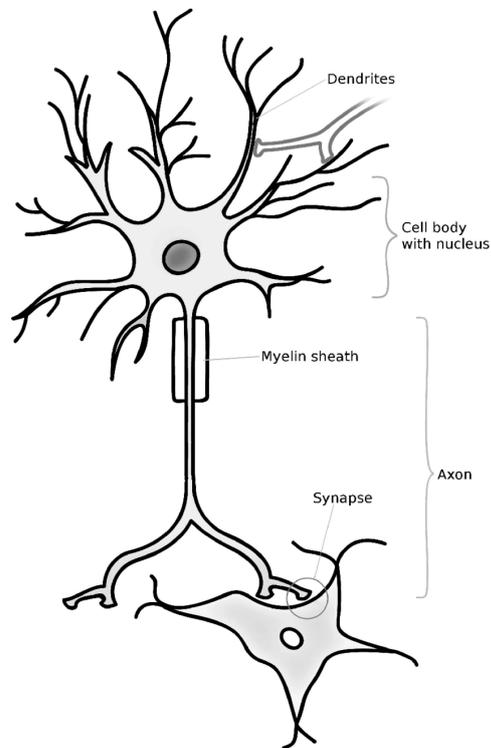


Figure 1.6. Pre- and post-synaptic neurons [D].

1.3.2 Studies based on ERPs

As mentioned earlier, auditory ERPs are now being applied in studies of many different pathologies.

The differences in amplitudes and latency of ERPs between normal subjects and patients could be used to inform models of the development of a pathology, as defining changes in cortical function in persons inheriting familial Alzheimer disease (FAD) [5]. The power spectral density of the signal could characterize the visual dependency and functional dynamics of cortical activation, for example during postural control [6] or it could demonstrate the effects of treatment, such as low frequency rTMS in chronic

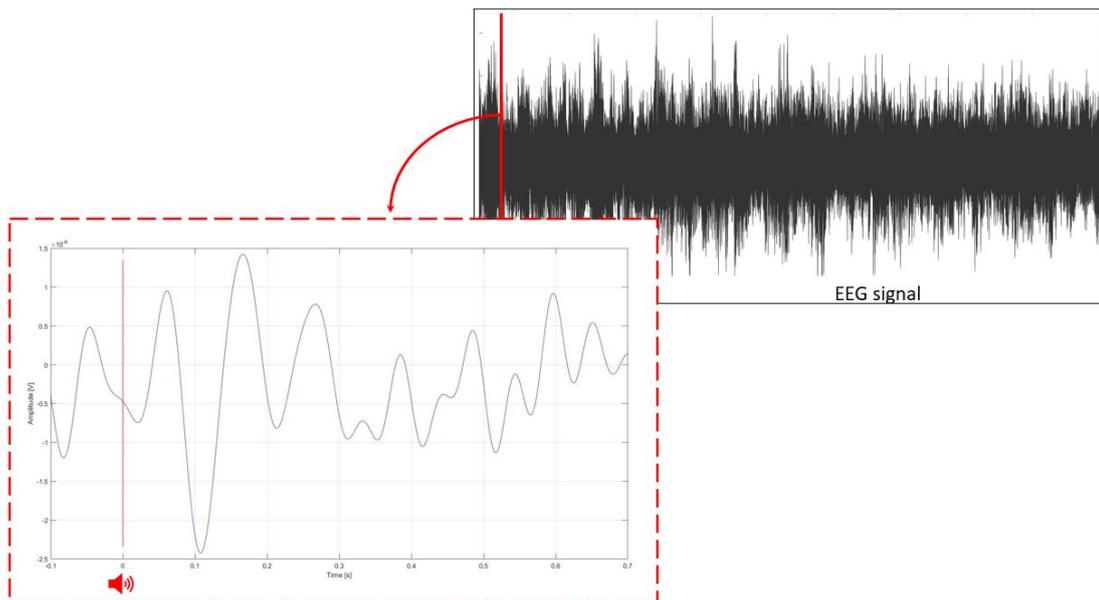


Figure 1.7. Event related potential.

tinnitus patients [7].

The application of ERPs in the AVH project aims to demonstrate the improvement of the cognitive dysfunctions in schizophrenia after rTMS treatments. The level of attention could modulate the sensory input and their evoked activity.

The main parameters used to describe ERPs are polarity, latency, and scalp distribution [8].

The voltage deflection of the component, expressed in μV , is called *polarity*, and it can be either positive or negative. It is related to the excitation or inhibition of cells during the transmission of the information through neurons.

ERP components are named with the letter 'P' for positive polarity or 'N' for negative polarity, and this letter is typically followed by a number that indicates the latency.

The *latency* is the number of milliseconds between the start point of the impulse (time-locked as 0 ms) to the event of interest. Some of the most important events will be discussed below.

The *scalp distribution* of an ERP component is the locations over the scalp where it highly appears during the processing of a stimulus. Different scalp distributions indi-

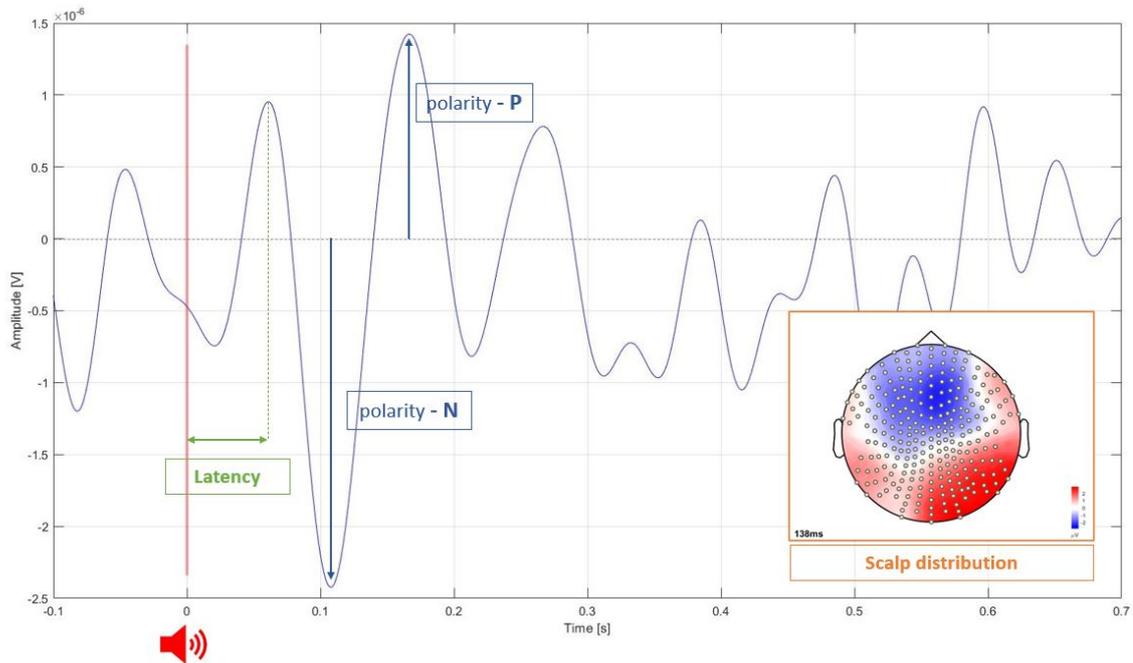


Figure 1.8. ERP parameters.

cate different cortical processing activity, and it can be represented by a graph (Figure 1.8).

The main component studied in this project is P300. The P300 first described by Sutton et al. (1965) is the leading ERP correlate of target discrimination [9] and it is mostly studied as a parameter of voluntary attention. It is the most positive peak occurring 300 ms after the stimulus and its amplitude is mostly used to obtain stimulus information, i.e. reduced P300 amplitude is an indicator of processing abnormalities in neuro behavioral disorders [10].

In many studies, P300 is related to N100, the negative deflection that occurs approximately 100 ms after the auditory stimulus, noticing a relation with working memory [11].

These two parameters were already studied in schizophrenia: in literature, the mean amplitudes of the auditory N100 and P300 responses decrease in schizophrenic patients in comparison to healthy participants [12]. The P300 is most commonly elicited using an oddball paradigm. In literature event-related potentials related to auditory oddball stimuli are largely studied in schizophrenia, finding irregularities in the brain response,

effect believed to be related to abnormalities in attentional and memory processes [13].

P50 has been also of high interest in psychiatric research to study dysfunction of sensory gating, specifically in schizophrenia [14]. It is a positive amplitude wave occurring approximately 50 ms after an auditory stimulus.

As anticipated before, generally experimental design in neuroscience consists in comparing one group of individuals that has the diagnosis of a disorder with another one with individuals that don't meet the criteria for any diagnosis (healthy subjects).

There are advantages and disadvantages to using ERPs as a diagnostic parameter for mental disorders. They are cheap and fast to collect (from the EEG), but many choices have to be made during data processing and analysis. The worst problem of the acquisition of the EEGs are artifacts related to the environmental noise, the electrical interference, ocular and body movements of the subject during the experiment and also the audio headphones used can cause electrical noise.

To avoid these complications, it is important to pre-process the signal before working on it, but this step could delete much important information.

1.4 Schizophrenia

1.4.1 General characteristics

Schizophrenia is a psychiatric syndrome that is mainly characterized by perceptual and cognitive deficits. Some of the main symptoms that could affect schizophrenic patients are delusions, verbal or acoustic hallucinations.

The late identification and intervention in the course of the illness could compromise the lives of these persons: people with untreated schizophrenia could experience a substantial decline that can be difficult to reverse. In worse cases, they become violent and could develop addiction to drugs and alcohol. This often happens in the case of people living in poverty or stressful surrounding, even more common if these situations start at young age.

A large amount of studies that aim to develop new treatments are emerging around the world. Diagnosis is still the first obstacle though: it requires several confirmations because signs can be mistaken as depression or other conditions.

In particular, schizophrenia symptoms can be divided into four categories:

- *Positive Symptoms*: delusions and hallucinations.
Delusions are stimulated by the belief that there are things that are not true. For example, a person thinks that a book, people on the radio or on TV are referring or talking directly to her or him. He/she thinks that someone is following, spying, or tormenting him/her or that the others are able to read and control his/her mind. Hallucinations are sensory perceptions that can be sensed only by the patient. They can be acoustic, visual, tactile, olfactory, or gustative. Hearing voices is common for people with schizophrenia.
- *Negative Symptoms*: having trouble experiencing affection, happiness and talking.
Subjects with these symptoms show no facial expressions (such as smiles), they talk a little and they have no interest in any activity.
- *Disorganized Symptoms*: thinking problems and strange behaviors.
Thoughts are disorganized and speeches are contradictory and inconsistent. The conversation can change from incoherent to incomprehensible. They can have childish behavior and bizarre movements.
- *Cognitive Symptoms*: difficulty in making decisions and understanding information, paying attention and having social interactions.
These symptoms can make it hard to have a job or take care of others (even of themselves).

The diagnosis of schizophrenia requires confirmation that patients meet established criteria for the disorder. Some of them are listed in the *Diagnostic and Statistical Manual of Mental Disorders*, for example when continuous signs of the disturbance persist for a period of at least 6 months, which must include at least 1 month of symptoms (or less if successfully treated) the subject may be affected by schizophrenia. Another one could be if the disturbance is not attributable to the physiological effects of a substance (e.g., a drug of abuse or a medication) or another medical condition, or if there is a history of autism spectrum disorder or a communication disorder of childhood onset, and the subject has prominent delusions or hallucinations, in addition to the other required symptoms or schizophrenia.

Many elements may cause schizophrenia, around 80% of cases are from heritable factors. Several studies identify a relation between the pathology and the immune system and synaptic functions [15]. As mentioned before, besides genetic factors, the environment may be involved. Exposure to viruses or nutritional problems before birth, having attended violent episodes during childhood or living in poverty are some circumstances that may cause schizophrenia [16].

1.4.2 AVH

People affected by auditory verbal hallucinations (AVH) are subjected to pathological hyperactivity at specific areas of the brain [17],[18]. This is the most common symptom of schizophrenia.

Patients experiencing AVHs usually describe hearing vague sounds, specific words, or conversations in which they are involved. These symptoms can become chronic, causing an impaired quality of life.

The pathophysiology of AVHs is still poorly understood but many studies have analyzed different neurological mechanisms involved. This study is based on the attentional skills among these subjects which may be related to deficits in affect perception [19]. Theoretically, deficient attentional skills could impair affect perception by interfering with the ability to decode facial stimuli.

The ability to sustain attention over time may be important because people must constantly follow the social dialogue to detect changes in emotional states. Attention problems may also affect many other higher cognitive abilities because attention is a necessary precursor for processing incoming information.

1.4.3 Treatments

There are two main types of treatment: antipsychotic medication and psychosocial treatments.

Antipsychotic medications aim to reduce the psychotic symptoms of schizophrenia. Each patient responds to the medication differently, and specific medicine, medication dose, and treatment plan are necessary. This is the reason why personalized medicine is fundamental in schizophrenia.

Medications could give some side effects, mainly the first days after the subject starts the treatment. They are divided into two types: traditional antipsychotics and second-generation antipsychotics. The first ones act blocking D2-like receptors (dopamine receptors), and they can be of low-, medium- or high-potency. The second-generation antipsychotics block dopamine receptors more selectively than the traditional ones, they have similar efficacy with low dose conventional antipsychotic and there are fewer extrapyramidal side effects (EPS) at effective doses with these drugs [20].

Psychosocial treatments are associated with antipsychotic medication. In this case, the family of the patient, friends, and colleagues are involved: doctors should teach them how to cope with the illness. But also the patient has to learn about schizophrenia and manage it in everyday life. The patient should also be followed by therapist and self-help groups, which provide support from other people with the illness. However, these treatments for schizophrenia remain unsatisfactory. The current available antipsychotic drugs don't treat many symptoms of the disorder and cause significant side-effects. The development of new treatments has a decisive role in the cure of this pathology.

Repetitive transcranial magnetic stimulation (rTMS) is a novel treatment which provides alleviation of symptoms of schizophrenia, especially reducing symptoms of auditory hallucinations (AH) [21].

RTMS

Transcranial magnetic stimulation (TMS) is a non-invasive method used over the past 25 years in the treatment of neurobehavioral disorders [22]. It is a neurostimulation technique that uses alternating magnetic fields to induce an electrical current in the cortex of the brain. During the application of TMS, an electromagnetic coil is placed on the scalp and transforms electrical activity to pulsed magnetic energy (*Figure 1.9*). It passes through the cranium and induces an electrical field in the cortex, depolarizing neurons and generating action potentials.

Low frequencies of the pulse have inhibitory effects on neural circuits [23], [24], high frequencies generate excitatory effects. Pulses can be regulated as single, paired, or in a series, called a train. The latter is called *repetitive TMS (rTMS)*. While single and paired pulse TMS are used for neurodiagnostic purposes, rTMS has therapeutic benefit in psychiatric disorders [23], [24].

Usually in schizophrenia, a minimum of 10 sessions of rTMS in 1-2 weeks is carried out [25], but the number can vary according to the choice of the doctor and the pulses of each session. Anyway, the effects of rTMS on AVH after one month are no longer significant [26].

Anesthesia is not necessary, but side effects can include headache, scalp pain at the site of the stimulation. The most serious potential adverse effect of TMS is the induction of seizure, which is rare.

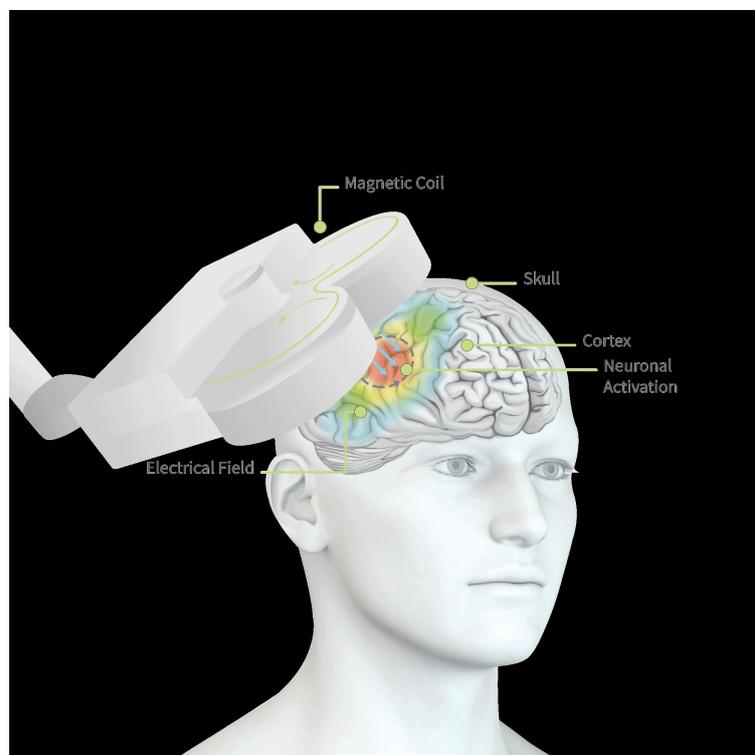


Figure 1.9. Repetitive Transcranial Magnetic Stimulation applied to the head of a subject [E].

