Bachelor Project

Czech Technical University in Prague

F3
Faculty of Electrical Engineering
Department of Circuit Theory

Analysis of deep brain stimulation recordings from emotional task

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Supervisor: Mgr. Tomáš Sieger Ph.D.
Field of study: Medical Electronics and Bioinformatics
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I. Personal and study details

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II. Bachelor's thesis details

Bachelor's thesis title in English:

Analysis of deep brain stimulation recordings from emotional task

Bachelor's thesis title in Czech:

Analýza elektrofyziologických záznamů z hluboké mozkové stimulace: emoční úloha

Guidelines:

1. Familiarize yourself with i) the treatment of Parkinson's disease (PD) using deep brain stimulation (DBS), ii) the neurophysiological signals recorded during the DBS implantation: microelectrode recordings (MER) and local field potentials (LFP), and iii) with the emotional task executed during the implantation, in which patients were presented with a series of happy, fearful, and neutral human faces.
2. Explore and clean supplied MER and LFP. Try to remove the activity of nearby neurons from MER, yielding the activity of distant neurons. Visualize signals in the time and frequency domain with respect to the presented task.
3. In each of the signals, estimate whether the neuronal activity was specific to different parts of the task using an appropriate statistical approach: could you find specific traces of activity related to neutral, happy, or fearful faces in the signals? Does activity in those three categories differ from each other?
4. Summarize your findings, try to aggregate them with respect to the type of neuronal activity, recording location, hemisphere, etc.
5. Implement the methods used in steps 2-4 in the form of well-documented and commented Matlab functions.

Bibliography / sources:

[1] Agnesi F, Johnson MD, Vitek JL. Deep brain stimulation: how does it work? Handb Clin Neurol. 2013;116:39-54. doi: 10.1016/B978-0-444-53497-2.00004-8. PMID: 24112883.
[2] Buzsáki G, Anastassiou CA, Koch C. The origin of extracellular fields and currents--EEG, ECoG, LFP and spikes. Nat Rev Neurosci. 2012;13(6):407-420. Published 2012 May 18. doi:10.1038/nrn3241
[3] Krauss JK, Lipsman N, Aziz T, et al. Technology of deep brain stimulation: current status and future directions. Nat Rev Neurol. 2021;17(2):75-87. doi:10.1038/s41582-020-00426-z
[4] Hamani C, Saint-Cyr JA, Fraser J, Kaplitt M, Lozano AM. The subthalamic nucleus in the context of movement disorders. Brain. 2004 Jan;127(Pt 1):4-20. doi: 10.1093/brain/awh029. Epub 2003 Nov 7. PMID: 14607789.
[5] Wild J. Automatic processing of neuronal single-cell recordings from deep structures of human brain. Diploma Thesis. Czech Technical University in Prague, Faculty of Electrical Engineering, 2008.

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III. Assignment receipt

The student acknowledges that the bachelor's thesis is an individual work. The student must produce her thesis without the assistance of others, with the exception of provided consultations. Within the bachelor's thesis, the author must state the names of consultants and include a list of references.

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Date of assignment receipt

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Student's signature

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In addition, I would like to thank my family and friends for their help and support during writing of this thesis.

Declaration

I declare that the submitted work was developed independently and that I made a list of all used sources of information.

Prague, 20. May 2022

Magdaléna Kůtová

Abstract

The human brain is a highly complex structure, and some of its functions are still a mystery. The biggest problem with studying its function is gaining the data because non-invasive methods are not sufficiently strong to record neuronal activity from depth brain structures like basal ganglia. However, in some situation where invasive microelectrode recordings can be used to gain valuable information about neuronal activity inside the brain, for example, deep brain stimulation.

In this thesis, neuronal activity from the subthalamic nucleus is analyzed. The recordings were made during electrode placement for deep brain stimulation. During the surgery, patients fulfill the emotion task to see if there is a specific neuronal activity for different emotions - happiness, fear, and neutral. The methods for processing the recordings and their statistical evaluation were studied and implemented as software tools.

The results of the analysis of given recordings show that although the subthalamic nucleus is mostly motoric centrum, some neurons had significantly different reactions based on emotion.

Keywords: Parkinson's disease, deep brain stimulation, microelectrode recordings, emotion task, signal processing, subthalamic nucleus (STN), t-test, bootstrap, chi-square test, Fisher's Exact test

Supervisor: Mgr. Tomáš Sieger Ph.D.

Abstrakt

Lidský mozek je velmi komplexní struktura a některé jeho funkce jsou pro nás i nadále záhadou. Největší problém při jeho studiu je získávání dat, protože neinvazivní metody nejsou dostatečně silné, aby zaznamenaly aktivitu neuronů z hlubokých mozkových struktur jako jsou například bazální ganglia. Nicméně jsou situace, kdy takovou data můžeme získat pomocí invazivních mikroelektrod, například hluboká mozková stimulace.

V této práci je analyzována neuronová aktivita v subthalamickém jádře. Nahrávky byly získány pří umisťování elektrod při hluboké mozkové stimulaci. Během operace, pacienti prováděli emoční úlohu, abychom mohli posoudit, jestli je nějaká specifická odpověď na různé druhy emocí – radost, strach, neutralita. Prostudované metody pro vyhodnocování byly implementovány jako softwarové nástroje.

Výsledky analýzy dostupných dat ukazují že ačkoliv je subthalamické jádro primárně motorické centrum, některé neurony vykazovaly významně rozdílné reakce na základě emocí.

Klíčová slova: Parkinsonova choroba, hluboká mozková stimulace, emoční úloha, mikroelektrodové záznamy, zpracování signálů, subthalamické jádro, t-test, bootstrap, chi kvadrát test dobré shody, Fisherův exaktní test

Překlad názvu: Analýza elfz. záznamů z hluboké mozkové stimulace: emoční úloha

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Chapter 1

Introduction

Deep brain stimulation is a way to treat, for example, Parkinson's disease when the medicaments do not help. But it also has the potential to treat other psychiatric disorders where we know which changes are made in the brain, but we do not understand why so we can cure just symptoms. To make this possible, we have to know what exactly is happening in the brain when we insert electrodes there. Because the brain is a complicated structure and we still do not understand the functions of every connection, before we place some foreign body into it, we have to know all possible advantages and, most importantly, disadvantages.

It is hard to get signals from humans' brains for research because there is no non-invasive method to record them from deep brain structures. However, we gain many rare electrophysiologist signals we can process during the insertion of electrodes for deep brain stimulation. From these recordings, we can learn a lot about the function of subcortical brain structures. Also, patients do some easy tasks during the surgery to gain as much information as possible. One example is an emotional task where different images are shown to the patients while the neuronal activity is recorded.

The motivation for examining recordings from the emotional task is that some patients who undergo deep brain stimulation have a change in emotions. However, electrodes are placed in the motoric structure during the treatment of Parkinson's disease. For better treatment and understanding of our brain, it is essential to find out if the stimulation of the motoric centrum can also influence the patients' emotions. In this work, we will examine if there are different reactions to different emotions - happiness, neutral, and fear.

The objectives of this thesis are the following:

1. To explore

   - The treatment of Parkinson's disease (PD) using deep brain stimulation (DBS)
   - The neurophysiological signals recording during DBS implantation - microelectrode recordings (MER) and local field potentials (LFP)
   - The emotion task

2. Visualize signals in the time and frequency domain, and to get the activity of the distant neurons, remove the activity of nearby neurons in MER signals.

3. Decide if specific traces of activity based on the type of shown image - neutral, happy, fearful can be found in the signals.

4. Summarize findings and decide if there is dependence on the type of neuronal activity, recording location, or hemisphere.

5. Implementation of the methods used in steps 2-4 in the form of well-documented and commented Matlab functions.

Chapter 2

Background

2.1 Neurons and neurotransmiters

The nervous tissue consists of two main types of cells - neurons and glial cells. More types of glial cells exist, and we divide them according to their function. Some of them support or protect the neurons, and others eliminate damaged neurons. Glial cells are more numerous than neurons in the nerve tissue and also, they are capable of cell division[1].

Neurons consist of three parts (Figure 2.1):

1. Soma = cell body, which is responsible for receiving, sending, and creating the information.

2. Axon = long filament. Each cell has just one axon, which transmits the information efferent (from the cell body), for example, from the soma to the muscle. It could be longer, even more than 1 meter.