Negative symptoms appear to be associated with hypoactivity of the dorsolateral prefrontal cortex of the brain, while positive symptoms appear to be associated with hyperactivity in the left temporo-parietal cortex. This part of the cortex is related to language perception areas (i.e., Wernicke's area) and auditory hallucinations may be due to abnormal activation of these areas [27].

There are two protocols of the treatment: high-frequency rTMS to the left dorsolateral prefrontal cortex to treat negative symptoms, and low frequency rTMS to the left temporo-parietal cortex (TPC) for the specific treatment of AH. The latter works by decreasing cortical excitability and possibly inhibiting dopamine release.

In this project, a group of participants affected by AVH was treated with an inhibitory frequency of stimulation of 1Hz to reduce the assumed hyperactivity of the TPC.

1.4.4 Psychometrics

Psychometrics is a scientific discipline concerned with the construction of assessment tools, and formalized models referred to psychological measurements. It focuses on the measurement of abilities, behaviors, personality traits.

Psychometric techniques are widely used across the sciences and have found applications in educational testing, behavior genetics, sociology, political science, and neuroscience. In this project, three scales were used to collect clinical information: Psychotic Symptom Rating Scales (PSYRATS), Quality of life (QoL) and Depression Anxiety Stress Scales (DASS).

PSYRATS

Psychotic Symptom Rating Scales (PSYRATS) is used to evaluate the seriousness of hallucinations and delusions in adults with schizophrenia. It is an interview using 11 items rated on a five-point ordinal scale (0-4). The scale measures the severity of AVH for the past week on eleven dimensions which are: frequency, duration, location, loudness, beliefs about origin, negative content, intensity of negative content, amount of distress, intensity of distress, disruption of life and control. PSYRATS has shown excellent inter-rater reliability and good discriminant and convergent validity for both chronic and first episode psychosis ([28]; [29]).

QoLS

Quality of life (QoL) consists of 16 items assessed on a 7 points self-report scale, from "delighted" to "terrible". It consists of five conceptual domains of quality of life:

material and physical well-being, relationships with other people, social community and civic activities, personal development and fulfilment, and recreation. The scale has been shown to have good test-retest reliability and good convergent and discriminant validity ([30]).

DASS

The Depression Anxiety Stress Scales (DASS) is a measure of mental health focusing on the three traits of depression, anxiety and stress. It consists of 42 items, rated on a four-point Likert type scale of how much that symptom occurred in the last week. In clinical samples, the scale has shown excellent internal consistency and temporal stability as well as excellent discriminant validity and good convergent validity ([31]).

Chapter 2

Previous studies

This work is part of the ongoing “Icelandic AVH-TMS” project [45], whose goal is to identify changes in psychometric scales and neurophysiological measurements between baseline and in three different times after the treatment with repetitive transcranial magnetic stimulation applied to the left posterior temporal lobe area to patients with AVH [45].

The first steps towards this objective were done through the time analysis [32]. The data extracted from the same group of subjects studied in this project had been analyzed in the time domain: P50 suppression and N100-P300 complex were studied to investigate their changes between cohorts.

In the P50 protocol, the paired-click paradigm was performed [32] to test the ability of the brain to inhibit, or gate, its response to a repeated stimulus. This protocol is studied in AVH patients because the auditory problems are often related to the lack to filter extraneous noises, with consequent diminishing ability to focus attention [33].

P50 suppression was calculated as the ratio of the mean value of the amplitude of the second click (S2) to the mean value of the amplitude of the first click (S1).

P300 response was measured with an auditory oddball paradigm attention task, which is explained in the next chapters.

N100-P300 complex values were calculated as the difference between the most negative voltage value and the most positive voltage value within the time range of 80-500 ms.

Marcu et al. divided the scalp into 7 regions of interest (ROI), according to the choice made with the neurophysiologists. Each ROI was represented by 15 electrodes

(105 electrodes out of 256): Left Anterior (LA), Left Posterior (LP), Medial Anterior (MA), Medial Central (MC), Medial Posterior (MP), Right Anterior (RA) and Right Posterior (RP) (*Figure 2.1*).

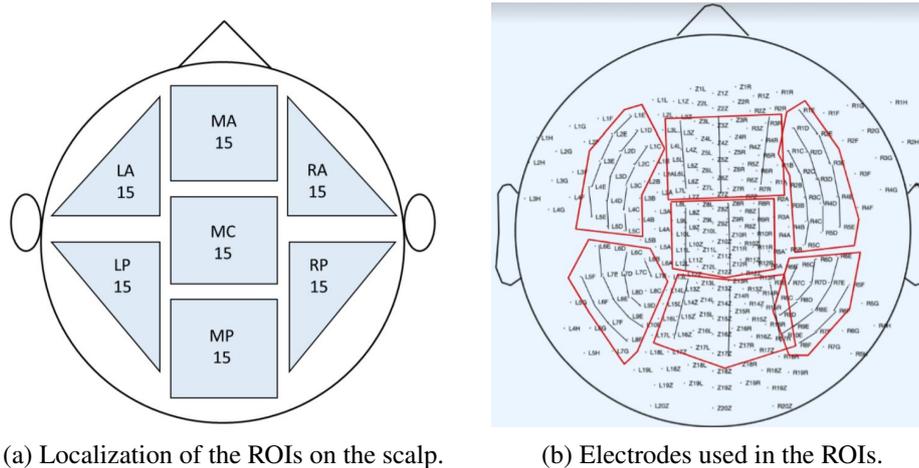


Figure 2.1. ROI creation.

P50 suppression ratio and N1-P3 wave's signals were represented as the average of the fifteen channels of every ROI.

The preliminary data showed a major P50 suppression in healthy subjects than in patients groups (*Figure 2.2*).

Patients showed higher ratios on the left anterior and posterior regions (as shown in *Figure 2.3*, which represents the P50 gating in three healthy participants and three patients with schizophrenia).

Healthy subjects had higher ratios on the left anterior and left temporoparietal cortex.

N1-P3 complex was reduced in patients before the rTMS treatment and higher after the treatment.

The main limitations of that study were the conflicting results obtained in some patients' data, which didn't follow the group's behavior. These contradictions had a high

impact on the last results because of the small number of samples available. This is the reason that brought us to move forward the time analysis and to start a new project studying the data in the frequency domain. Besides, only 105 of 256 electrodes available from the HD EEG cap were used in the last methodology, losing a large amount of valuable information that was included in this study.

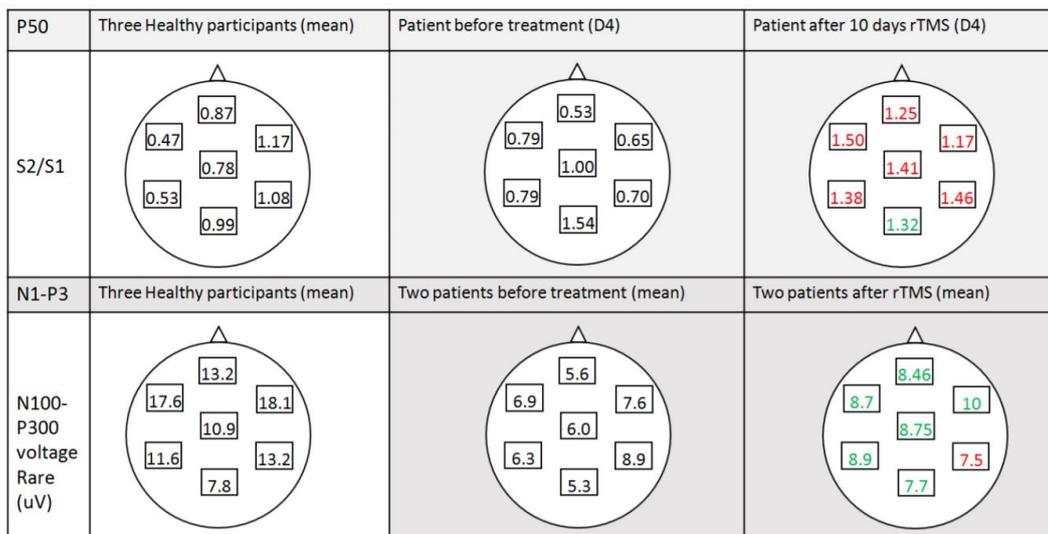


Figure 2.2. P50 suppression ratio and N100-P300 voltage measurements.

P50 suppression ratio in three healthy subjects and one patient with schizophrenia before and after rTMS treatment. N100-P300 voltage measurements in three healthy subjects and two patients with schizophrenia. Data was calculated (uV) by averaging 15 channels responses corresponding to 7 ROI: left anterior, left posterior, medial anterior, medial central, medial posterior, right anterior and right posterior.

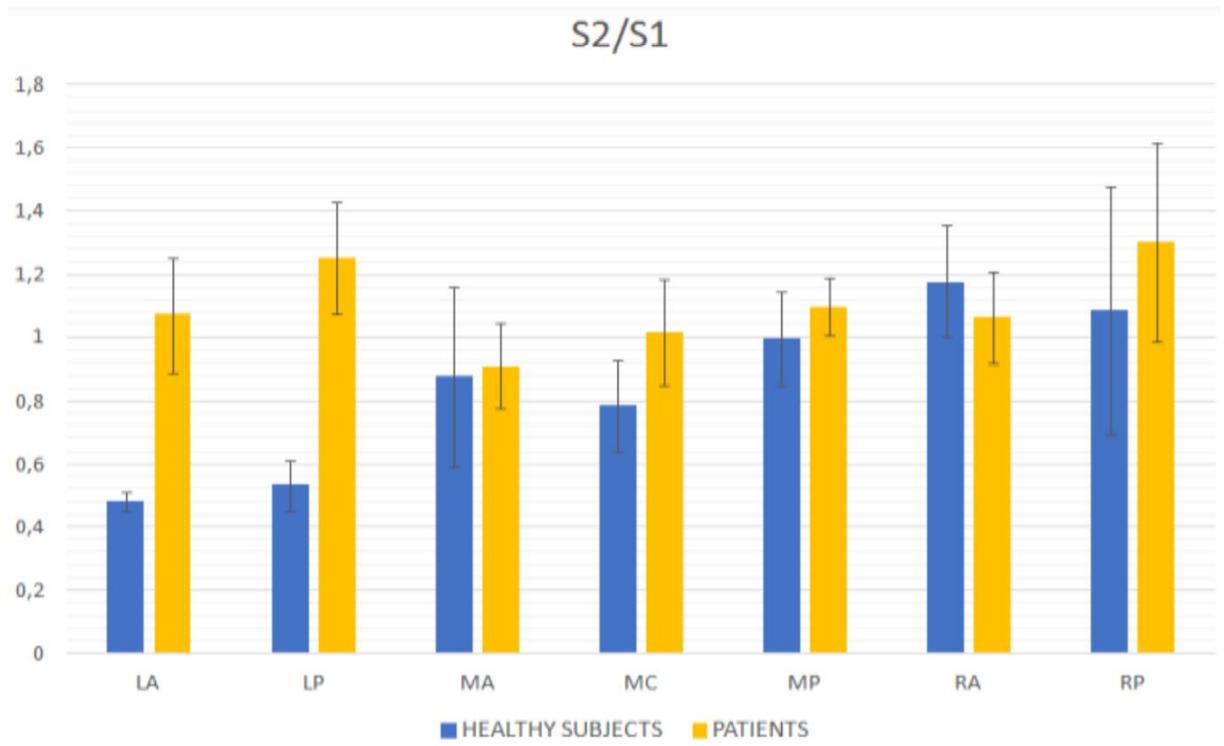


Figure 2.3. P50 suppression in three healthy participants and three patients with schizophrenia (baseline).

Chapter 3

Experimental Set up

3.1 Participants

The patients were recruited from the psychiatric wards and outpatient clinics of the National Hospital of Iceland. They were diagnosed with schizophrenia, following the ICD-10 (International Statistical Classification of Diseases and Related Health Problems 10th Revision) schizophrenia classification (F20) [46]. Permission from the Health Research Ethics Committee at the University Hospital of Iceland was obtained (approval no. 21.2018).

Inclusion criteria

1. Patients between 18-55 years of age
2. with treatment resistant AVH due to schizophrenia or schizoaffective disorder
3. for at least 1 year, and
4. experiences AVH at least once per hour.

Treatment resistance AVH refers to two pharmacotherapy attempts that used recommended dosage for at least 6-8 weeks.

Exclusion criteria

1. History of epilepsy,

2. daily Cannabis use,
3. use of other hard drugs within one month prior to the study or during the study,
4. drinks more than three units of alcohol daily,
5. uses benzodiazepine daily,
6. uses antiepileptic agents,
7. meet any of the exclusion criteria on the rTMS safety screening list,
8. left handedness (assessed with the *Edinburgh Handedness Inventory*)

Left-handed patients were excluded because different studies hypothesize a relation between the handedness and the neuronal response [34].

Patients that met the inclusion criteria were offered to take part of the study. The rTMS protocol was clearly explained to them both orally and written form. Written informed consent was obtained before proceeding (Appendix A). All patients were able to discontinue the treatment at any point during the study without any influence on their current treatment at the University Hospital of Iceland.

Ten patients randomly divided into two groups (TG and CG) and five healthy subjects were recruited:

TG - Treated Group Five patients ranged in age between 30 and 48 (four men and one woman, mean age 35.2, standard deviation = 5.12) were included in the real treatment group.

They received ten daily sessions of 15 minutes 1Hz frequency rTMS (900 pulses/session) at 100% of abductor pollicis brevis RMT (resting motor threshold) applied at T3-P3 location.

CG - Control Group Five patients ranged between 26 and 39 (three men and two women, mean age 29.6, standard deviation = 3.92) were included in the second group of control patients.

They received ten daily sessions of 15 minutes 1Hz frequency rTMS (900 pulses/session) at 100% of abductor pollicis brevis RMT (resting motor threshold) applied at the vertex, Cz EEG location.

HS - Healthy Subjects A third group of five healthy subjects aged between 24 and 43 (three men and two women, mean age 31.2, standard deviation = 6.64) served as a comparison cohort.

They did not receive rTMS treatment, only their EEG signal was acquired.

They were recruited by convenience sampling and went through Mini-International Neuropsychiatric Interview (MINI), a diagnostic interview developed for ICD-10 psychiatric disorders [32][35].

To compare the development of the pathology after the treatment, the data were acquired twice in each patient group: before the rTMS treatment (pre-treatment) and within one week after completing the ten sessions of rTMS treatment (post-treatment). This produced 25 datasets: five healthy subjects (HS), five pre-TMS TG, five post-TMS TG, five pre-TMS CG and five post-TMS CG. Three scales were used to collect clinical information pre- and post-treatment: Psychotic Symptom Rating Scales (PSYRATS), Quality of Life Scale (QoLS), Depression Anxiety Stress Scale (DASS). *Figure 3.1* represents the experimental set-up and workflow designed for this study.