3. Dendrite = short filament, which transmits the information afferent (to the cell body), for example, from receptors.

Synapses transmit information between neurons or between neurons and non-nervous cells. Synapses are places where the neurons are in touch. The synapses could be chemical (usually) or electrical. The means of the chemical
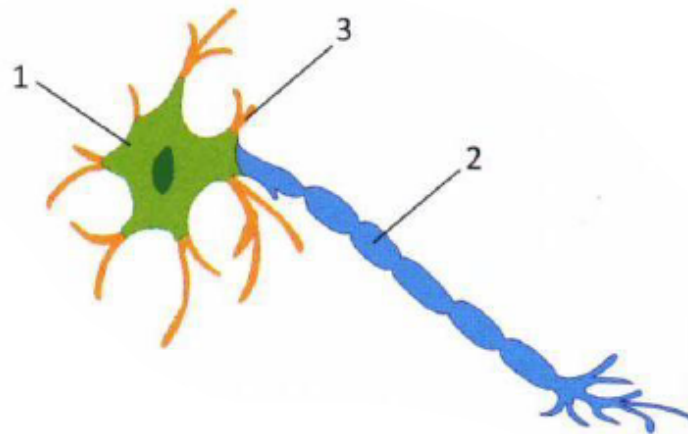
[1]

Figure 2.1: Structure of nerve cell (neuron). 1 - Soma, 2 - Axon, 3 - Dendrite

synapses are neurotransmitters, also called mediators. Neurotransmitters, chemical substances created in neurons, are released on the neuron membrane, touching the axon. The mediator breaks the electrical and chemical equilibrium of the other neuron, which causes the transmission of the signal. The chemical composition of neurotransmitters determines if the signal is excitatory or inhibitory. The primary excitation neurotransmitter is glutamate. On the other hand, the primary inhibition neurotransmitter is GABA (= gamma-aminobutyric acid). [2]

The principle of the neuronal connection is simple, but when we look at the whole neuronal network, it is very complicated and has great plasticity. Because one neuron can be connected through synapses with other 20 - 30 thousand neurons, and the estimated number of neurons is 25 billion, the amount of all synapses in the neuronal network is really high.

2.2 Basal ganglia

Basal ganglia are subcortical structures made of grey matter - somas of neurons. They are placed deep in the telencephalon. They are composed of several independent structures of different origins (Figure 2.2). One of the biggest parts is the caudate nucleus. However, there are also smaller but not less important nuclei like the striatum, putamen, nucleus subthalamic, substantia nigra (pars compacta and pars reticularis), or amygdala. [2]

The function of the basal ganglia is not exactly clear, but we know that they are involved in motoric circuits. So far, we assume their function is something like a motoric filter that lets just some motoric patterns proposed by the cortex to the thalamus. When the patterns get to the thalamus, we will do that movement [1]. Whether the basal ganglia allow the movement depends on the amount of dopamine. An abundance of dopamine causes Huntington's disease.

On the other hand, if there is a lack of dopamine Parkinson's disease occurs. [3] Because of their involvement in motoric circuits, basal ganglia are the primary object for deep brain stimulation for Parkinson's disease treatment. The electrodes are mostly placed near or inside the subthalamic nucleus because it is an important output modulator of basal ganglia. [4]
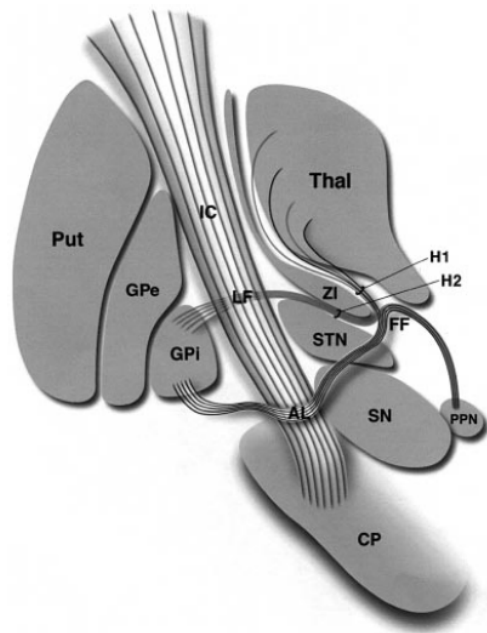
[4]

Figure 2.2: Basal ganglia. AL = ansa lenticularis; CP = cerebral peduncle; FF = Fields of Forel; GPe = globus pallidus externus; GPi = globus pallidus internus; H1 = Field of Forel (thalamic fasciculus); IC = internal capsule; LF =lenticular fasciculus (H2); PPN = pedunculopontine nucleus; Put = putamen; SN = substantia nigra; STN = subthalamic nucleus; Thal= thalamus; ZI = zona incerta.

2.3 **Parkinson's disease**

Parkinson's disease (PD) is a neurodegenerative movement disorder. The first symptoms usually occur during the fifth or sixth decade of life and worsen over time. Unfortunately, we do not know what causes it. It is probably the results of genetic mutations and some environmental factors that are currently unknown.

Patients with PD have dopaminergic neuron loss in substantia nigra, part of the basal ganglia - a structure made of grey matter in the telencephalon. This loss of neurons causes a decrease in dopamine. Dopamine is a chemical substance responsible for transmitting signals from substantia nigra to create smooth conscious movements. If there is not enough dopamine, basal ganglia refuse to do the movement (a part of the basal ganglia called the striatum is responsible for this decision)[3]. So the significant symptoms are motoric.

Four main symptoms are:

- Tremor - is a rhythmic movement of the muscles with the frequency of 3 to 6 per second. Usually, hands, foot, or jaw are affected. It is obvious mainly when the hands are at rest. Tremor disappears during sleep or voluntary movement.

- Rigidity - the muscles are constantly tensed and contracted.

- Bradykinesia - spontaneous and automatic movements are slowing down, making everyday tasks like dressing or washing dishes more difficult and slower.

- The postural inability - it causes problems with balance.

As the disease worsens, other symptoms like difficulty in swallowing and chewing, speech changes, urinary problems, constipation, and more, occur. But Parkinson's disease also has many non-motoric symptoms, for example, emotional changes or depression. [5].

■ 2.4   Treatment of Parkinson's disease

Because we do not know the precise cause of the PD, we are able to treat just symptoms. Nowadays, we have two approaches to treat its symptoms.

The first is using levodopa medication. Because levodopa is a precursor of dopamine, this approach alleviates symptoms caused by the loss of dopamine in substantia nigra, especially the very troublesome bradykinesia. Despite this fact, as the disease progresses, the beneficial effect of levodopa decreases. Another problem with levodopa medication is that most dopamine is crated from levodopa in other parts of the brain, where there is enough dopamine. An abundance of dopamine in other brain structures can cause several side effects, such as loss of appetite, sleep disturbances, changes in mood, and more [3].

The second approach is surgery. In the past, ablative techniques were used. However, nowadays, it is replaced by deep brain stimulation (DBS). Because it is an invasive method and we still do not have all possible side effects, this approach is only used when the medicaments do not help [6]

■ 2.5   Deep brain stimulation

Deep brain stimulation is not a new technique; in fact, it has been known for many years. But the first electrical stimulation used to alleviate tremors was made in the 1950s by Hessler and colleagues [7].

Deep brain stimulation is a surgical treatment belonging to neuromodulation treatments that use electrical stimulation to modulate brain function. The effects of DBS stimulation are immediate, reversible, and do not cause permanent tissue lesions such as ablative techniques.

It is commonly used for the treatment of movement disorders such as Parkinson's disease or dystonia. However, it can also be used to treat other neurological diseases such as pain syndromes or epilepsy. Furthermore, it has the potential to treat psychiatric disorders like obsessive-compulsive disorder, depression, addiction, and lots of others. Nevertheless, the treatment of psychiatric disorders is still in the study phase [6].

Deep brain stimulation is a fully implantable system (Figure 2.3). It consists of one or more electrodes placed inside the brain, which deliver electrical impulses to the focal brain regions [6]. The exact location depends on the treated disease. For example, for treating Parkinson's disease, the electrodes are placed in the STN (subthalamic nucleus), or another possible target is globus palidus internus [7].

The second part of the system is the neurostimulator. It is a power source whose primary function is to generate pulses. That is why the device is also called an internal pulse generator (IPG). The construction of the neurostimulator is similar to the cardiac pacemaker, and as well as the pacemaker is placed under the collar bone. These two parts - electrodes and neurostimulator are connected by an extension lead. [6]
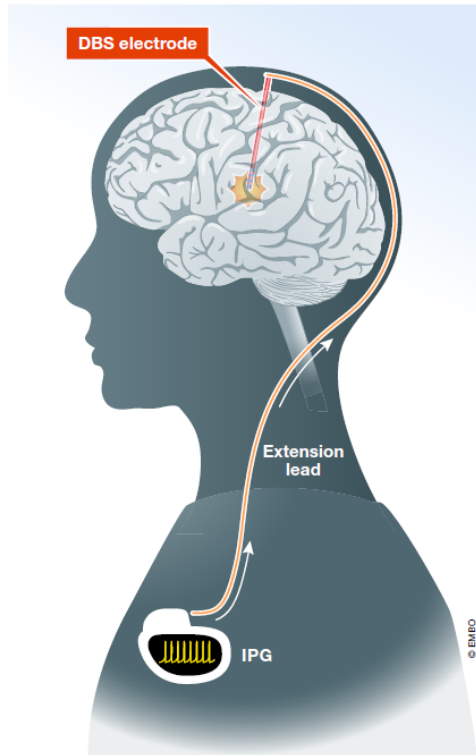
[6]

Figure 2.3: DBS system used for clinical applications

The future goals for improvements of the device are to construct it without extension cables and improve properties of IPG such as battery life or wi-fi connection for better control. However, the improvement can bring some dangers which have to be solved. For example, when the neurostimulator has internet or Bluetooth connection, it will help patients manage the treatment according to their individual needs. Because of it, the therapy will be more personalized and thus more effective. On the other hand, it raises the risk of

brain hacking and maybe a possibility of controlling somebody's brain, so it has to be really secure[8].

2.5.1 Neurosurgery

For deep brain stimulation, we need to place electrodes inside the brain. Placing the electrode is a difficult task because the target is inside the brain, and along the way, there should not be any other part of the brain tissue damaged.

Firstly, the patients get a local anesthetic to numb the skin on the top of the skull because two burr holes are made there. General anesthesia is not needed because there are no pain receptors in the brain. Furthermore, patients must report any sensory changes during the surgery, and after the electrode is successfully placed, the effect of the stimulation has to be tested.

The most challenging problems for this neurosurgery are location and navigation. STN is a small structure ($20 - 30mm^3$), and its location can slightly differ from patient to patient. The target's location is set based on scanning the patient's brain. Magnetic resonance imaging (MRI) and computed tomography (CT) are used to do this scanning [9].
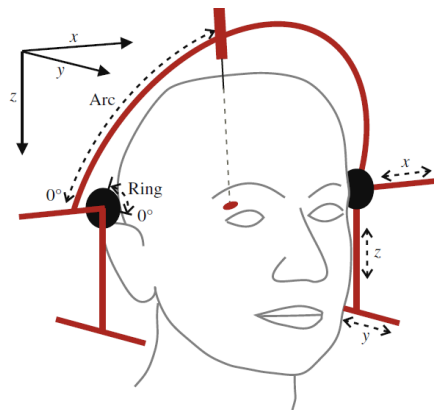
[10]

Figure 2.4: Schematic image of stereotactic frame used for navigation

The navigation is solved by a stereotactic frame (Figure 2.4). The stereotactic frame is a semicircular arc attached over the patient's head. Moreover, a movable holder for auxiliary microelectrodes can be found on the arc. Its primary purpose is to generate a coordinating system in the brain which allows neurosurgeons to navigate there. [6]

■ 2.5.2   Effects on the brain

Deep brain stimulation has an electrical impact on individual neurons and neuronal networks. Overall it causes enhancement of the activity in the basal ganglia and thus equalizes the dopamine loss. But DBS does not have just electric effects. Studies revealed that it also influences the chemical environment of neurons. Immediate neurochemical effects are changes in the firing patterns of the nerve circuits. Nevertheless, these changes probably affect neurotransmitters, so DBS causes long-term effects. [6]

Electrodes themselves can cause changes in the brain's chemical composition as well. Especially the resistance of the tissue can change. The resistance primarily changes because of tissue edema, which emerges around the electrodes after their implantation. But that is not a problem because it disappears by itself four to six weeks after the surgery.

■ 2.5.3   Emotions and Deep brain stimulation

Deep brain stimulation of the subthalamic nucleus, like any other treatment, does not have just positive effects which suppress the symptoms of motoric disease (like Parkinson's disease). Side nonmotor effects also occur, like impulsivity, irritability, mania, depression, or body weight gain. The reason why this is happening is still unknown. [11]

According to the studies made in primates, we can divide STN into three parts: sensorimotor, associative, and limbic territories. Other anatomical studies in monkeys and rodents located the limbic area in the ventral part of STN. Also, studies in humans showed that neuronal activity in STN changed during motor and emotional information processing. These researches lead us to the theory that the ventral part of STN is connected to a limbic brain network, and thus it is involved in emotion processing [12].

One of the hypotheses of its function is that STN generates emotional patterns and coordinates the activity of the cortical and subcortical structures. Because the research findings showed that STN activity does not depend on the type of emotion or even valence (positive or negative), it should have a more general function. [13]

Several studies already exist about the impact of STN on emotional processing. Nevertheless, they do not discover the exact function of STN. That is probably because there are a lot of other influences. For example, the medicaments that PD patients take are omitted just one night before the surgery, so we cannot fully neglect their effect. Furthermore, the location of the electrodes could change a bit, and even a little difference can cause a significantly different impact. Last but not least reason is that all information we know about these processes is gained from DBS. People who undergo it are not neurologically healthy, so we have to consider that our data could be changed because of it. [13]

■ 2.6 Signals from neurons

Electric currents from all cell processes, which are simultaneously active in the range of the whole brain, are overlapped and generate the electrical potential [14]. Potential V_e is a scalar quantity, and we have to determine it relative to a reference potential. The electric field arises because of the difference in electrical potential between two brain areas. This electric field can be measured using several approaches.

The first of them is electroencephalography (EEG). With the help of EEG, we can measure potential V_e from the surface of the head. We have to place the non-invasive electrodes on the head surface to record the data. The electrodes are small with a diameter of 4 to 10 millimeters and are usually made from silver [15]. We have to ensure that the electrodes do not move. For this reason, special electrode hats (Figure 2.5) are created, so the electrodes stay in the correct position. The two most used electrode connection systems are 10-20 and 10-10. The number means the percentage of the distance between electrodes. Locations of the electrodes are in figures 2.6 2.7

From EEG, we can find out a lot of information that can be used in a wide range of examinations. For example, according to signals from EEG, we can determine epilepsy, some types of brain damage, or for monitoring of sleep processes [15].

EEG is one of the oldest methods we have for investigating the brain's electrical activity. Hans Berger made the first measurement of EEG on humans in 1929. Nevertheless, electrical brain activity was discovered earlier in animals. The first person who described it was Richard Caton in 1875 [17].
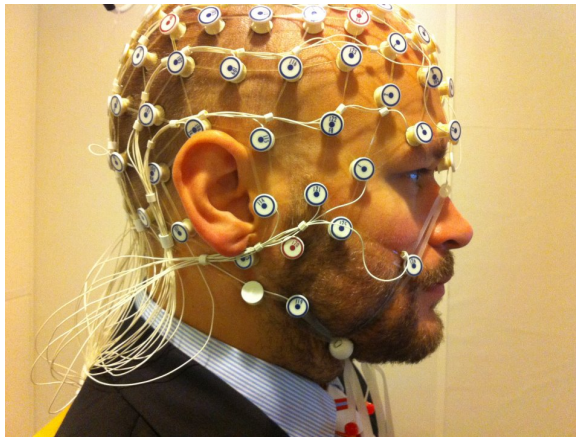
[16]

Figure 2.5: A man undergoing EEG with the electrode hat
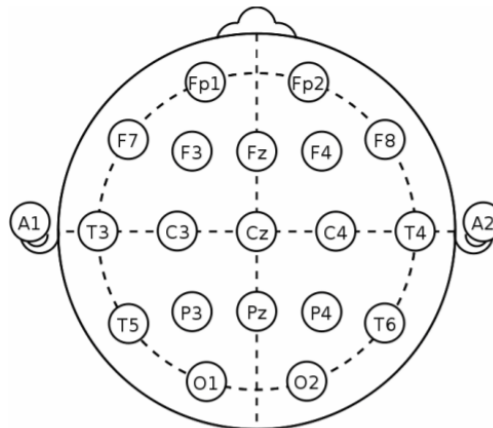
[15]

Figure 2.6: Position of the electrode system 10-20. A – auricular (ear lobe) C – central region P – parietal (parietal lobe) F – frontal (frontal lobe), Fp – frontopolar (frontal lobe), O – occipient (occipital lobe), T – temporal region. Numerical indexes are used for indication of the position lateral, even numbers are in the right and odd numbers are in the left.
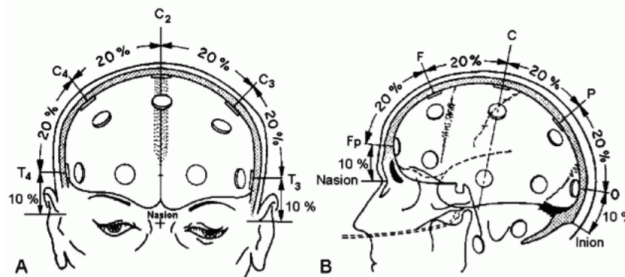
[15]

Figure 2.7: Locations of electrodes 10-20. A – frontal view, B - side view.