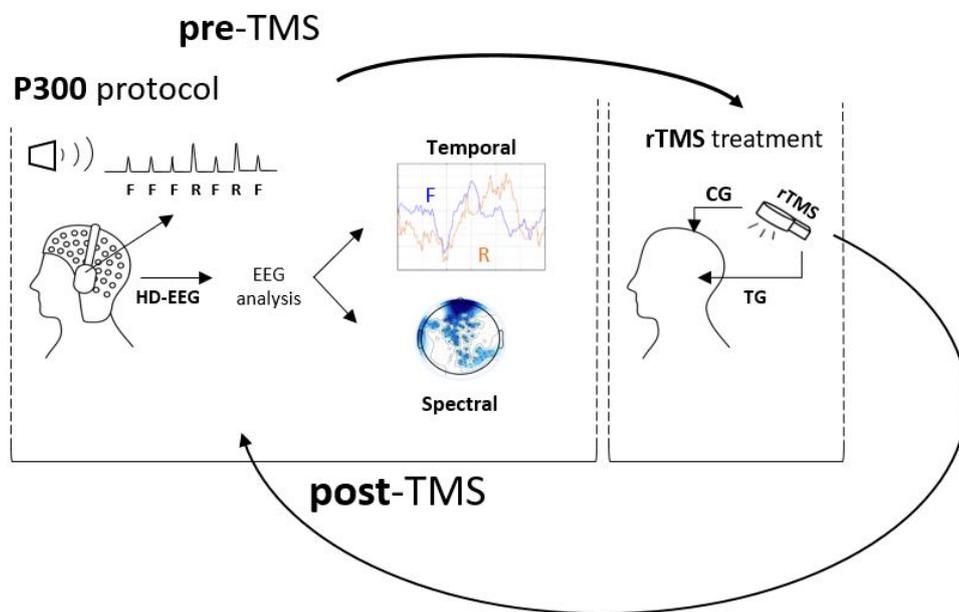


Figure 3.1. Data acquisition and processing workflow.

3.2 P300 recordings

P300 experiment was measured with an auditory *oddball paradigm* attention task, wherein two stimuli are presented in a random order, with one occurring more frequently than the other. The recordings took place between 11h00 and 14h00. The subjects were sitting with their eyes closed. The frequent (F) and the rare (R) auditory stimuli were presented binaurally through headphones at an interstimulus interval between tones of constant 1.1 sec. For each subject, there was one EEG trial with 200 tones presented. Rare stimulus occurred randomly with a probability of 0.2, so each trial had 160 frequent stimuli and 40 rare stimuli [32]. It was required the participants to focus on the rare stimuli without counting or moving a finger.

The P300 was identified as the most prominent peak around 300 ms after the stimulus, as shown in *Figure 3.2*.

P300 is an ERP that is elicited when people are paying attention to a specific stimulus. It is thought to be connected to updating information in working memory.

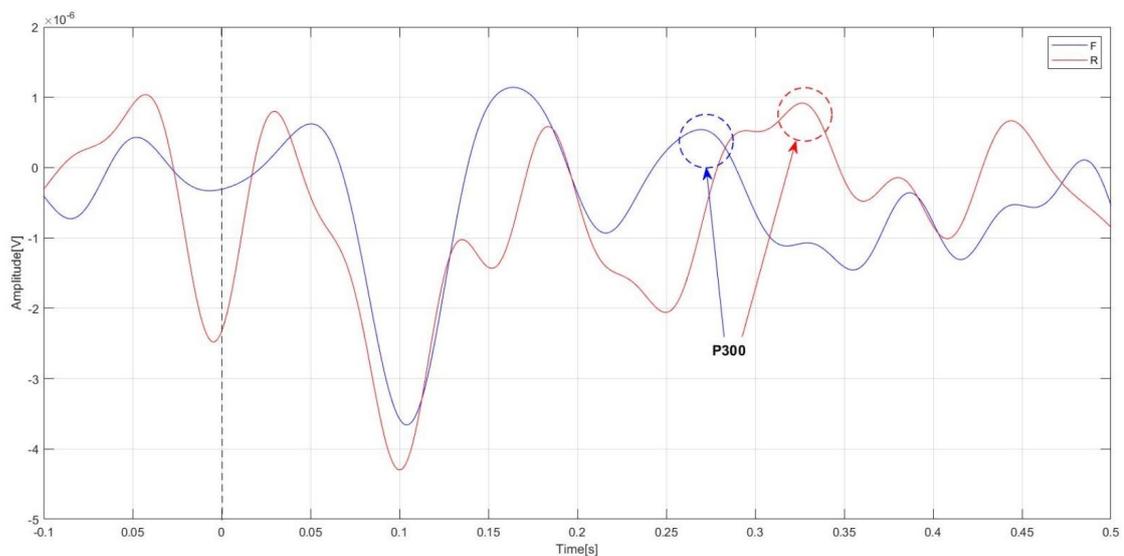


Figure 3.2. Example of P300-peaks for frequent (F) and rare (R) stimuli.

Chapter 4

Material and Methods

4.1 Acquisition

The EEG was recorded by neurophysiologists in the Icelandic Center for Neurophysiology laboratory located at Reykjavik University, in collaboration with Landspítali department of science. A 256 channel system (ANT Neuro, Netherlands) was used with an electrooculogram (EOG) electrode placed below the right eye and a ground electrode placed on the left side of the neck (*Figure 4.1*).

Ag/AgCl electrodes were used, and impedance was kept below 10 K Ω . They were positioned on the scalp according to the international 10/20 system.

4.2 EEG pre-processing

In these following sections, my contribution to the AVH TMS Project at Reykjavik University is explained.

Data pre-processing and analysis were performed with Brainstorm [36] and MATLAB 2019b (MathWorks, Inc., Natick, 158 Massachusetts, USA).

4.2.1 A brief introduction to pre-processing

Biomedical signals, and electroencephalogram in particular, are very difficult to process.



Figure 4.1. ANT Neuro System.

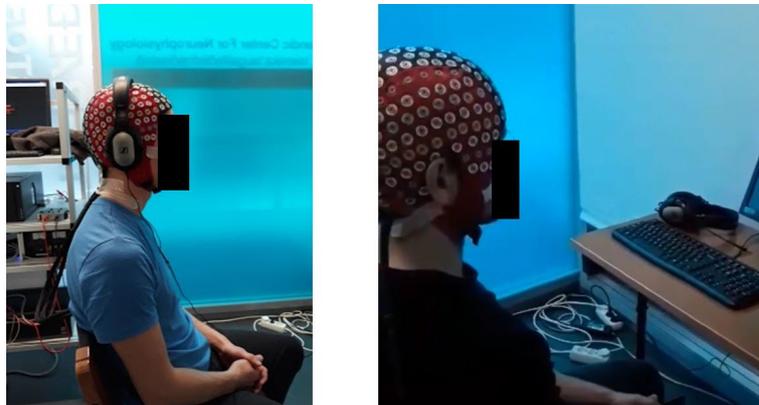


Figure 4.2. Experiment set-up.

First of all, the acquisition system interfaces with biological tissue and the result is instrumental noise added to the pure signal. Specifically to the EEG, electrodes of a cap are positioned on the scalp of the subject: hair and several layers of the skin interpose between them and the neurons, reducing the quality of the output. Moreover,

numerous signals from other parts of the body are usually co-present (e.g. eye blink). As we are generally interested in a single component, it is necessary to pre-process the signal of interest removing the additional noise superimposed. This operation can be done using *filters*.

On the other hand, biomedical signals are not stationary. To reduce the computational complexity, the processes are divided into short *epochs*, in which the signal can be assumed to be stationary.

Noise

Noise is an important class of stochastic signals [37]. It is always present during the acquisition of physiological processes, so they can be considered as realizations of stochastic (or random) mechanisms.

A simple model that can be used to describe the final output $x(t)$ of the measurement of a biological signal is given by the sum of the original signal $s(t)$ and a noise component $n(t)$:

$$x(t) = s(t) + n(t) \quad (4.1)$$

The parameter that measures the quality of the signal $x(t)$ is called *signal to noise ratio* (SNR) and it is defined

$$SNR = 10 \log_{10} \frac{P_s}{P_n} (dB) \quad (4.2)$$

where P_s is the power of the signal and P_n is the power of the noise.

This ratio can be increased by processing the signal, reducing the content of noise. The standard method that is used to improve the quality of the data is based on *filtering*. This is a process used when the noise is separated from the pure signal in the frequency domain [37].

4.2.2 Protocol

The pre-process protocol was performed with Brainstorm [36]. Data were sampled at 1024 Hz and re-referenced to the average of left and right mastoid electrodes (R19R, L19L). The steps included in the protocol are:

- Filtering

- Outliers and bad time sequences removing
- Interpolation of bad channels
- Import into epochs

This protocol is based on the one proposed by Pegolo in her thesis' project [38]. It is described in detail in Appendix B.1.

Filtering

Two filters were applied:

- **Band-pass filter** between 0.5-70 Hz (*Figure 4.3*) to keep the frequency bands of interest.
- **Notch** of 50 Hz (*Figure 4.4*) to remove the line interference.

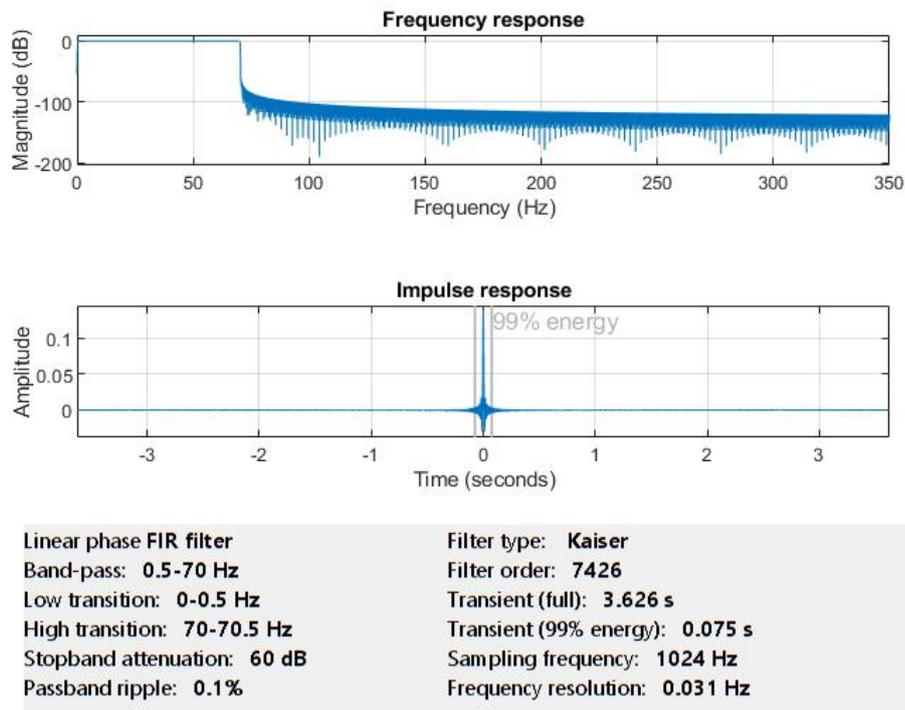


Figure 4.3. Band-pass filter.

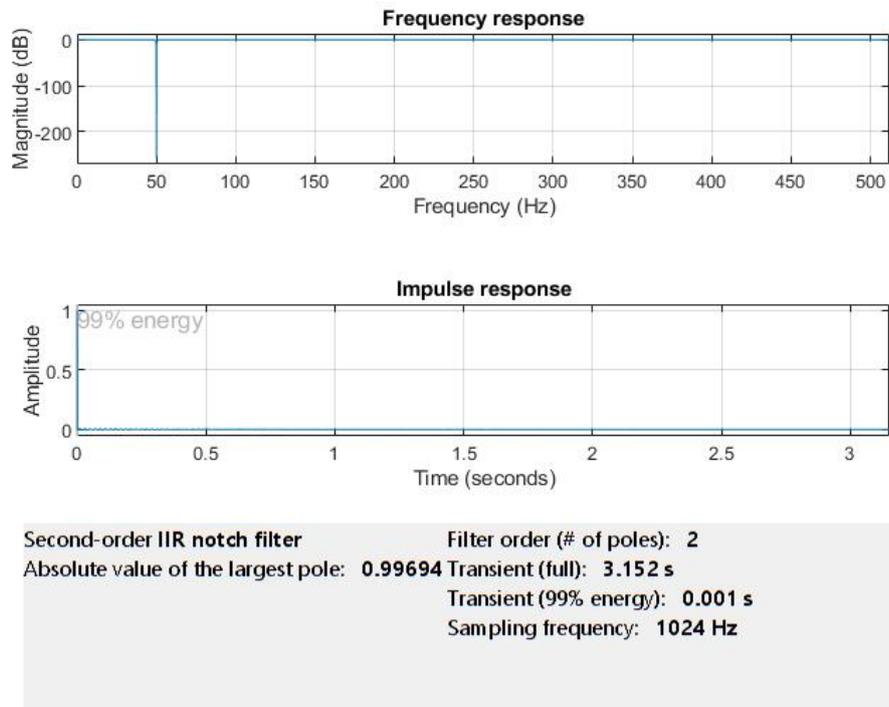


Figure 4.4. Notch filter.

Outliers and bad time sequences removing

After filtering, a further data cleaning was manually performed. It consisted in *removing outliers*, i.e. bad channels with anomalous behavior (represented in red in Figure 4.5).

Thus, time sequences of bad signals were highlighted (Figure 4.6) and subsequently interpolated (operation explained in the next paragraph).

A signal was considered *bad* if its amplitude exceeded $\pm 80 \mu\text{V}$. This choice was made to remove eye movements or other artifacts.

If more than 10% of the channels showed too much noise or incorrect signal, the whole trial was rejected.

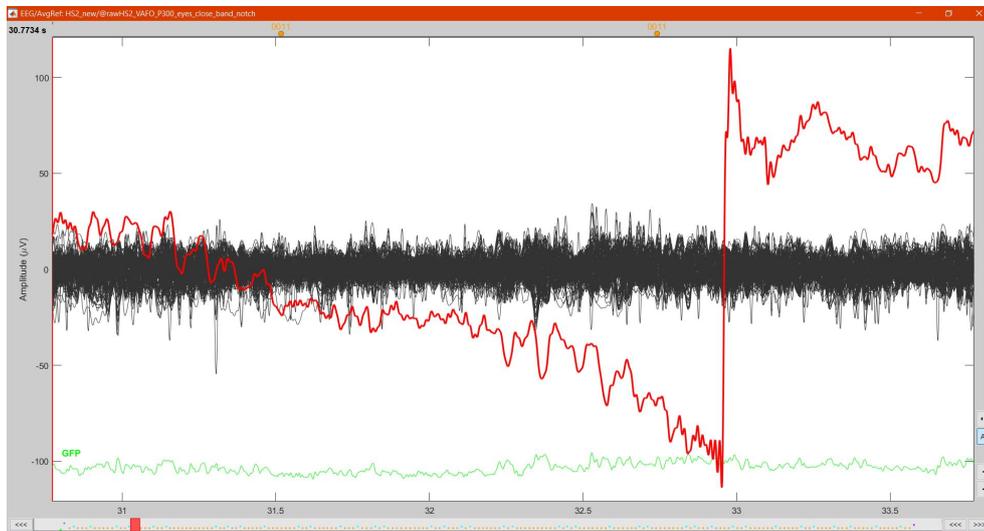


Figure 4.5. Outliers.

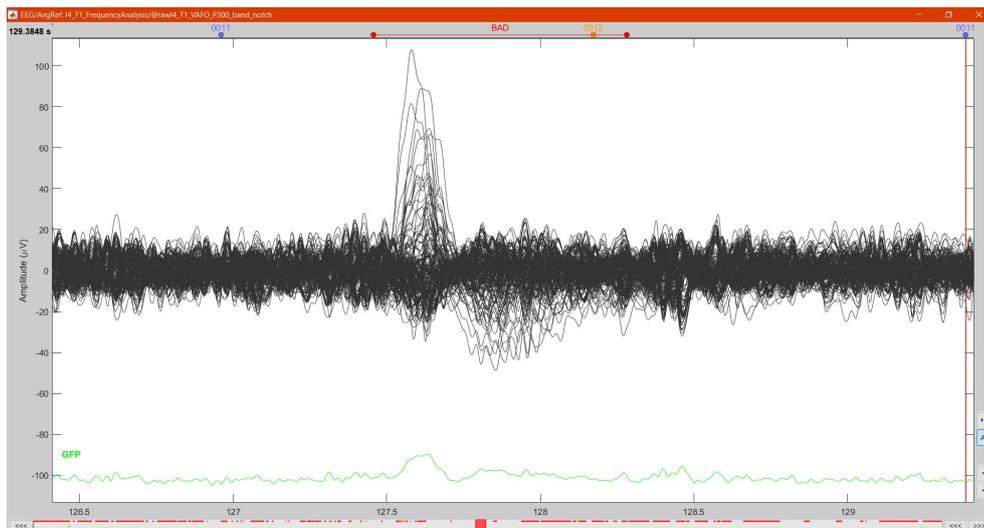


Figure 4.6. Bad time sequence.