The next approach is electrocorticogram (ECoG). Using ECoG, we can measure potential V_e directly from the surface of the cerebral cortex. For that, we use electrodes mostly made from platinum-iridium. Because we record signals subdural they are not noisy like when using EEG. That is the reason why the popularity of ECoG is rising recently. Nevertheless, the price for this is that ECoG is an invasive method [14].

Another approach is local field potential (LFP). This method is also known as macro-EEG. While in the previous two approaches, we record neuronal activity from the brain's surface, in this case, we record in the deeper parts of the brain. LFP records the activity of the neuronal populations (group of neurons). Those signals are most valuable for studying cortical electrogenesis [14].

A similar approach as LFP is microelectrode recording. It is also an invasive method because the recording electrodes are placed deep in the brain. But with this approach, we gain extracellular electric activity of individual neurons. [18]

Other recordings methods for extracellular events are megnetoencephalogy and voltage-sensitive dye imaging. Magnetoencephalogy (MEG) measures the magnetic field of neurons outside of the brain. Superconducting quantum interference devices (SQUIDs) that are used for measuring are very accurate. The most significant advantage is that this method is non-invasive. Unlike the EEG, MEG is not disturbed by the conductivity of the extracellular space. The voltage-sensitive dye imaging (VSDI) method enables us to detect changes in the voltage of neurons by a camera. [14]

Another display method is fMRI (Figure 2.8), an abbreviation for functional magnetic resonance imaging. Functional MRI, as well as EEG and MEG, is a non-invasive method that allows us to display which part of the brain is active during some tasks. The task could be, for example, to move the index finger. It is based on the fact that brain activity in a specific area causes a transient increase in the blood supply. As a result, the BOLD (Blood Oxygen Level Dependent) signal arises. This means that oxygen increases in the brain area responsible for the performed action. Because oxygen is a paramagnetic substance, we can use magnetic resonance for imaging. Nevertheless, the response in the form of increased blood flow is not immediate.

Moreover, that is not the only disadvantage of this method. Another problem is that we have to average images in time, so the time resolution is bad. On the other hand, we have good space distinction, and also, we can use this method in vivo because it is non-invasive. So that is the most significant
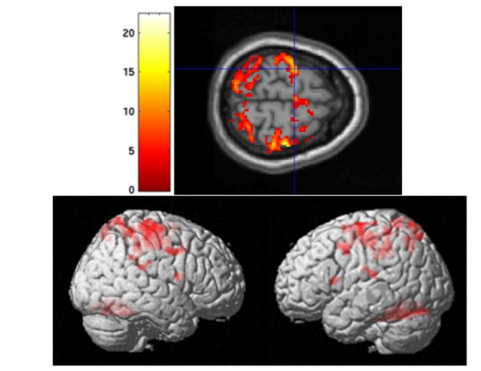
[19]

Figure 2.8: fMRI used to study the regions of the brain responsible for bilateral finger tapping

advantage over micro-EEG. [19]

█ 2.7 Extracellular field

The extracellular field is not created by one type of cell. In fact, all excitable membranes in the brain contribute to it. The excitable membranes in the brain are dendrites, axons, or axon terminals. The resulting extracellular field arises as a superposition of all ionic processes.

Extracellular currents have several sources. The primary source is the synaptic activity of the neurons. The influx of cations (Na^+ and Ca^{2+}) from the extracellular into the intracellular area occurs during synaptic transmission. These changes, mediated by neurotransmitters, give rise to the extracellular current.

Another important source of the extracellular current are fast action potentials. The fast action potentials is detected as spike activity, and it is generated by Na ions. Although it is the source of the strongest current, it is hard to detect because it has a short duration, even less than 2ms.

Calcium spikes are the next source of the extracellular current. They are also non-synaptic and are generated by Ca^{2+}. Unlike the fast action potential, the calcium spike has a long duration.

Nevertheless, other sources of the extracellular currents exist. For example, intrinsic currents and resonances, spikes after polarization and down states, gap junctions, and neuron-glia interaction or ephaptic effects [14].

Chapter 3

Data

For this experiment, were used 105 records from 22 patients. The data were collected from surgeries for deep brain stimulations done between the years 2016 to 2018. For each patient, several recordings were done in different depths of the electrodes in the brain (Figure 3.1). Each of them was recorded with a sampling frequency of 25 kHz.
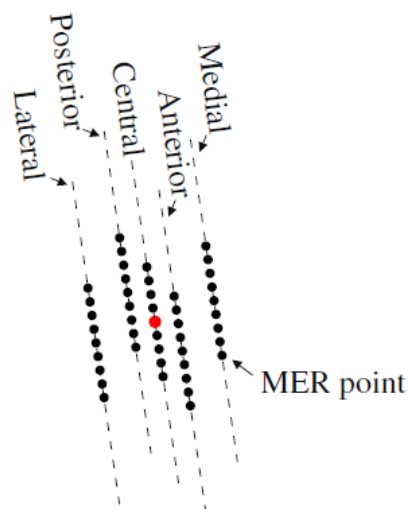
[10]

Figure 3.1: Schematic image of microelectrodes for recording (MER). MER points labeled depths where was the signal recording

We aimed to reveal if DBS has some impact on emotions and if that impact depends on the type of emotions. During recordings, images of human faces were shown to the patients during the surgery for 500, 550, or 600 ms. Because of that, we selected the 550 ms after the picture occurred as significant parts of the signal for further processing. So the brain has enough time to process it, but it is not expected that the reaction persists after the image is gone. These pictures were chosen to evoke some emotional reactions in patients. The reaction could be pleasant, neutral, or unpleasant to allow us to see the impact of different emotions in the signals. The examples of used images are shown in the figure 3.2.

Figure 3.2: Examples of the shown pictures. From the left it is happy, fearful and neutral images.

During each of the recordings, 50 happy, 50 fearful, and 50 neutral images were shown to each patient. So enough data was collected for statistical testing.

From the recorded data, we process signals from microelectrodes recordings denoted as LFPx and MERx, where x is an index of the channels, from one to five (there are five recording electrodes in the brain). So we have ten different channels from each patient's recording for processing. According to their position, the electrodes were labeled as medial, anterior, central, posterior, and lateral.

We were only interested in electrodes that were active in STN (Figure 3.3). If the electrode is active, it records the activity of STN. And the neurosurgeons determined if the electrodes were active in STN or not.

Recordings denoted as LFPx are broadband signals of local field potentials. The method of local field potentials is described in the section 2.6. Recordings denoted as MERx are broadband signals from single-unit recordings. The single-unit recording is a method to get action potentials from individual neurons in the brain. [20]

[10]
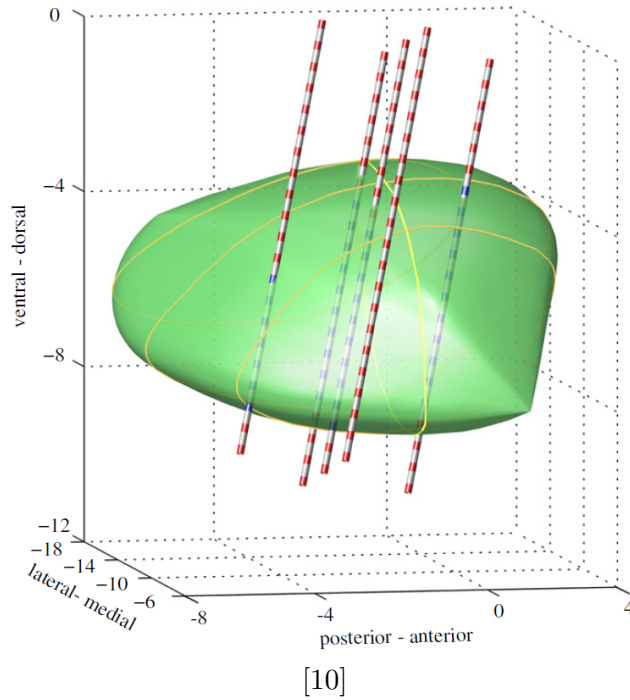
Figure 3.3: Schematic image of microelectrodes (MER) for recording in STN

To prove if there are any responses to the emotion in the STN, we statistically tested the amplitudes of signals after the different types of images were shown. That means that we tested signals after fearful (F) images against signals after happy (H) images, signals after neutral (N) images against signals after fearful (F) images, and signals after neutral (N) images against signals after happy (H) images (Figure 3.4).
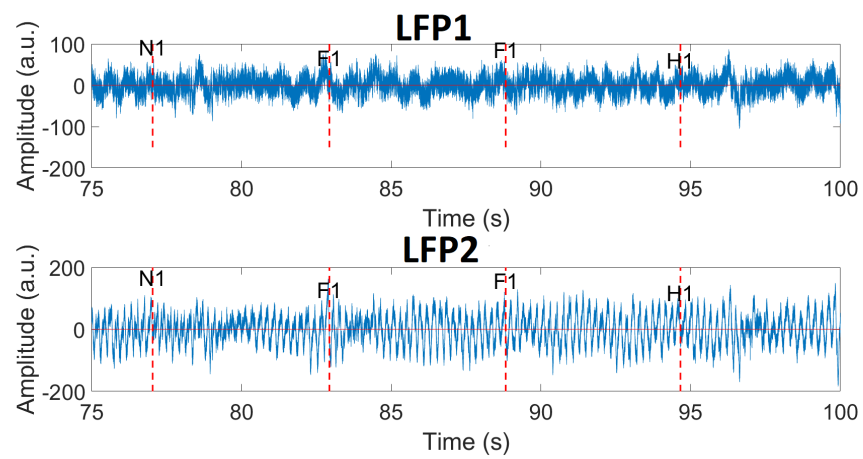
Figure 3.4: Image of the recorded signal with tags from 2 LFPx recordings. Where N1 is tag for neutral image, F1 for fearful image and H1 for happy image.

Chapter 4

Methods

This chapter will briefly introduce all the methods we used for processing the data.

4.1 Frequency domain

In the world around us, it is quite common for artificial and even natural signals to oscillate. From the biological signals, it is, for example, voice. So to obtain more information about them, it is useful to decompose them into sinusoids and learn something about the signal's frequency, phase, and amplitude. The decomposition of the signals is done by Fourier analysis. Because the signals are discrete in this case, the Discrete Fourier Transform is used.

4.1.1 The Discrete Fourier Transform

The Discrete Fourier Transform (DFT) transforms discrete signals from the time domain into the frequency domain. The transformation is made according to the equation 4.1. This equation is called direct Discrete Fourier Transform. The equation 4.2 is called inverse Discrete Fourier Transform and transforms a signal from the frequency domain back to the time domain.

$$X[k] = \sum_{n=0}^{N-1} x(n) e^{-j\frac{2\pi}{N}nk}, k = 0, 1, 2, ..., N - 1, \qquad (4.1)$$

$$x(n) = \frac{1}{N} \sum_{k=0}^{N-1} X[k] e^{j\frac{2\pi}{N}nk}, n = 0, 1, 2, ..., N - 1 \qquad (4.2)$$

In practice, the Fast Fourier Transform (FFT) is used to compute DFT instead of direct calculation from the equation 4.1. This is because FFT is much faster than solving this equation. The main idea behind this algorithm is to decompose one signal with N samples into the N signals of one sample. Then, for each individual signal, its frequency spectrum is computed. The frequency spectrum of signal with one sample is equal to the sample itself, so no system of linear equations needs to be solved. And that is why FFT makes the computing of DFT faster. And to gain the frequency spectrum corresponding to the original N point time-domain signal, it synthesizes the N frequency spectra. It is essential to synthesize them in the same order as they were decomposed. In each step, the part of the signal is divided into two. The first half contains the samples at even positions and the second half samples at odd positions (Figure 4.1).
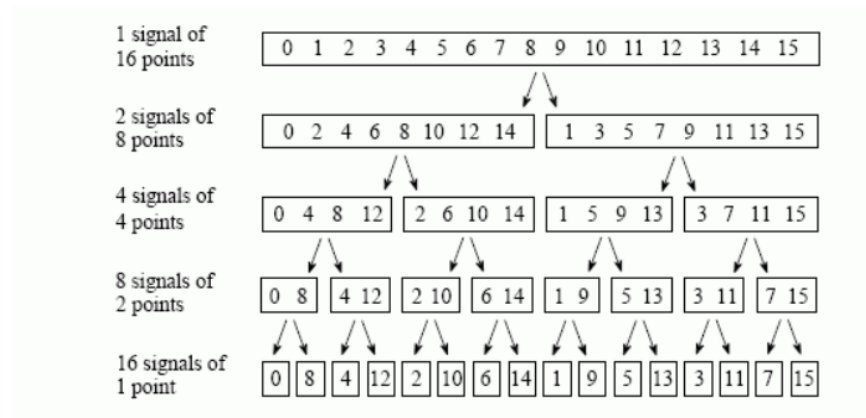
[21]

Figure 4.1: The FFT decomposition. The signal of 16 samples is divided into 16 signals of one samples.

◼ 4.1.2 Spectrogram

The spectrogram shows how the frequency changes during the time. The signal is divided into segments, and for each of them, the spectrum is computed. The

spectrum represents the signal in the frequency domain. Then these spectra are placed side by side to describe the development in the time domain.

In the frequency spectrum, the frequency resolution depends on the duration of the signal. So to have a better frequency resolution, we can extend the signal with zeros. Zeros do not change the shape of the spectrum; they only change the length of the signal to create more samples in the frequency domain.

Another problem is when the signal is not entirely periodic. That means it contains a sinusoid with a frequency different from the basis function. This will be reflected in the spectrum as a peak with tails that expand it. The signal is multiplied by a Hamming window before DFT to solve this (Figure 4.2). On the other hand, the usage of the Hamming window reduces the resolution of the spectrum.
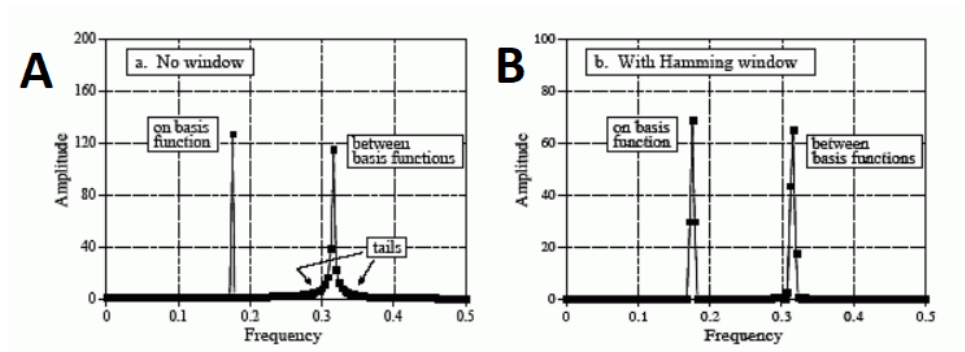
[21]

Figure 4.2: Spectral analysis of a signal consisting of two sine wave. One have a frequency equal to a basis function, the second does not. (A) is without using Hamming window, (B) with.