Interpolation of bad channels

The interpolation function repairs bad or missing channels in the data using the information given by the good channels. Data can be replaced with 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 [39].

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).

Import into epochs

Signal samples were analyzed in specific epochs (*table 4.1*).

The time window of each epoch was chosen to start hundreds of milliseconds before the acoustic stimulus presentation (at 0 s). This choice was made to include the *baseline* time range. The mean value of every channel in this time range was subtracted from the channel at every time instant to remove the baseline.

P300 protocol		
	F	R
Duration	1200 ms	1200 ms
Time range	-500 ms to 700 ms	-500 ms to 700 ms
Baseline	-500 ms to -100 ms	-500 ms to -100 ms

Table 4.1: Epochs creation

At this point, the epochs were re-referenced, an operation that consists in a linear combination of the data. As mentioned above, in this project the channels of reference were the “L19L” and the “R19R” which represent the mastoids. The montage used as the new reference was the bimastroid of Brainstorm.

4.3 Data Analysis

4.3.1 A brief introduction to PSD and Statistical Analysis

Power Spectral Density

In 600 B.C., Pitagora launched what nowadays is the most important instrument to study complicated and non-deterministic signals. His laws of musical harmony created the basis to the wave equation, which was finally solved in 1822 by Fourier.

Fourier obtained a method to decompose any signal into a linear combination of sinusoidal functions

$$u(x) = \sum_{k=1}^{\infty} (A_k \cos k\alpha x + B_k \sin k\alpha x) \quad (4.3)$$

where the coefficients A_k and B_k :

$$\begin{aligned} A_k &= \frac{2}{\pi} \int_0^{\pi} u(x, 0) \sin(kx) dx \\ B_k &= \frac{2}{\pi} \int_0^{\pi} u(x, 0) \cos(kx) dx \end{aligned} \quad (4.4)$$

Equation 4.3 works as long as the signal has finite energy.

The Power Spectrum Density (PSD) of a signal in the time domain is the distribution of its power into its frequency components. The PSD can be computed through different techniques, and in this project Welch's method was used. It is based on the periodogram spectrum estimate, which is a non-parametric method based on the Fourier transform of the time series of interest.

The PSD can be calculated under the assumption of wide sense stationary (WSS) and ergodic signal.

A process is WSS when its statistical mean is a constant in time and when its autocorrelation function depends only on the delay but not on the specific time instant. A WSS stochastic process is ergodic with respect to a function if its statistical mean is equal to the time mean estimated from a single realization.

If the serie $x[n]$ is WSS and ergodic, the power spectral density $P_{x,x}(f)$ estimated through the periodogram can be written as:

$$P_{x,x}(f) = \lim_{M \rightarrow \infty} E \left\{ \frac{1}{(2M+1)T} \left| T \sum_{n=-M}^M x[n] e^{-j2\pi f n T} \right|^2 \right\} \quad (4.5)$$

which is the discrete Fourier transform (DFT) of the time series.

The method of Welch is based on dividing the time series in a specific number of sub-series that have an overlap of more or less 50%, and averaging the periodograms of each of them.

Statistical Analysis

A qualitative visual inspection could not be enough to fully understand the results of an experiment and the statistical analysis could overcome this limitation.

The t-test gives an idea of how significant the differences between two groups are. The t value is calculated by the means of group one \bar{x}_1 and group two \bar{x}_2 , the variance s_1^2, s_2^2 and the number of samples of each group n_1 and n_2 :

$$T = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \quad (4.6)$$

Every t-value has a correspondent p-value (a value between 0-100%, usually written in decimals, i.e. 0-0.1), which is the probability that the result from the sample data occurred by chance. Low p-values mean that data don't occur by chance.

Paired t-test is a specific type of t-test used to study two measurement on the same item. In this case p-values can be obtained through the t-table, using the degrees of freedom (this value can be obtained by subtracting 1 to the sample size) and the alpha level (the probability of making the wrong decision when the null hypothesis is true).

4.3.2 Protocol

The data analysis was computed partially with Brainstorm [36] and MATLAB 2019b (the complete protocol is explained in Appendix B.2).

4.3.2.1 Brainstorm: Power spectral density computing

Epochs of each channel were averaged, obtaining 254 averaged data.

The power spectral density (PSD) was computed with Welch's method. The power in the following frequency bands: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), low beta (13–18 Hz), high beta (18–30 Hz), low gamma (30–70 Hz) was then divided for each frequency band by the associated bandwidth to get the final PSD.

4.3.2.2 Matlab: Comparison within cohorts

Comparison between frequent and rare stimuli - pre and after the treatment for the patients group - of the same cohort were done.

Matrices creation As explained in section 3.1, three cohorts were created: HS (healthy subjects), TG (treated group), CG (control group).

The PSD of each subject was computed in Brainstorm, exported in Matlab as matrix 254×6 (Figure 4.7), being 254 the EEG channels (2 channels were used as reference) and 6 the number of final PSD values in the six frequency bands (from delta to low gamma); each matrix was saved in the folder of the cohort to whom the subject belonged.

Each subject had four matrices 254×6 :

		frequency bands					
		delta	theta	alfa	low beta	high beta	low gamma
channels	1	*	*	*	*	*	*
	2	*	*	*	*	*	*
	...	*	*	*	*	*	*
	254	*	*	*	*	*	*

* = Averaged PSD

Figure 4.7. PSD matrix.

- PSD_F_T1 : PSD of the averaged epoch of the frequent stimulus (F), before the treatment (T1);
- PSD_R_T1 : PSD of the averaged epoch of the rare stimulus (R), before the treatment (T1);
- PSD_F_T2 : PSD of the averaged epoch of the frequent stimulus (F), after the treatment (T2);
- PSD_R_T2 : PSD of the averaged epoch of the rare stimulus (R), after the treatment (T2).

for the patients, and two matrices 254×6 :

- PSD_F : PSD of the averaged epoch of the frequent stimulus (F);

- PSD_R : PSD of the averaged epoch of the rare stimulus (R).

for healthy subjects.

After the data exporting, a new structure called *matr_ave* was created in MATLAB 2019b. It contained all the matrices of the subjects of the same cohort.

Statistical Analysis Paired t-test was performed between subjects within the same cohort to obtain a single matrix 254×6 for each group ($F_{T1}, R_{T1}, F_{T2}, R_{T2}$). Channels whose p-value was less than 0.05 were considered *significant*.

Bonferroni correction and false discovery rate were also computed, but the results are not presented in this project because no valuable information was obtained.

PSD values of only significant channels were compared between groups, obtaining four topographical maps of the 6 frequency bands. However, only three frequency bands were considered informative for our study: alpha (8-13 Hz), low beta (13-18 Hz), high beta (18-30 Hz). Significant values were enhanced within those plots (*Figure 4.8*).

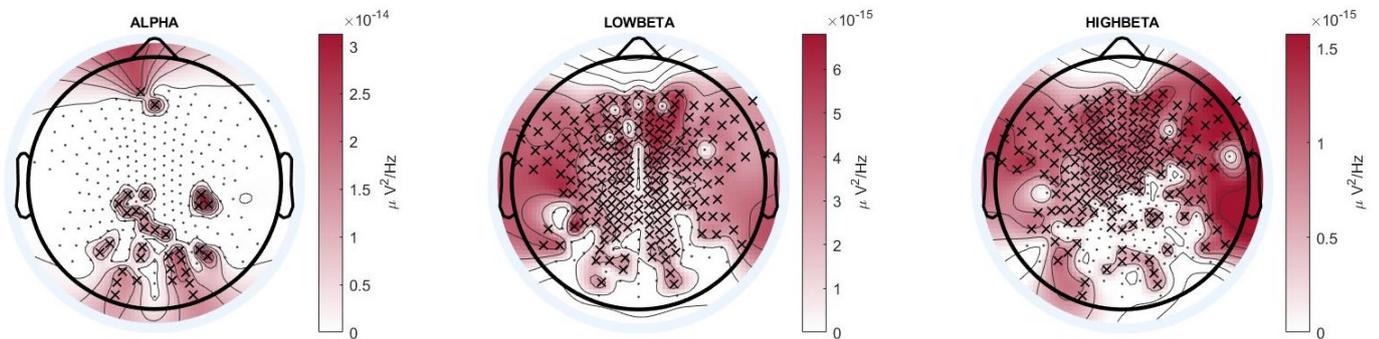


Figure 4.8. Example of plots of the same cohort's PSD values of significant channels (marked with 'x').

4.3.2.3 Matlab: Comparison between cohorts

The different cohorts were compared to understand the behavior pre and post-treatment, the behavior between healthy subjects and the patients with schizophrenia and the different responses to frequent (F) and rare (R) stimuli.

Groups creation The difference between groups were computed for the comparison. Ten comparisons were performed:

- Healthy - Treated groups before the treatment (for the frequent and the rare stimuli);
- Healthy - Treated groups after the treatment (for the frequent and the rare stimuli);
- Healthy - Control groups before the treatment (for the frequent and the rare stimuli);
- Healthy - Control groups after the treatment (for the frequent and the rare stimuli);
- Control group after the treatment - Treated group after the treatment (for the frequent and the rare stimuli);
- Healthy frequent - rare cases;
- Treated group frequent - rare (for before and after the treatment);
- Control group frequent - rare (for before and after the treatment);
- Treated group before - after the treatment (for the frequent and the rare stimuli);
- Control group before - after the treatment (for the frequent and the rare stimuli);

Statistical Analysis Statistical analysis (paired t-test) was computed between subjects of the same cohorts. Only channels that were significant both in the two groups of the comparison were studied: they were intersected and the averaged PSD difference calculated (*Figure 4.9*).

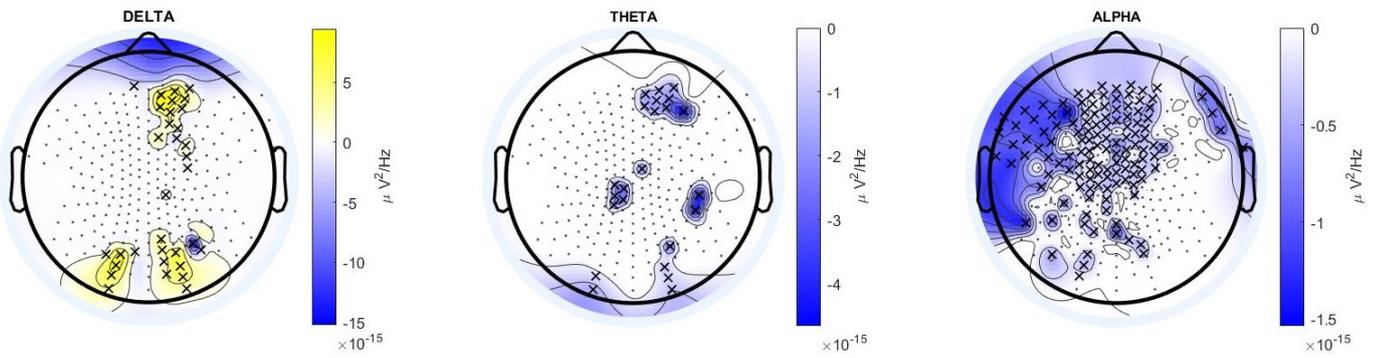


Figure 4.9. Example of plots of PSD subtraction between significant channels (marked with 'x') of different cohorts.

Chapter 5

Results

5.1 Frequent - rare

Figure 5.1 shows the values of the subtraction between the PSD of the frequent and the rare stimuli of the same cohort: **A.** healthy subjects, **B.** treated group before the treatment, **C.** control group before the treatment. Only the channels that were significant in both subjects groups were plotted. In the image 'X' represents the place of a channel which is significant. The marked areas evidence the most significant regions of the scalp (F: frontal; T: temporal; P: parietal; C: central; O: occipital).

For the healthy subjects, a higher PSD for the rare stimulus could be observed for every band, especially in the alpha band, which had higher values than the other bands. The beta band presented a higher number of significant channels, most of them in the frontal and the central parts of the scalp. Specifically, for low beta, significant channels were located in the right temporal and parietal parts, whereas for high beta, they were located in the right temporal and parietal parts. The alpha band had few significant channels in the frontal and occipital parts of the scalp, but the PSD of the rest of the electrodes was not significant.

For TG patients, as for the healthy subjects, the PSD was higher for the rare stimulus, mainly in the alpha band. The alpha band also presented more significant channels all over the scalp. At the same time, low beta had two significant channels in the right parietal part and high beta presented few channels spread in the right frontal-parietal and left temporal-parietal parts of the scalp.

For CG patients, the rare stimulus showed a higher PSD for both alpha and low beta

bands. The difference between PSD of rare and frequent stimuli in high beta band was lower, but it had a higher number of significant channels. Low beta presented a higher PSD in the left parietal part. Other significant channels were in the occipital, right temporal and parietal parts. The alpha band presented a high number of significant channels in the right frontal and left frontal-temporal and parietal parts of the scalp.

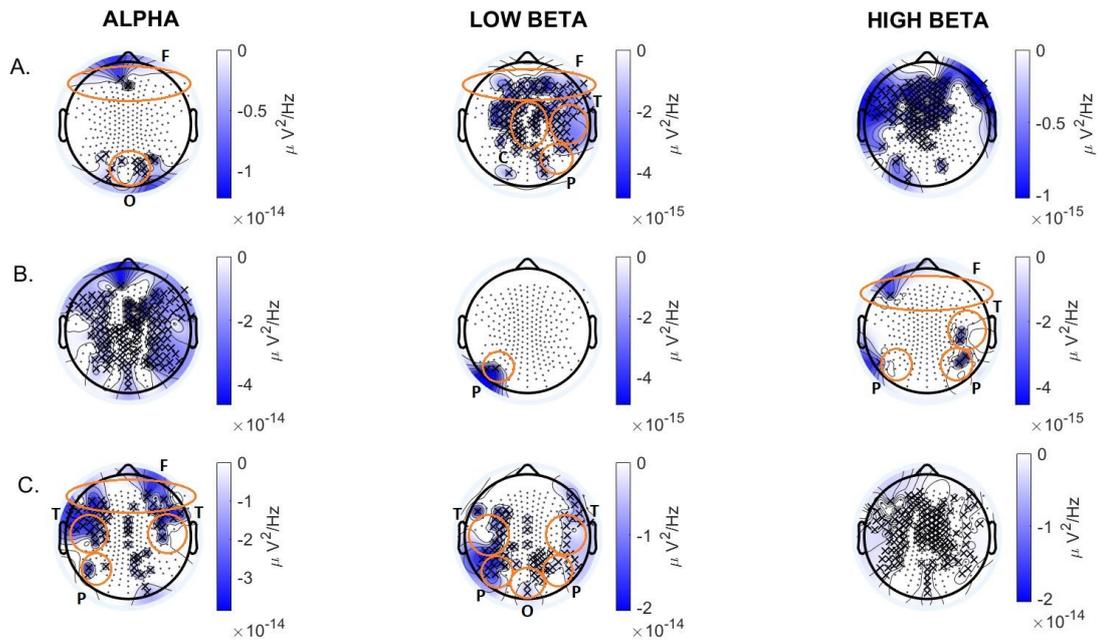
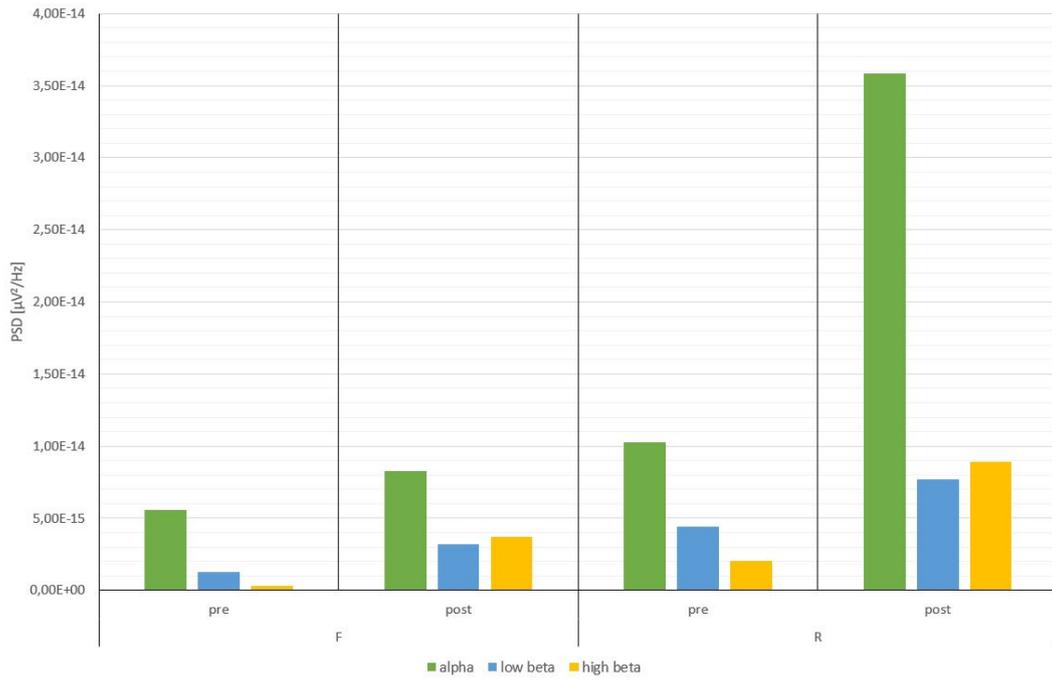


Figure 5.1. PSD Subtraction of significant channels: Frequent - Rare stimuli.

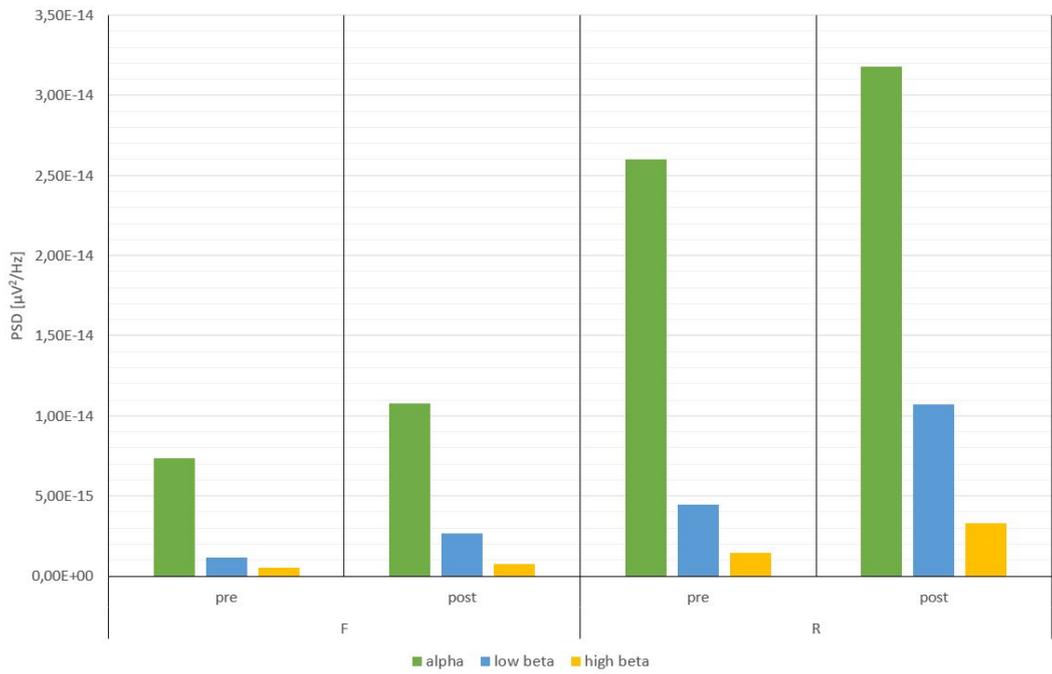
(A) healthy subjects cohort; (B) TG cohort; (C) CG patients cohort. The marked areas evidence the most significant regions of the scalp (F: frontal; T: temporal; P: parietal; C: central; O: occipital).

Both F and R stimuli had a major amplitude after the treatment, in TG and CG. *Figure 5.2* shows the PSD values of one patient of each patient group (I4 = treated patient *Figure 5.2a*, D2 = control patient *Figure 5.2b*). Only significant channels (obtained from the statistical analysis between subjects of the cohort) were averaged to plot the image.

The rare stimulus presented a higher raise after the treatment than the frequent stimulus in both subjects, especially in the alpha band. Taking into account this behavior, it was decided to focus on the rare stimulus for the following comparisons between groups.



(a) Patient of TG.



(b) Patient of CG.

Figure 5.2. PSD values of a single patient.

5.2 Post-TMS - pre-TMS

To analyse quantitatively the results of *Figure 5.1*, the averaged PSD difference between post and pre treatment of TG and CG patients were computed and presented in *table C.1*, in Appendix C.

The PSD differences post - pre including only significant channels in alpha, low beta and high beta bands, for **A.** treated group and **B.** control group are presented in *Figure 5.3*. Only the results of the rare stimulus are shown.

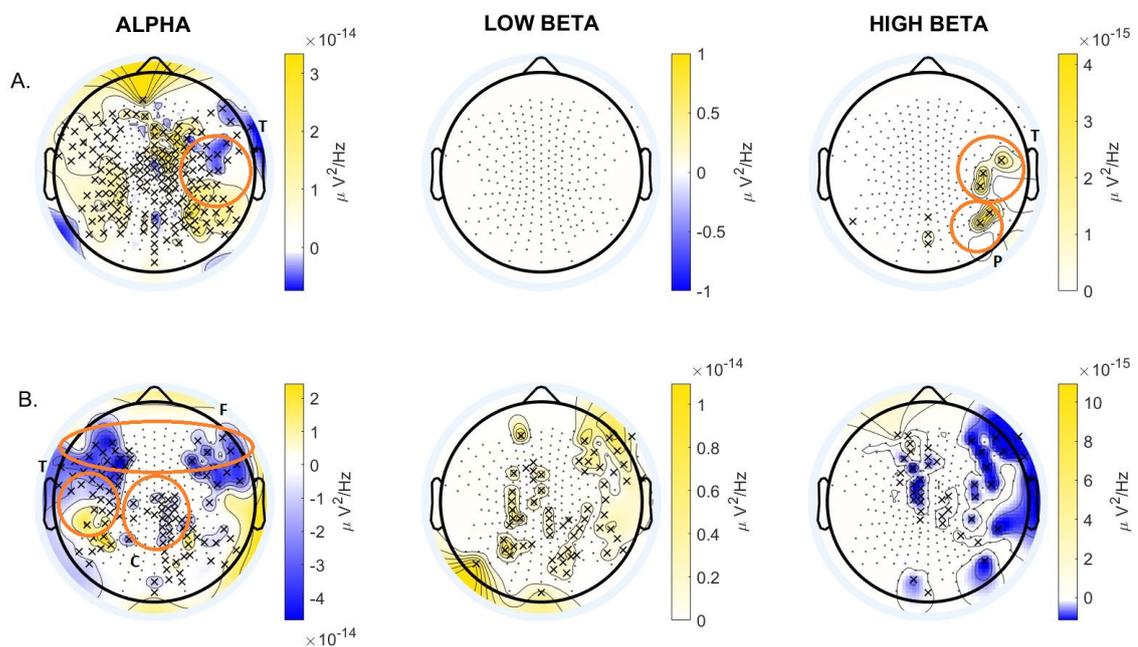


Figure 5.3. PSD Subtraction of significant channels: post - pre TMS.

(A) TG cohort; (B) CG patients cohort, of the rare stimulus. The marked areas evidence the most significant regions of the scalp (F: frontal; T: temporal; P: parietal; C: central; O: occipital).

For TG patients, slightly higher PSD post-TMS was seen for the rare stimuli in most areas of the scalp for the alpha band, where a lot of significant channels could be observed. In the low beta band, there was no information and in high beta, there were too few significant channels in the right temporal-parietal part to discern any behaviour.

Regarding the rare stimulus of CG patients, in the alpha band, the PSD decreased in the post-TMS condition for the frontal and right parietal parts and remained quite stable for the rest of the scalp, in particular in the central part. For the low beta, the PSD increased after TMS; for high beta, PSD decreased. For both low beta and high beta, significant channels were observed mostly on the right area of the scalp.

5.3 Healthy - patients comparison

The results included in the *table C.1* showed that the TG response was higher pre-TMS and that the difference between healthy and patients was even higher post-TMS, for each band.

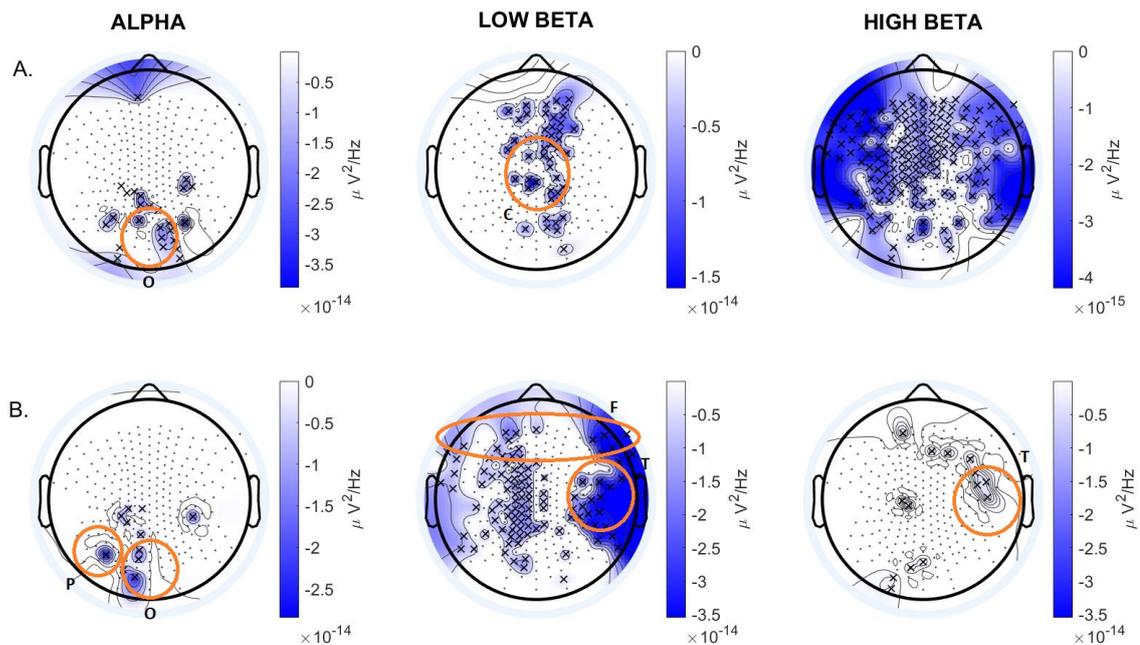


Figure 5.4. PSD Subtraction of significant channels: HS cohort - TG cohort.

(A) pre TMS; (B) post TMS, of the rare stimulus. The marked areas evidence the most significant regions of the scalp (F: frontal; T: temporal; P: parietal; C: central; O: occipital).

It confirms what is seen in *Figure 5.4*, where the averaged PSD difference between healthy subjects cohort and treated group of the rare stimulus was plotted. The alpha band pre-TMS (*Figure 5.4.A*) had significance mostly in the occipital region, and for the low beta, there were significant channels in the whole central area, whereas for high beta, there were significant channels in almost all parts of the scalp.

The post-TMS (*Figure 5.4.B*) presented few significant channels in the occipital and right-parietal lobe for the alpha band. High beta had several significant channels but a more negative PSD in the left frontal and temporal part of the scalp. The low beta band of post-TMS was not relevant; there were few significant channels, mostly located in the right temporal part.

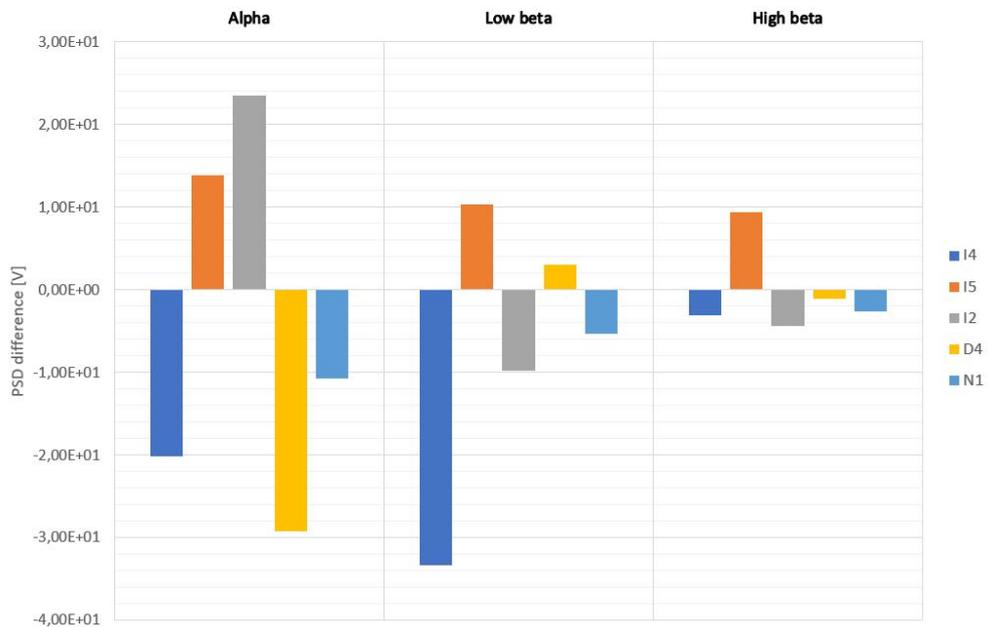
The same behavior was observed for pre-TMS CG, where the PSD was higher than the HS group, but the difference was lower post-TMS for the alpha and high beta bands.

5.4 Variability between subjects of the same cohort

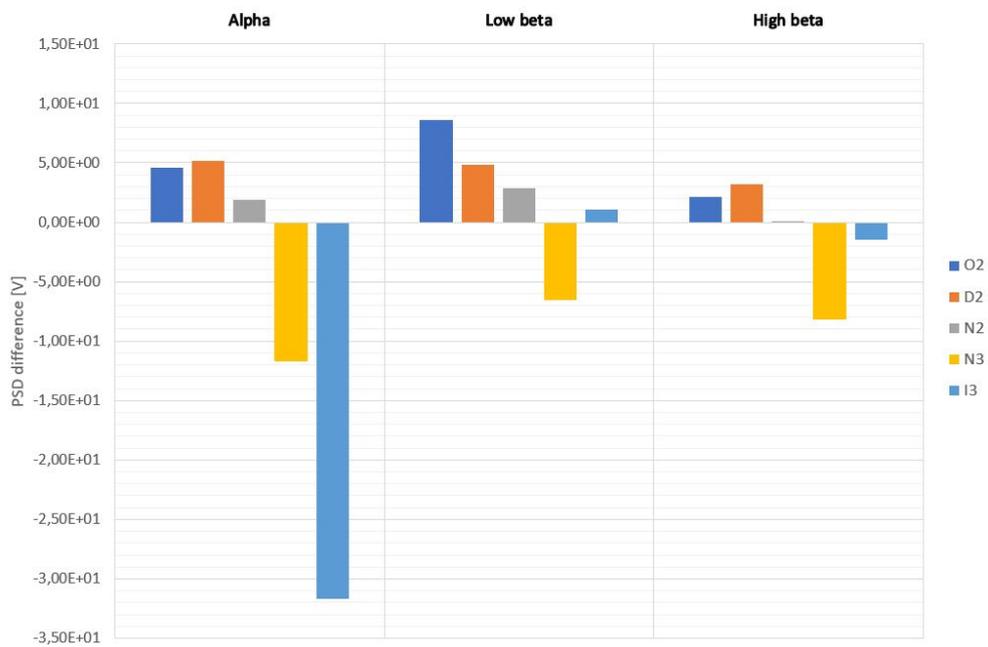
Figure 5.5 represents the difference between the PSD post and pre treatment of each subject of TG (*Figure 5.5a*) and CG (*Figure 5.5b*). PSD values of significant channels of pre and post groups were averaged separately and then subtracted, for each frequency band.

The subjects of the treated group present a high variability: in the alpha band, the PSD difference of subjects I5 and I2 is positive, while the results of the other subjects are negative; in the low beta band subject I5 and D4 have a different behavior; in the high beta band only I5 have a positive PSD difference.