The spectrogram of the signal is created in several steps:

1. Divide the signal into segments

2. Extend each segment of the signal with zeros

3. Multiply each segment of the signal with the Hamming window

4. Computed DFT for each segment

[21]
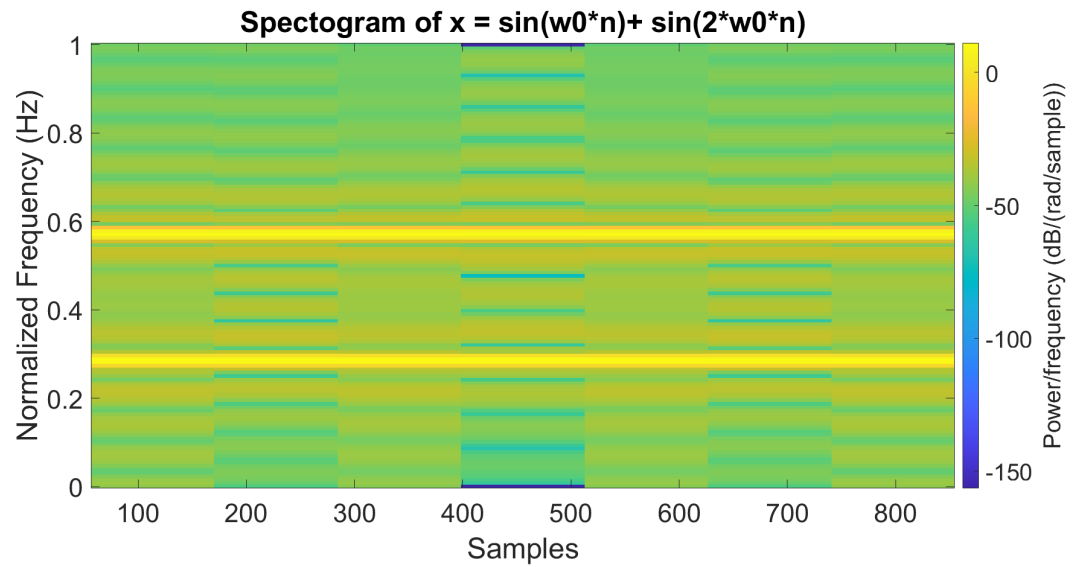
Figure 4.3: Spectogram of the signal x = $\sin(\frac{2\pi}{7}\mathrm{n}) + \sin(\frac{4\pi}{7}\mathrm{n})$

4.2 Filters

The main objective of the filter is to suppress the determined frequency component and pass the others. Using that, we can, for example, remove the noise from the signal so it cannot distort the result of the following signal processing.

One of the most used filters is a linear time-invariant filter (LTI). A Linear filter means that the principle of superposition applies, so the sum of the system's responses to a part of the signal is equal to an overall system's response. And when the system is time-invariant, the delay of the input causes the same delay in the system's output.

Filters can be described based on the responses to the unit sample sequence, also known as the impulse response. Unit sample sequence is defined as 4.3. And the response of the system can be computed as a convolution of the input and the response of the filter to the unit sample sequence - using formula 4.4. [22]

$$\delta[n] = \begin{cases} 0, n \neq 0 \\ 1, n = 0 \end{cases} \tag{4.3}$$

24

$$y(t) = \int\limits_{\infty}^{-\infty} u(\tau)h(t-\tau)d(\tau) \tag{4.4}$$

◼ **4.2.1** **Classification of filters**

Filters can be classified by the duration of their response to the unit sample sequence into two groups: filters with finite impulse response and with infinite impulse response.

A filter with finite impulse response (FIR) is stable in contrast to a filter with infinite impulse response (IIR), so its design is easier. Their stability is caused by settling their impulse response to zero in finite time. Another parameter that describes the filter is the filter's order, which indicates the filter's steepness. The filter's steepness directly impacts how the filter muffles the required frequencies. The bigger the steepness, the bigger the filter efficiency. The filter steepness is related to the impulse response duration, so as indicated from the name of the filter, IIR muffles better than FIR with the same order.

Another way to classify filters is by which frequencies they pass. There are four main types (Figure 4.4):

1. Low pass filter - pass lower frequencies and suppress the higher ones.

2. High pass filter - pass higher frequencies and suppress the lower ones.

3. Band-pass filter - pass interval of frequencies, which means that frequencies lower or higher than the interval are suppressed.

4. Band-stop filter - suppress interval of the frequencies, so the lower and higher frequencies are passed.

[23]

25
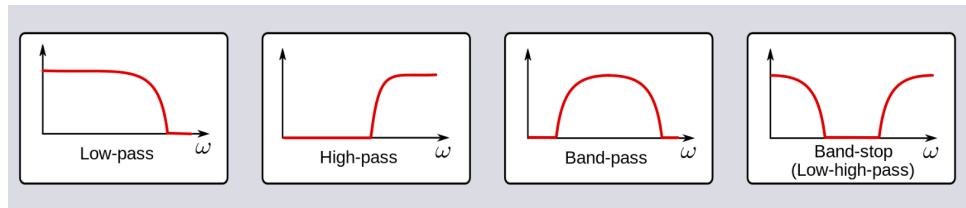
[24]

Figure 4.4: Filter response for different types of real filters. From left to right: low-pass, high-pass, band-pass and band-stop filter

■ 4.2.2 Application of filters

In our data, it was important to remove the noise caused by electromagnetic interference, which affects the signal during the whole measurement. We use a comb filter (Figure 4.5), an FIR filter that can filter 50 Hz and its multiples.[25]. But because it's an FIR filter and the sampling frequency is high, its order must be high too to achieve the required steepness of the filter. So it takes a long time to compute it.
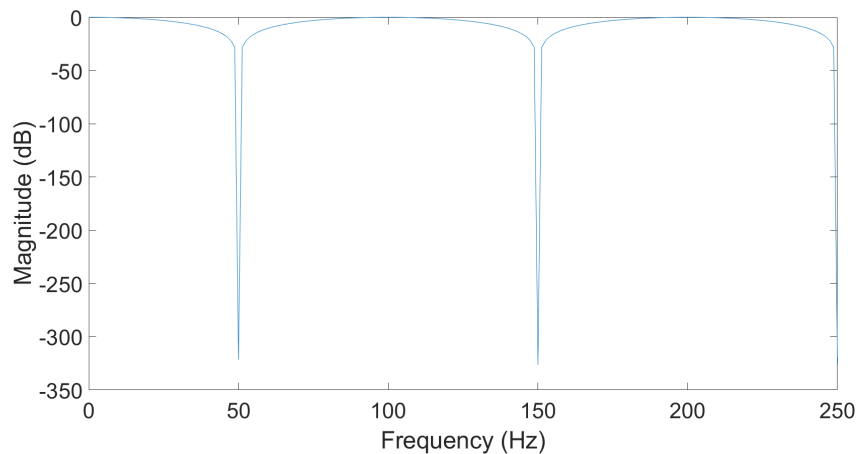
Figure 4.5: Frequency response of comb filter

The same problem with the filter order is in our second filter application, where we use a high pass filter to supress the noise in the low frequencies.

4.3 Resampling

When we record a continuous signal, we get its representation in a discrete time. So we have a sequence of samples. How many samples we can record in one second is given by the sampling frequency. The sampling frequency is also called the sampling rate. The sampling frequency is not a fixed value; we can manually change it - increase it or decrease it by an integer factor.

4.3.1 Decimation

Decimation or also downsampling is the reduction of the sampling frequency by an integer factor. So our sampling frequency will be lower, and we will have fewer signal samples in one second. For example, if we want to reduce the sampling rate twice, we will take every other sample from the original signal. So, in the end, we will have half of the samples for the same time interval (Figure 4.6).
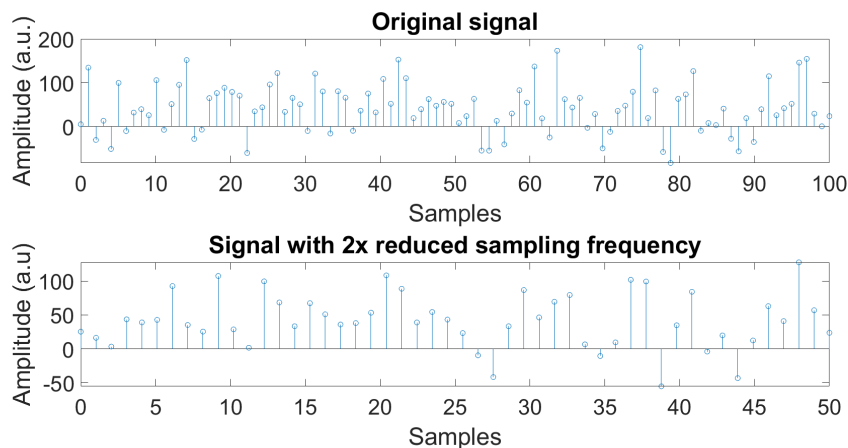
Figure 4.6: Decimation of the signal by factor 2

However, it is essential to comply with the Nyquist theorem 4.1.

Theorem 4.1. *For reconstructing a periodic signal from its samples, the signal sampling rate must be at least twice its highest frequency component.*

According to the theorem, the sampling frequency can be reduced to a maximum of twice the highest frequency signal component. Otherwise, aliasing occurs.

27

Aliasing (Figure 4.7) is an overlap in the signal's frequency spectrum, so it loses some information, and the original signal cannot be restored. We can first reduce the frequency range to avoid it, so after decimation, the new sampling frequency compiles with the Nyquist theorem. [22]

[26]
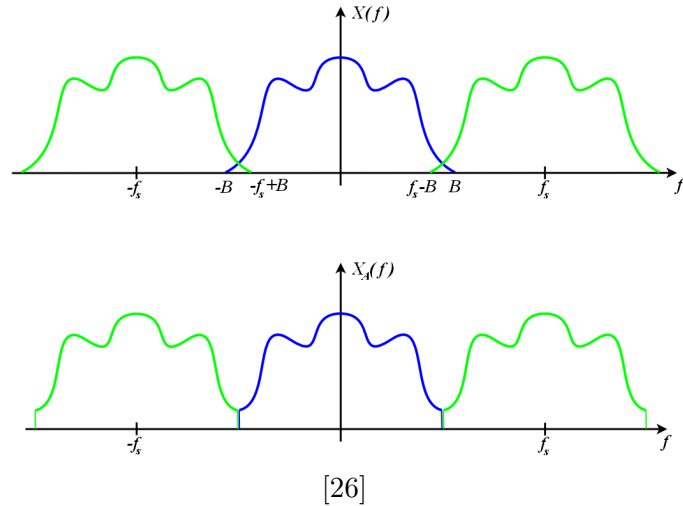
Figure 4.7: An example of aliased spectrum

■ **4.3.2 Interpolation**

Interpolation or also upsampling is increasing the sampling rate by an integer factor. In interpolation, we insert zeros between signal samples, so we enhance the amount of the samples in one second, and due to it, we increase the sampling frequency. The increase factor depends on how many zeros we insert between signal samples. For example, if we want to increase the sampling rate twice, we insert one zero between each signal sample (Figure 4.8). After inserting the zeros, we have to use a lowpass filter to get the exact shape of the signal in the time domain [22].

■ **4.4 WaveClus**

WaveClus is an algorithm that can identify the action potentials of individual neurons in the signals. Action potentials, or in different words, spikes, rise due to electrical neuronal activity. Moreover, WaveClus is not only for unsupervised spike detection from recordings but also for their sorting, making it a very powerful tool for processing neuronal signals.
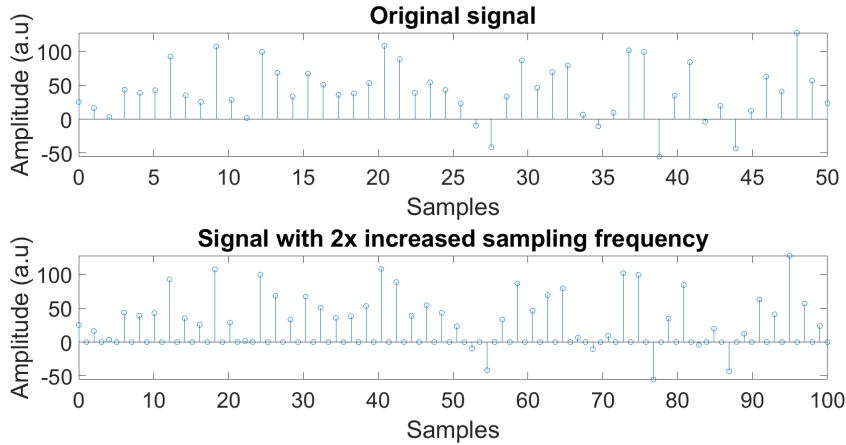
Figure 4.8: Interpolation of the signal by factor 2

This method has three stages (Figure 4.9):

1. Detection of the spikes

2. Selection of spike features

3. Clustering

The spike detection is done by automatic amplitude thresholding. It is used after the signal is filtered by a bandpass filter at 300 - 6000 Hz. The thresholding runs automatically, so the spike detection threshold is set according to the equation 4.5.

$$Thr = 4 \cdot median\{\frac{|x|}{0.6745}\} \tag{4.5}$$

Where x is the filtered signal, and $median\{\frac{|x|}{0.6745}\}$ is an estimation of the background noise.

In the second stage, it is needed to compute the wavelet transformation of the signals. Wavelet transformation is a time-frequency representation of the signal. Sixty-four wavelet coefficients for describing one spike are obtained from this method. To select the spike features, it needs to choose a few wavelet coefficients that best describe the spike and distinguish it from others. That is done automatically by the Lilliefors modification of a Kolmogorov-Smirnov test for normality. The WaveClus algorithm applies ten wavelet coefficients with the most significant distinction from normality.
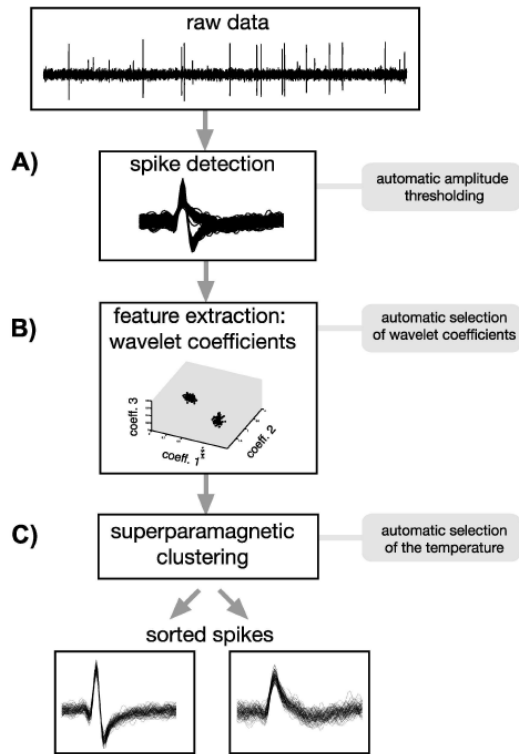
[27]

Figure 4.9: Overview of the automatic clustering procedure. (A) spikes are detected by setting an amplitude threshold. (B) A set of wavelet coefficients representing the relevant features of the spikes is selected. (C) The SPC algorithm is used to cluster the spikes automatically.
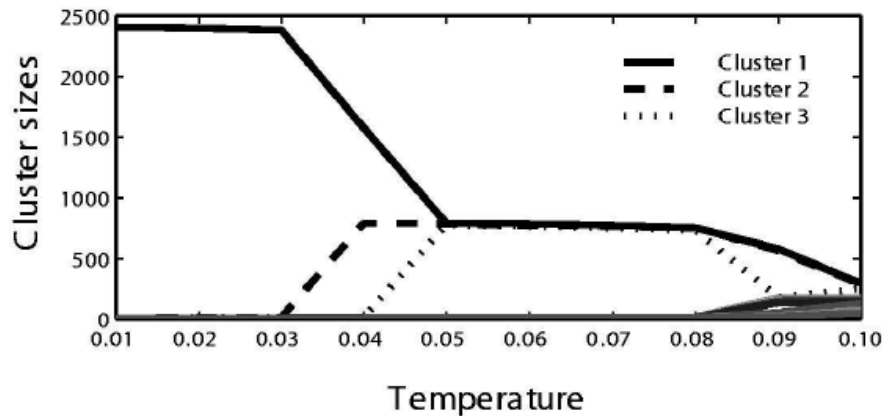
[27]

Figure 4.10: Cluster size vs. temperature. At temperature 0.05, the occurring of the transition to the superparamagnetic phase can be seen and then the three clusters are separated.