For the subjects of the control group: in the alpha band, subjects N3 and I3 have a negative PSD difference, while the results of the other subjects are positive; in the low beta band, only N3 presents an opposite behavior from the other subjects; high beta band, as for the alpha band, has I3 and N3 with a positive PSD difference, while the other subjects have a positive value.



(a) TG patients.



(b) CG patient.

Figure 5.5. PSD difference post - pre treatment (rare stimulus) of subjects of the same cohort.

5.5 Psychometric

In TG, patients D4, I2, I4 and I5 presented a positive response to the treatment (*Table 5.1*). In particular, I2 and I4 showed an improvement in their psychometric score post-treatment: increased quality of life post-treatment, decreased DASS after TMS and decreased PSYRATS post-treatment. N1 presented a decrease in its psychometric score post treatment: the quality of life remained the same, but the DASS increased and the PSYRATS slightly increased.

In CG, patients D2 and N2 presented a positive response to the treatment. In particular, N2 showed an improvement post-treatment. O2 presented a decrease in its psychometric score post-treatment. I3 presented a stagnation in its psychometric score post-treatment. N3 presented inconsistent scores with worst quality of life, decreased DASS and PSYRATS remained the same after the treatment.

TG	QoL	DASS	PSYRATS	CG	QoL	DASS	PSYRATS
D4	↑	-	↓	D2	-	↓	-
I2	↑	↓	↓	I3	-	-	-
I4	↑	↓	↓	N2	↑	↓	↓
I5	-	-	↓	N3	↓	↓	-
N1	-	↑	↑	O2	↓	↑	-

Table 5.1: Increase (↑), decrease (↓) or constancy (-) of the psychometric of each subject of TG and CG after treatment

5.6 Discussion

The present work aimed to design a methodological tool for data measurement and analysis. Its second goal was to define if the chosen ERP component (P300) and the related power spectral density can be used as an effective biomarker of the neural response in patients with schizophrenia and AVH submitted to 10 days of left temporoparietal 1Hz rTMS treatment.

According to the literature, the alpha and beta bands were identified as the most relevant to investigate in this case, fitting with the fact that those bands rely on attention,

focus and cognitive process [40].

The results seen on *Figure 5.1* showed a higher PSD for the rare stimulus than for the frequent one and confirmed what is described in the literature, i.e. a higher focus on uncommon events. For the TG (*Figure 5.3.A*), the PSD was mostly higher in the alpha band, known for characterizing focus and attention [40] and higher in the few significant channels of high beta. If no definite assumption can be made regarding the impact of TMS, the results both on the figure and the values (*table C.1*) suggest a higher neural response post-treatment, related to focus and attention task. For the CG (*Figure 5.3.B*), the PSD post-TMS was higher only in low beta and lower in alpha and high beta frequency bands. This is confirmed by the difference values (*Table C.1*).

The difference between HS and TG pre-TMS (*Figure 5.4.A*) and post-TMS (*Figure 5.4.B*) (*table C.1*) showed a slightly higher neural response after the treatment for each band. In both cases, the significant channels were located in the occipital region for the alpha, probably in relation to the eyes-closed alpha occipital synchronization, and in the central region for low beta.

The previous analysis presented averaged data. This choice was made to represent the general behavior of the group, but to understand if this data is representative, it is necessary to see if the subjects of the same cohort act in a way that can be considered analogous.

Looking at *Figure 5.5a*, it is important to notice that subject I5 presents in every frequency band an opposite behavior from the other subjects, as for subject N3 in *Figure 5.5b*. Because of the small number of subjects, these values give a heavy contribution to the average, changing the effective result of the group.

In *table 5.1* there are some indications of improvement in the psychometric scores, in four out of five subjects in TG and three out of five in CG. This means that patients felt a better quality of life and a decrease of symptoms of schizophrenia. This data is not justified by the spectral analysis, and it could depend on the fact that psychometric are self-report measures, not always reliable and consistent.

Chapter 6

Conclusion

The spectral analysis suggests a different brain electrical activity after the rTMS treatment in frequencies between 8 Hz and 30 Hz: alpha (8-13 Hz), low beta (13-18 Hz) and high beta (18-30 Hz) bands. These bands are known to reflect focus and attention. These response results are different between TG and CG patients. An impact can be noticed for TG patients, with a higher neuronal response for rare stimulus post-treatment, possibly indicating a positive change in focus abilities. For CG patients, no remarkable changes are visible to enable interpretation of the TMS effect. This difference of response is consistent with Hoffmann's observation [41] of more effect with T3-P3 rTMS, compared with vertex stimulation. Considering this, PSD of the P300 response in the alpha and beta bands can potentially be a decent biomarker of the TMS neural response in patients with schizophrenia and AVH.

However, this study has some limitations. The patients were under their usual antipsychotic, or sometimes benzodiazepine, treatment. This did not change between pre- and post-rTMS conditions but might have influenced the background neural activity and the generation of the ERPs [43]. The experimental procedure was long (1 hour) and tiring and some patients had difficulty cooperating and maintaining task engagement. Muscle and movement artifacts added noise to the EEG signal, requiring an averaging operation between the samples. We encourage similar experiments with the patients lying down to reduce the noise and improve the data quality, making them easier to process and analyze.

Another constraint was the small number of subjects and acquisitions in each cohort. Augmenting the number of individuals for each cohort would help quantify and assess

more certainly the effectiveness of the rTMS treatment. The developed methodology allows for processing of many more subjects and can be used in the future experiments.

Chapter 7

Future Goals

The team of Icelandic Center for Neurophysiology at Reykjavik University is working on new analyses of these data, trying to solve the limitations explained in the previous chapter.

Taking into account the small number of subjects involved in this experiment, we are trying to combine what we already obtained with clinical outcomes such as symptom severity scales and psychometric tests. Considering the fact that psychometric are a semi-self assessment evaluation of patients condition, they would help to establish reliable biomarker of the neural response to TMS in patients with schizophrenia and AVH.

The new study is considering also the gamma band (30 -100 Hz), because gamma oscillations also participate in cognitive processes such as attention and working memory [44].

As said before, averaged results for a sample size of 5 might not be suitable. Individual analysis are being performed, creating a portfolio for each patient.

Brain connectivity might also be studied to integrate the spectral analysis, obtaining a specific outcome of the interaction between neurons during the stimulation.

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Appendices

Appendix A

Informed consent

A.1 Invitation and introduction form



Repetitive Transcranial Magnetic Stimulation as a Treatment for Persistent Auditory Verbal Hallucinations

Clinical applied research in Iceland

INVITATION & INTRODUCTION FORM

Dear recipient

The Clinical Neurophysiology Unit, National University Hospital of Iceland and the Icelandic Center for Neurophysiology at Reykjavik University are looking for participants for a research project on repetitive transcranial magnetic stimulation as a treatment for persistent auditory verbal hallucinations.

Participants

Participants are healthy individuals and will be found by sending an e-mail to students at Reykjavik University or by direct contact.

The goal of the study

To study the effect of repetitive transcranial magnetic stimulation (TMS) as a treatment for persistent auditory verbal hallucinations. The goal is to reduce auditory verbal hallucinations and by doing so, increase emotional well being and quality of life of the patients.

We invite you to participate in order to create a database of healthy controls based on brain activity parameters.

The study and research is totally noninvasive, there is no treatment and what the researchers do is recording electroencephalograms while subjects are listening to sounds or assess the normal inhibitory mechanism of the brain using a TMS pulse (see below).

What is involved in participation?

You will be required to come to the National University Hospital of Iceland at Fossvogur and undergo a 15 minute neuropsychiatric interview. Participants will also be asked to answer a background information questionnaires. Next, neurophysiological measures of brain activity will be taken using TMS pulse. Participants will then arrive at Reykjavik University where an electro cap will be placed upon the head and 256 electrodes will be attached to your scalp with a special gel which will take about 30 minutes. The study will then proceed and you will be required to finish three different tasks which will take about 30 minutes. During the tasks you will be required to listen and discriminate between different kinds of click sounds.



Repetitive Transcranial Magnetic Stimulation as a Treatment for Persistent Auditory Verbal Hallucinations

Clinical applied research in Iceland

INVITATION & INTRODUCTION FORM

Participants confidentiality

Information that will be gathered in this study will be kept at the University Hospital of Iceland in a database that only the researchers will have access to. All data will be encrypted to ensure that no information can be traced back to any single participant.

Risk and benefit

The study is very safe but some questions might cause emotional instability. You are reminded that you can discontinue participation at any stage of study without any repercussions. If the interview or any other measures in the study might cause you any distress or psychological harm then you may request a psychological interview with a psychologist, free of charge.

Your participation will help us develop a treatment for persistent auditory verbal hallucinations.

Researchers and permission

The study is part of Viktor D. Jónasson master project, Aníta Ó. Georgsdóttir bachelor project, Fabio Barollo doctoral project and Ovidiu C. Banea doctoral project. All are students at Reykjavik University. Ethics Committee of the National University Hospital of Iceland and Landspítali medical director approved the study and the applied research has been submitted to the Icelandic Data Protection Authorities.

For any further information please contact:

Brynja Björk Magnúsdóttir: phone 543-4062, Email: brynjabm@landspitali.is

Ovidiu Constantin Banea: phone 543-4454, Email: ovidiuc@landspitali.is

Viktor D. Jónasson, Email: viktord@landspitali.is

Aníta Ósk Georgsdóttir, Email: anitag16@ru.is

The responsible person for the project is Brynja Björk Magnúsdóttir, Clinical Neuropsychologist at the National University Hospital of Iceland and Assistant professor at Reykjavik University, 101 Reykjavík, Phone: 543-4068, e-mail: brynjabm@landspitali.is

The research is done with the permission of the Ethics Committee and the Icelandic Data Protection Authorities has been notified. If you have any questions about your rights as a participant then you can contact the hospital Ethics Committee, Fossvogi, 108 Reykjavík. Phone: 543-7465, fax: 5432339, Email: sidanefnd@landspitali.is

A.2 Information form for participants in the healthy control group



Informed consent for participation in a clinical applied research study - Healthy control group

Repetitive Transcranial Magnetic Stimulation as a Treatment for Persistent Auditory Verbal Hallucinations

1. With my signature I confirm my decision to participate in the study of *repetitive transcranial magnetic stimulation as a treatment for persistent auditory verbal hallucinations* that is conducted by the Clinical Neurophysiology Unit, National University Hospital of Iceland and Icelandic Center for Neurophysiology, Reykjavik University. The responsible person for the project is Brynja Björk Magnúsdóttir, clinical neuropsychologist.
2. I confirm that I have read and understood the informed consent form and I have been given a chance to read the introduction form about the purpose and procedure of the study. I have been given a chance to ask questions about any items that were unclear to me and I have received satisfactory answers to my questions.
3. I understand that I can leave the study at any time without giving any reason and without any repercussions.
4. I agree to participate under the condition that all research that will be done and the results and information that will come out of the study is in accordance with current law and rules of the scientific ethics committee and all data processing will obey the rules of the Icelandic Data Protection Authorities.

Participant's Name & Signature

Date

Researcher's Name & Signature

Date

Appendix B

Protocol

B.1 Brainstorm: P300 Protocol

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

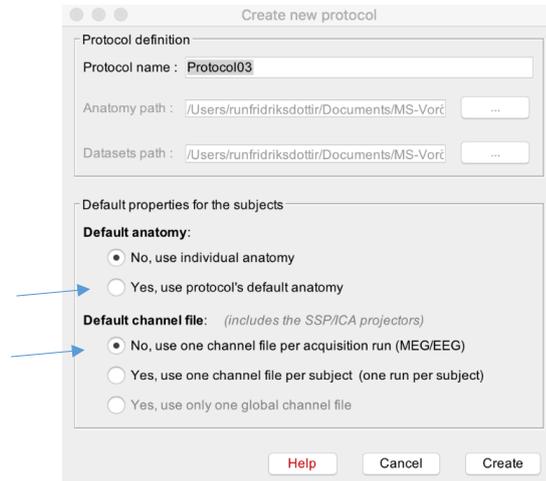
» Name your protocol.

Default anatomy

» Yes, use protocol's default anatomy

Default channel file

» No, use one channel file per aq. run



Go to the functional data window



Right click on the protocol

» New subject » Name the Subject

Right click on the Subject

» Review Raw file

» open the *.cnt file

(ANT EEPROBE format)

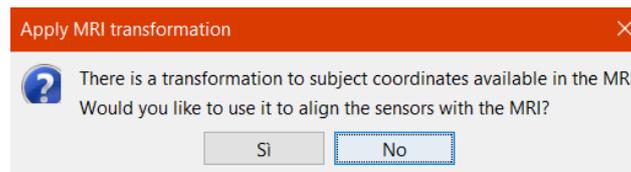
Core Registration

Right click on the Subject folder

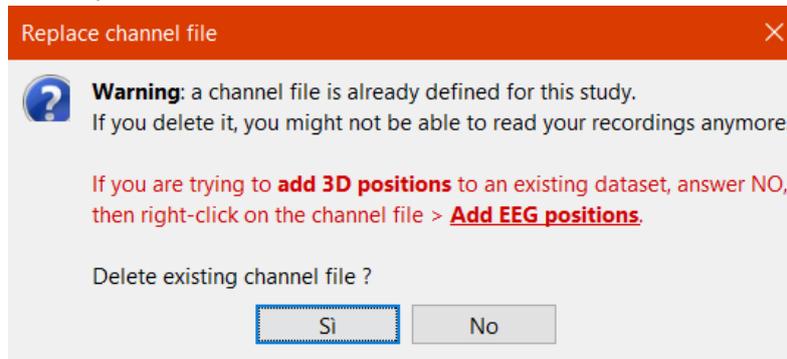
» import channel file (Warning appears, click yes to overwrite)

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

» Warning appears: first click no,



» then click yes.

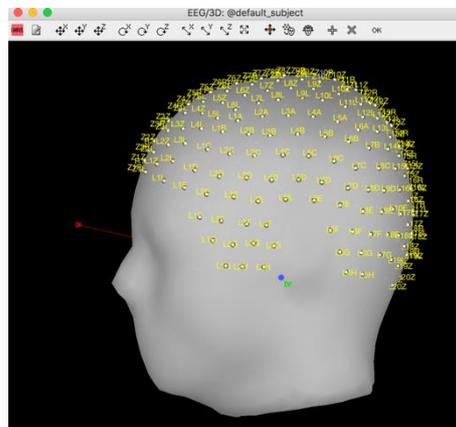
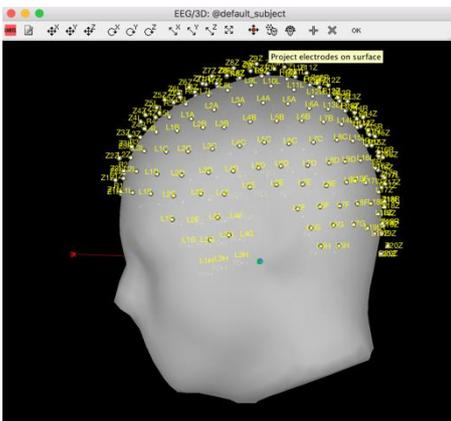


Right click on ANT Xensor under the Common Files folder

» MRI registration

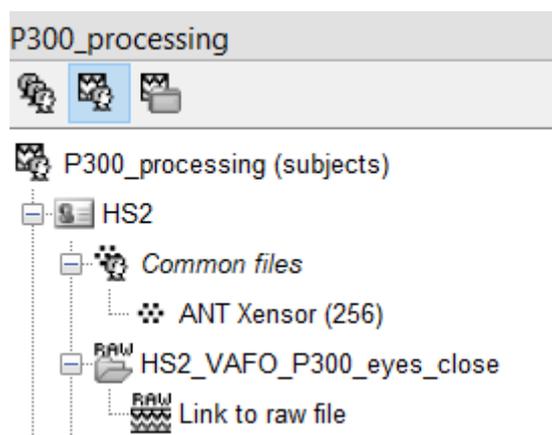
» Edit

» Project electrodes on surface » Refine registration » OK



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

The Subject window should look similar to this:



Select

» run » add process » preprocess » band-pass filter (0.5 Hz – 70 Hz) » run

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

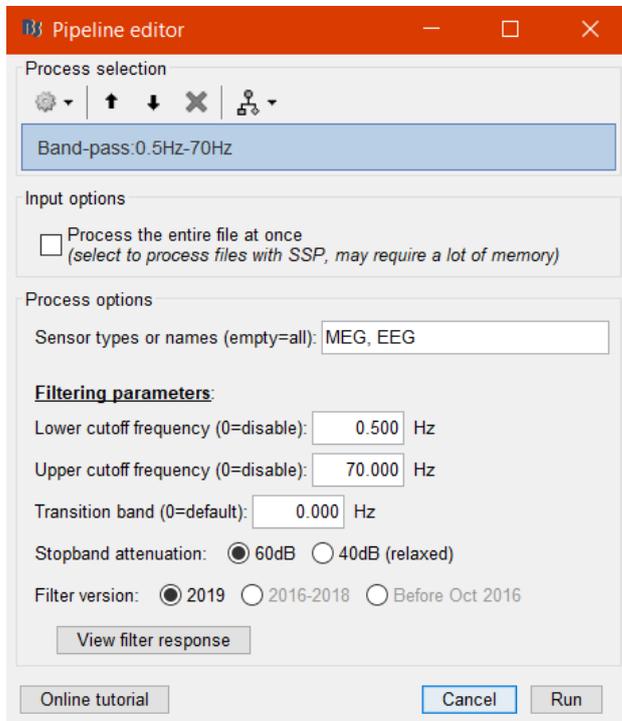
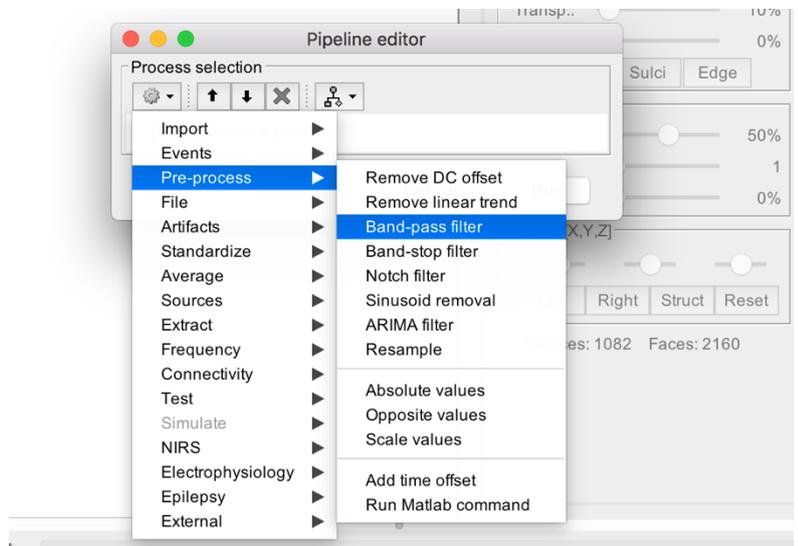
A new file folder '| band' is created for each sub folder.

Select

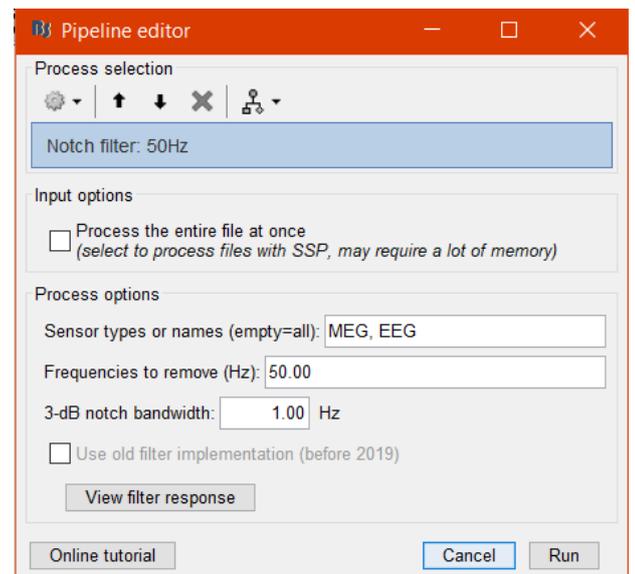
» run » add process » preprocess » notch filter » run

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

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



Band-pass filter (0.5-70Hz)



Notch filter (50 Hz)

Reference

Create a new reference: BIMASTOID

» Double click on the raw file 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)

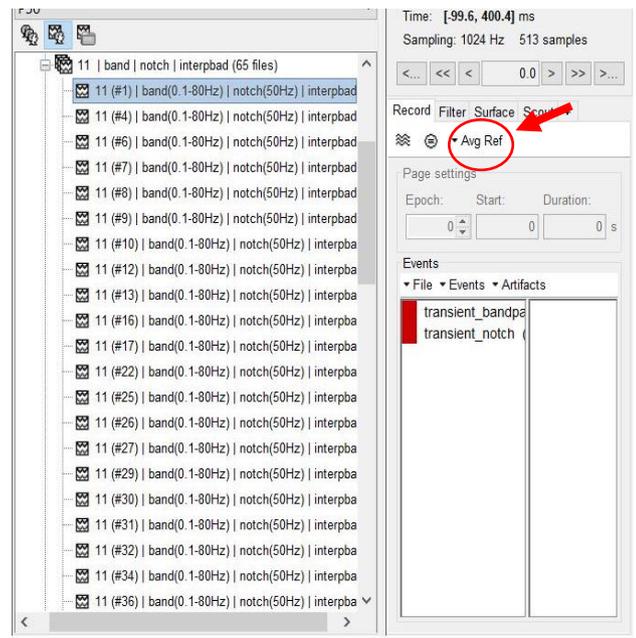
Click OK on the warning message

» New montage name: 'Bimastoid'

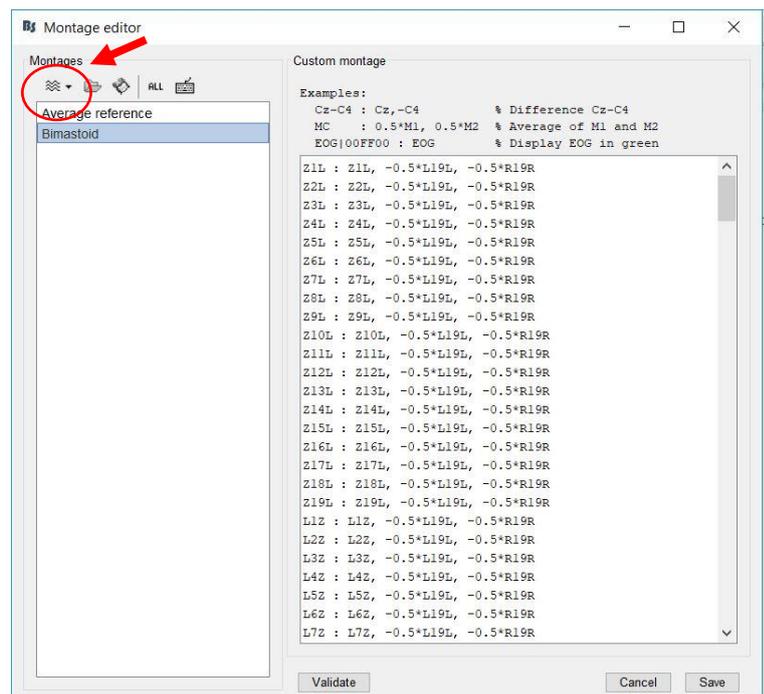
» Select the first reference channel from the list (R19R) » OK

» 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).



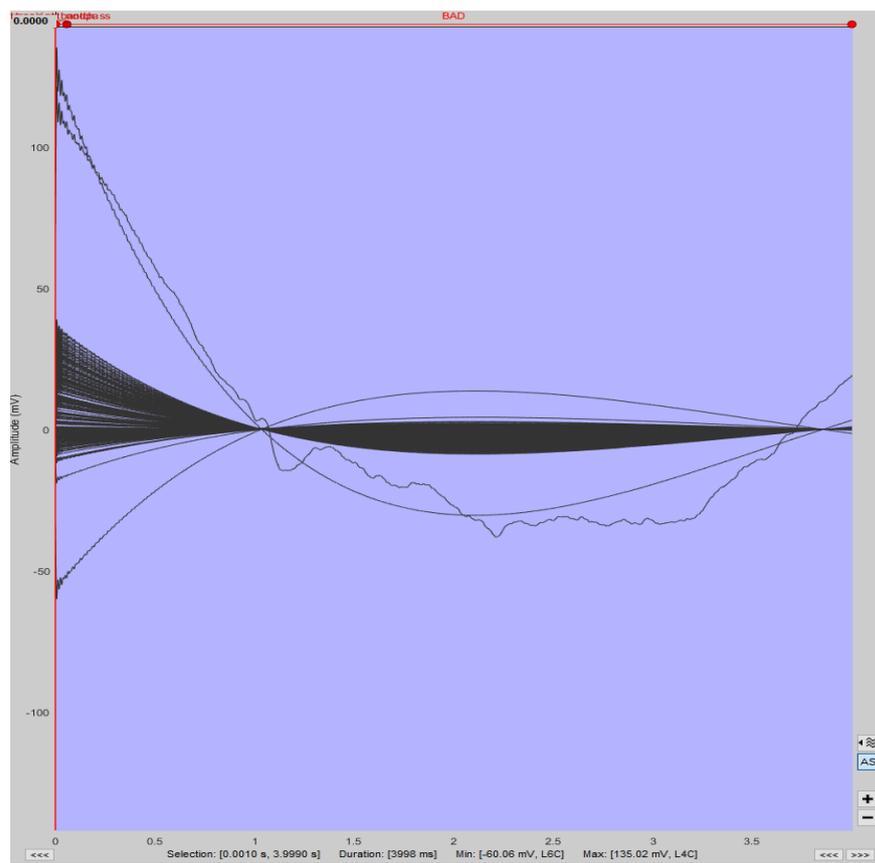
New montage

Visual inspection : removing bad channels and bad time segments

Visually check the file to detect bad time segments

» Double click on the raw file that has been filtered » select the bad time segment » right click » Reject time segment (or press B)

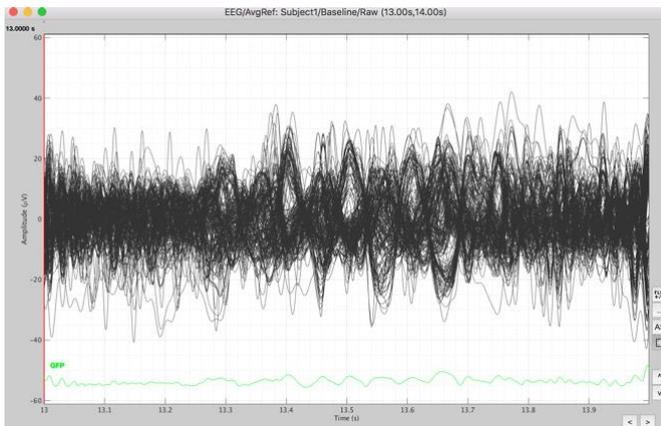
Go through the whole raw file to identify and reject all the bad segments



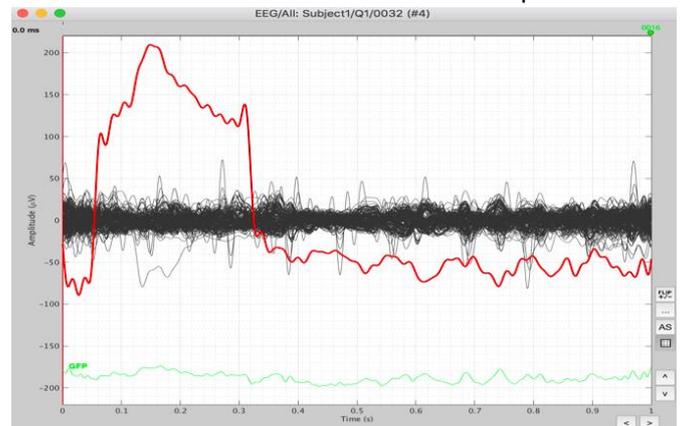
Visually check the raw file to detect weird channels.

» Double click on the raw file that has been filtered » click on the bad channel (becomes red) » right click » channels » mark as bad (or press delete button on keyboard)

Good channels



A channel that should be interpolated



Go through the whole file, and delete every channel that is weird.

Interpolate bad channels

We remove outliers or channels over $\pm 80\mu\text{V}$, if the outlier is just for a short period ($< 0.2\text{ ms}$) keep it.

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 | 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.

Import into epochs

Select the interpolated raw file. Right click » import in database.

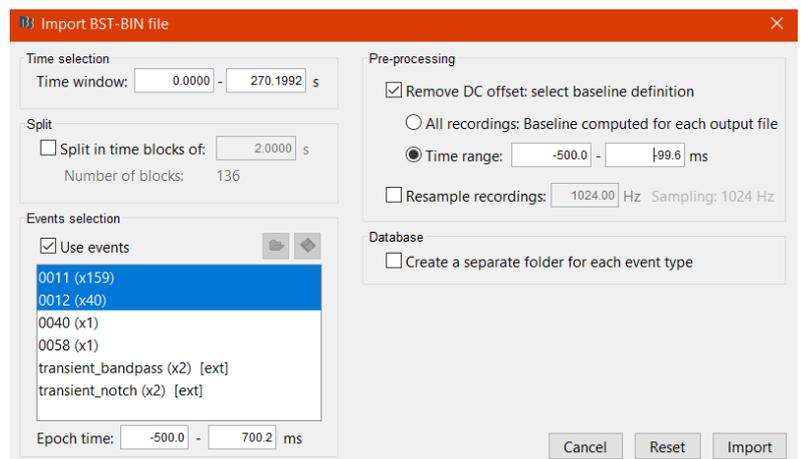
P300 :

Chose the time window of the file to import. When importing events, choose events (select 011 and 012) and epoch time (-500ms to 700ms)

Remove DC offset: select baseline definition

» Time range: [-500 ms -100ms]

Click import



Remove DC Offset: Check this option, select Time range: [-500, -100] ms.

For each epoch, it will: Compute the average of each channel over the baseline (pre-stimulus interval: [-500,-100] ms) and subtract it from the channel at every time instant (full epoch interval: [-500,700] ms).

This option removes the baseline value of each sensor.

Averaging and process

Select the folders created for the events 011 and 012 and drag them to the processing panel below.

Click Run >> Average >> Average files >> Run

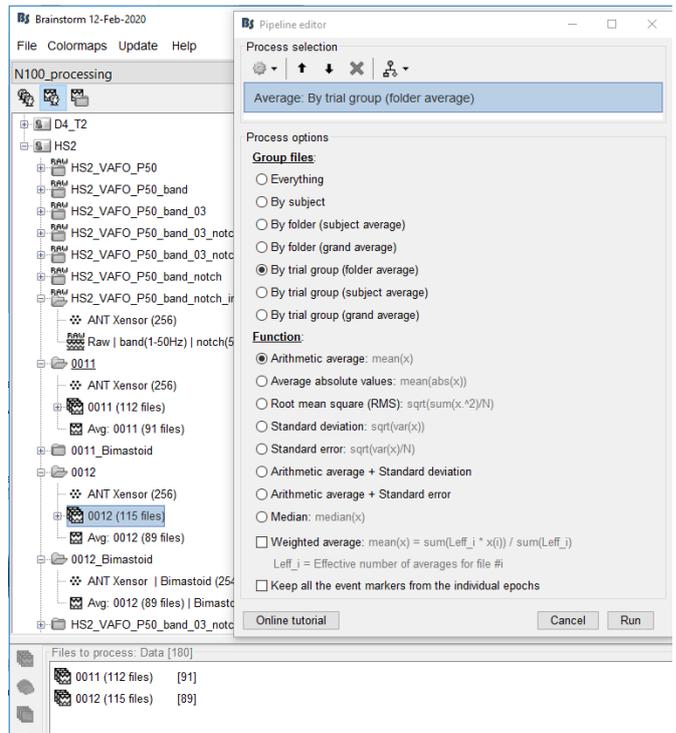
Two new files are created : Avg : 0011 and Avg : 0012

Drag those 2 files in the processing panel

Run >> Standardize >> Apply montage >> Bimastoid (Check create new folders) >> Run

A new folder is created with the 2 files inside.

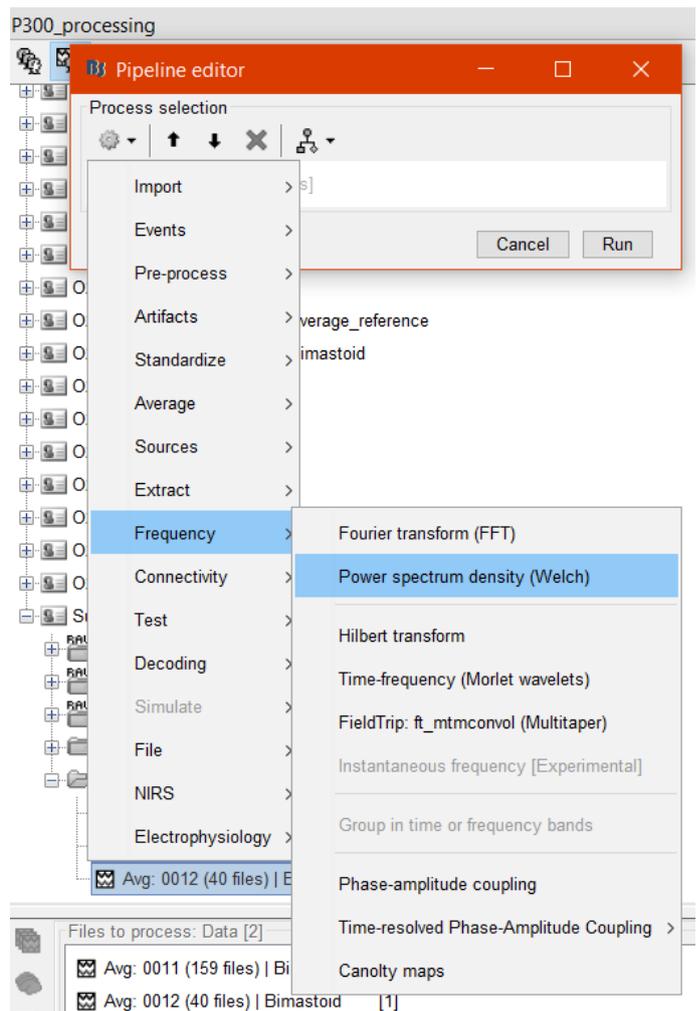
Avg : 0011 | Bimastoid and Avg : 0012 | Bimastoid



PSD computation

Select

» run » add process » frequency » power spectrum density » run



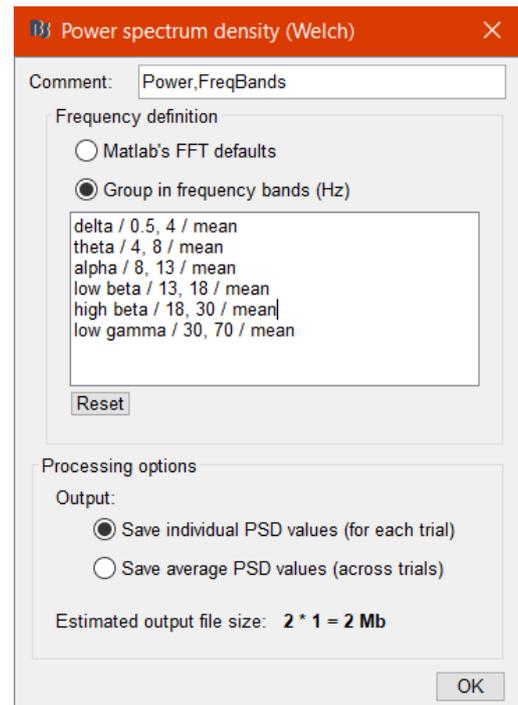
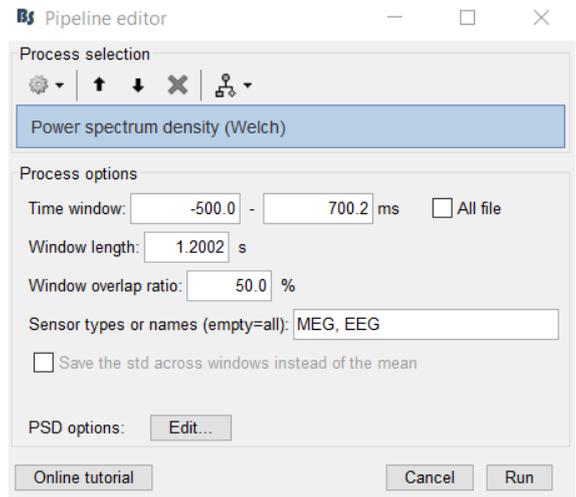
A new window will appear

» click Edit...

Change the frequency range of the bands:

- delta = [0.5 – 4] Hz
- theta = [4 – 8] Hz
- alpha = [8 – 13] Hz
- low beta = [13 – 18] Hz
- high beta = [18 – 30] Hz
- low gamma = [30 – 70] Hz

» click OK



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 P300 data averaged and referenced to the bimastoid are saved in the folder: *brainstorm_db\P300_processing\data\HS2_Bimastoid*. The files we're interested in are .mat files and they are named with the date and time in which they're created.

 timefreq_psd_200422_1430	22/04/2020 16.30	MATLAB Data	32 KB
 timefreq_psd_200422_1431	22/04/2020 16.31	MATLAB Data	32 KB

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

 PSD_HS2_F	22/04/2020 16.30	MATLAB Data	32 KB
 PSD_HS2_R	22/04/2020 16.31	MATLAB Data	32 KB

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

» TF contains the PSD of each channel averaged for the different trials of the 6 frequency bands;

» Vector *Time* contains the time samples;

Workspace	
Name ^	Value
Atlas	[]
ColormapType	[]
Comment	'PSD: 1/1200ms ...
DataFile	'HS2_FrequencyA...
DataType	'data'
DisplayUnits	[]
Freqs	6x3 cell
GridAtlas	[]
GridLoc	[]
HeadModelFile	[]
HeadModelT...	[]
History	1x3 cell
Leff	125
Measure	'power'
Method	'psd'
nAvg	125
Options	1x1 struct
RefRowNames	[]
RowNames	1x254 cell
Std	[]
SurfaceFile	[]
TF	254x1x6 double
TFmask	[]
Time	[-0.5000,0.7002]
TimeBands	[]

B.2 Matlab: Data analysis Protocol

Comparison within cohort

- Copy the data from brainstorm to the new folder of the subjects (e.g. D4) in 'MATRIX_PSD' folder and change their name as 'PSD_sub_T(1/2)_(F/R)' (T1_F, T2_F, T1_R, T2_R; T1: before the treatment; T2: after the treatment; F= frequent event (011) ; R =rare event (012)).

Nome	Ultima modifica	Tipo
D4	31/08/2020 11.50	Cartella di file
I2	31/08/2020 11.50	Cartella di file
I4	31/08/2020 11.50	Cartella di file
I5	31/08/2020 11.50	Cartella di file
N1	31/08/2020 11.50	Cartella di file

Nome	Ultima modifica	Tipo	Dimensione
PSD_D4_T1_F	27/04/2020 13.21	MATLAB Data	32 KB
PSD_D4_T1_R	27/04/2020 13.22	MATLAB Data	29 KB
PSD_D4_T2_F	27/04/2020 13.22	MATLAB Data	29 KB
PSD_D4_T2_R	27/04/2020 13.22	MATLAB Data	29 KB

- Go to the main folder and use the matlab code 'MATR_arrang_new.m' to create *matr_ave*. It is a structure with the averaged epoch of PSD of each group (T1_F, T2_F ...) of each subject: each subject of the structure has 4 matrixes 6x254.

Nome	Ultima modifica	Tipo	Dimensione
MATRIX_PSD	31/08/2020 11.50	Cartella di file	
corrections	23/03/2020 12.38	File R	3 KB
correzione_selection	27/04/2020 13.49	MATLAB Code	2 KB
eeglab-electrodes_position.elc	23/03/2020 12.39	File ELC	6 KB
MATR_arrang_new	27/04/2020 13.37	MATLAB Code	4 KB
NEW_plot_ttest_MARKED_T1	13/05/2020 12.28	MATLAB Code	6 KB
NEW_plot_ttest_MARKED_T2	13/05/2020 12.33	MATLAB Code	6 KB
reading_tables	27/04/2020 13.47	MATLAB Code	3 KB
Rstudio_matr_creation	27/04/2020 13.47	MATLAB Code	1 KB
Sign_PSD_T1_creation	29/04/2020 18.26	MATLAB Code	3 KB
Sign_PSD_T2_creation	29/04/2020 18.28	MATLAB Code	3 KB
ttest_T1	27/04/2020 13.40	MATLAB Code	3 KB
ttest_T2	27/04/2020 13.41	MATLAB Code	3 KB

- Use the matlab code 'ttestT1.m' or 'ttestT2.m' to do the statistical test between subjects in T1 and T2. It gives two structures *statT1* and *statT2* with the p values and the significant channels for each frequency band.
- Open 'R_studio_matr' to create the data that has to be imported on Rstudio to do the multiple corrections -Bonferroni and FDR. The following two steps should be done changing manually the data of the script. This part should be done four times: *statT1.freq*, *statT1.rare*, *statT2.freq*, *statT2.rare*.
- Run the script 'corrections.R' with Rstudio.

- Import the tables in matlab using 'reading_tables.m'. The data are imported in excel files and saved in four structures *bonft1*, *bonft2*, *fdrT1* and *fdrT2*.
- Save the channels which have the Bonferroni and FDR correction that are less than 5% with the script 'correzione_selection.m', creating four new structures *BON_T1*, *BON_T2*, *FDR_T1* and *FDR_T2*.
- Run 'Sign_PSD_creation.m' to create the PSD values associated only to the significant channels
- Run 'NEW_plot_ttest_MARKED_T1', 'NEW_plot_ttest_MARKED_T2' to plot the PSD associated to the significant channels for the ttest before and after the treatment, respectively.

Comparison between cohort

- Copy the data from brainstorm to the new folder of the subjects (e.g. I4) in 'MATRIX_PSD' folder and change their name as 'PSD_sub_T(1/2)_(F/R)' (T1_F, T2_F, T1_R, T2_R – T1: before the treatment; T2: after the treatment; F= frequent event (011) ; R=rare event (012)).
- Go to the main folder and use the matlab code 'MATR_arrang_new.m' to create *matr_ave*. It is a structure with the averaged epoch of PSD of each group (T1_F, T2_F ...) of each subject: each subject of the structure has 4 matrixes 6x254.

> Spectral Analysis > Difference TG frequent - rare

Nome	Ultima modifica	Tipo	Dimensione
MATRIX_PSD	31/08/2020 12.14	Cartella di file	
corrections	23/03/2020 12.38	File R	3 KB
correzione_selection	12/05/2020 12.11	MATLAB Code	2 KB
eeglab-electrodes_position.elc	23/03/2020 12.39	File ELC	6 KB
MATR_arrang_new	12/05/2020 13.02	MATLAB Code	3 KB
matr_diff_psd	12/05/2020 12.21	MATLAB Code	2 KB
NEW_ttest_plot_sign_T1	20/05/2020 14.14	MATLAB Code	6 KB
NEW_ttest_plot_sign_T2	12/05/2020 12.51	MATLAB Code	6 KB
reading_tables	12/05/2020 13.09	MATLAB Code	3 KB
Rstudio_matr_creation	12/05/2020 13.09	MATLAB Code	1 KB
Sign_diff	12/05/2020 12.45	MATLAB Code	2 KB
Sign_PSD_creation	12/05/2020 12.29	MATLAB Code	2 KB
ttest_F	12/05/2020 11.58	MATLAB Code	3 KB
ttest_R	12/05/2020 11.58	MATLAB Code	3 KB

- Use the matlab code 'ttestF.m' or 'ttestR.m' (or 'ttestT1.m' or 'ttestT2.m' depending on the comparison that is computing) to do the statistical test between subjects in one cohort and in another cohort. It gives two structures *statF* and *statR* with the p values and the significant channels for each frequency band.
- Open 'R_studio_matr' to create the data that has to be imported on Rstudio to do the multiple corrections -Bonferroni and FDR. The following two steps should be done changing manually the data of the script. This part should be done four times: *stat.Freq.T1*, *stat.Freq.T2*, *stat.Rare.T1*, *stat.Rare.T2*.
- Run the script 'corrections.R' with Rstudio.

- Import the tables in matlab using 'reading_tables.m'. The data are imported in excel files and saved in two structures *bonf* and *fdr*.
- Save the channels which have the Bonferroni and FDR correction that are less than 5% with the script 'correzione_selection.m', creating two new structures *BON* and *FDR*.
- Run the script 'Sign_diff.m' to identify the intersected channels between 2 cohorts.
- Run the script 'matr_diff_psd.m' to compute the difference between the 2 cohorts
- Run 'Sign_PSD_creation.m' to create the PSD values associated only to the significant channels
- Run 'NEW_plot_ttest_MARKED_T1', 'NEW_plot_ttest_MARKED_T2' to plot the PSD associated to the significant channels for the ttest before and after the treatment, respectively.

Appendix C

Supplementary data

Table C.1: Averaged PSD difference - cohort comparison : pre-post, F-R, Healthy-Patients.

Unit : $(\mu V^2/Hz) \times 10^{-15}$

Alpha	HS _F -HS _R	CG _F -CG _R	TG _F -TG _R	TG _{post} -TG _{pre}	CG _{post} -CG _{pre}	HS-TG _{pre}	HS-TG _{post}	HS-CG _{pre}	HS-T _{Spost}	CG _{post} -TG _{post}
Pre	-	-26,3	-5,63	-	-	-	-	-	-	-
Post	-	-22,19	-17,14	-	-	-	-	-	-	-
F	-	-	-	-3,15	-3,42	3,16	10,37	3,18	4,63	7,85
R	-	-	-	7,68	-11,22	-11,91	-12,69	-6,61	-1,66	-3,34
-	-4,20	-	-	-	-	-	-	-	-	-

Low Beta	HS _F -HS _R	CG _F -CG _R	TG _F -TG _R	TG _{post} -TG _{pre}	CG _{post} -CG _{pre}	HS-TG _{pre}	HS-TG _{post}	HS-CG _{pre}	HS-T _{Spost}	CG _{post} -TG _{post}
Pre	-	-2,76	-1,35	-	-	-	-	-	-	-
Post	-	-7,31	-4,53	-	-	-	-	-	-	-
F	-	-	-	-0,99	1,16	-1,98	-1,17	-0,86	-1,94	2,64
R	-	-	-	-	2,95	-8,82	-15,74	-1,63	-5,09	-8,82
-	-2,18	-	-	-	-	-	-	-	-	-

High Beta	HS _F -HS _R	CG _F -CG _R	TG _F -TG _R	TG _{post} -TG _{pre}	CG _{post} -CG _{pre}	HS-TG _{pre}	HS-TG _{post}	HS-CG _{pre}	HS-T _{Spost}	CG _{post} -TG _{post}
Pre	-	-1,61	-3,97	-	-	-	-	-	-	-
Post	-	-1,41	-3,47	-	-	-	-	-	-	-
F	-	-	-	1,04	-0,16	-0,51	-0,64	-0,46	-0,29	-0,61
R	-	-	-	1,94	-0,67	-2,16	-2,94	-1,47	-1,07	1,94
-	-0,67	-	-	-	-	-	-	-	-	-

Acknowledgements

I want to extend my deepest thanks to my supervisors, professors Visintin Monica and Gargiulo Paolo, who gave me the opportunity to join this wonderful project and who gave me valuable advice that I will treasure for the rest of my life.

A special thanks to Marco, Romain and all my colleagues at Reykjavik University, who let me be part of a beautiful team and patiently introduced me to my new life in Iceland.

Thanks to my friends, Eleonora, Manuela, Giulia and Silvia, who have always been there for me, sharing their love and support every time I needed it. Thanks to all my friends from university, who made me laugh so much and lighted up my lunch breaks and my pre-exams. Thanks to Martina, my childhood friend who gave me such good memories that have always made me smile.

My deepest thanks to Francesco, who helped me to overcome every obstacle and celebrated every good news with me. He gave me the courage to leave my city and start this wonderful experience.

At last, thanks to my family, who supported me since my first day of school: thanks to my grandparents and my uncles, for your infinite love and kindness; to my mum who passed down to me the courage to start every new adventure with positivity; to my father, who has always believed in me and supported my decisions; to my brother for being the shoulder I can always depend on and the best companion I could ever ask for.