The last step - clustering, is done by superparamagnetic clustering (SPC). SPC is inspired by spin glass. Spins are dependent on temperature. If the temperature is high, the spins switch randomly. But as the temperature gets lower and lower, the spins get grouped, and in these groups, the state will change simultaneously. This phase is called 'superparamagnetic.' In low temperatures, all spins are in one group and shift their states together. Similarly, it works in SPC. It tries to find a temperature that describes the superparamagnetic phase, so groups of points changing together will describe the clusters (Figure 4.10). This approach is also automatic because SPC can set the temperature by itself. [27]

4.4.1 The usage of WaveClus algorithm on the processed data

In our task, we use the WaveClus algorithm to expose spikes in MER signals. We want to focus on the activity of the distant neurons, so we want to remove the action potentials of the neurons close to the electrodes. And to achieve that, we use this algorithm to find the spikes, sort them, and gain the time when they are active. After that, we compute the median shape of spikes for each detected neuron to deduct it from the signal at the time when they occur there (Figure 4.11).
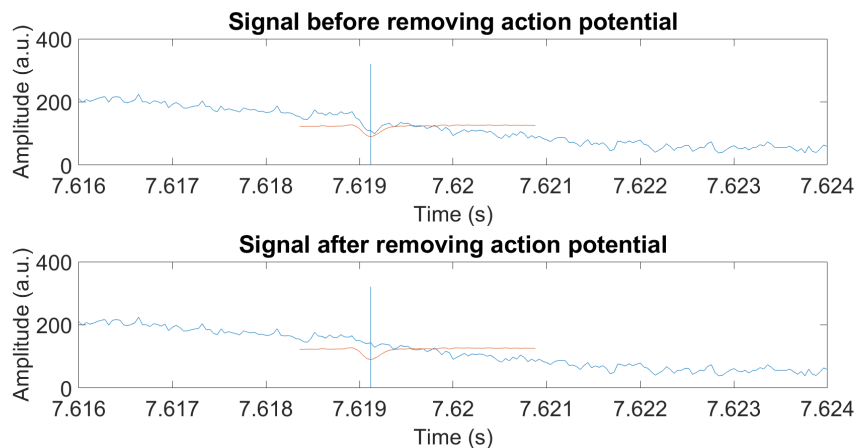
Figure 4.11: Example of removing action potential from the signal. The median of the action potential is shown in red.

■ 4.5 Hypothesis testing

The most important part of hypothesis testing is choosing the hypothesis we want to test. This hypothesis is called the null hypothesis, and it is tested against the alternative hypothesis. For example, we want to know if we should stop distributing the medicine based on its side effects. In this case, the null hypothesis is that this medicine does not increase the risk, and the alternative hypothesis is the opposite - the medicament is increasing the risk. [28]

But the results of hypothesis testing are not always correct. In fact, there can be two types of errors. A type I error occurs when we reject the null hypothesis, which is actually correct. Conversely, a type II error occurs when we accept the null hypothesis when it is not valid.

The main idea of the testing is that we try to figure out if there is sufficient evidence to reject our hypothesis. If we do not find it, the null hypothesis is accepted. Also, in hypothesis testing, the significance level must be chosen. The significance level is a number from zero to one and is denoted as alpha. The smaller the significant level is, the stronger evidence we need to have for rejecting the null hypothesis. The significance level also expresses the probability of a type I error. [29]

■ 4.5.1 P-value

Another important thing in hypotheses testing is a p-value.

Theorem 4.2. *P-value is the probability under the null hypothesis of obtaining results at least as extreme as, or more extreme than, the observed value*

P-value can be between zero and one. And we can also say that p-values show us how strong evidence against the null hypothesis is in the data - the closer the p-value is to zero, the stronger the evidence against the null hypothesis. Based on the p-value, we can decide if we reject or accept the null hypothesis by comparing it to the chosen significance level. We reject the null hypothesis when the p-value is lower than the significance level. [29]

■ 4.5.2 The t-test

T-test, also called Student t-test, is one of the possible approaches to test statistical hypotheses.

■ The t-test for one sample

The conditions in which we can use the t-test:

1. The data is from a normal distribution

2. The variance of the distribution is unknown

In the t-test, we test if the mean μ of the distribution with n samples is equal to the fixed mean μ_0. So we get a random variable T which has a t-distribution with $n-1$ degrees of freedom and is equal to 4.6

$$T = \frac{\sqrt{n}(\bar{X}_n - \mu_0)}{S_n} \tag{4.6}$$

Where samples are $(X_1, ..., X_n) \sim N(m, s^2)$, S_n is the sample variance of the samples $(X_1, ...X_n)$, μ_0 is the fixed mean, and $\bar{X}_n$ is the sample mean of the samples $(X_1, ...X_n)$.

■ The t-test for two samples

Another possible usage of the t-test is to compare two normal distributions again when the distribution variances are not known. In this case, we test if the mean of the first distribution (with m samples) is equal to the mean of the second distribution (with n samples). Here we get a random variable T which has a t-distribution with $m + n - 2$ degrees of freedom and is equal to 4.7.

$$T = \frac{\bar{X}_m - \bar{Y}_n}{S\sqrt{\frac{1}{m} + \frac{1}{n}}} = \frac{\frac{\bar{X}_m - \bar{Y}_n}{\sigma\sqrt{\frac{1}{m} + \frac{1}{n}}}}{\sqrt{\frac{S^2}{\sigma^2}}} \tag{4.7}$$

Where there are two independent groups of samples: $(X_1, ..., X_m) \sim N(m_1, s^2)$ and $(Y_1, ..., Y_n) \sim N(m_2, s^2)$, and $\bar{X}_m$ is the sample mean of the samples $(X_1, ..., X_m)$ and $\bar{Y}_n$ is the sample mean of the samples $(Y_1, ..., Y_n)$. S is a sample estimation of s^2, and it is computed according to equation 4.8.

$$S = \sqrt{\frac{(m-1)S_X^2 + (n-1)S_Y^2}{m + n - 2}} \tag{4.8}$$

Where S_X^2 and S_Y^2 are two estimations of the same value s^2, but S_X^2 is the sample variance of the samples $(X_1, ..., X_m)$ and S_Y^2 is the sample variance of the samples $(Y_1, ..., Y_n)$.

[28]

■ The usage of the t-test on the processed data

My null hypothesis was that the signals after two different types of images (for example, after neutral and happy images) come from the same distribution. This means that there were no specific reactions to different emotions. And the alternative hypothesis is the opposite - that the signals come from two different distributions, and the signals are affected by watching the different types of images. The significance level was chosen to be 5%, so the type I error equals 5%.

Time domain. In the time domain, we used a t-test to compare the signal's amplitudes in each time interval of the significant part of the signal (550 ms when the picture is shown to the patients). If we used it for each time interval, we would have to do 13750 tests because the sampling rate is 25 000 Hz.

But the samples are dependent on the surrounding samples, so the individual results of the t-tests are not independent. Also, if we do so many tests, the probability that at least one of the results is the type I error is high - almost 1. And because of that, to manage the error rate of 5%, we have to lower the significance level of each test, so the sum of them is still 5%. So the level of significance of the each t-test is 0.05/the number of the tests.

If we compare each signal sample, the significance level will be absurdly low. So to avoid this complication, we reduced the number of samples. We divided the signal into 55-time intervals, each with 250 signal samples. Each time interval is represented by only one value computed as an average of the signal samples in the time interval (Figure 4.12). In this case, the level of significance for each t-test was set to $\frac{0.05}{55}$.
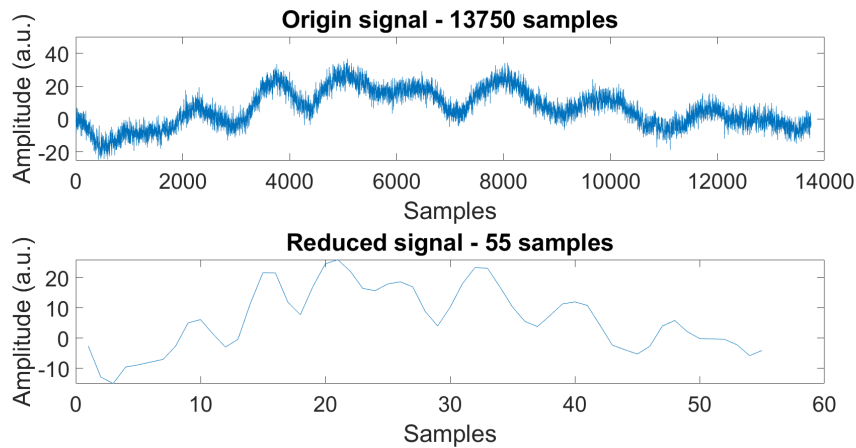
Figure 4.12: Example of reducing number of samples for t-test

Frequency domain.   In the frequency domain, we compare the power spectral density of two signals at the same time and frequency interval. In medical signals, the difference is expected to be in the lower frequencies, so we decided to process just frequencies lower than 200 Hz. Here is a similar problem with the number of tests as in the time domain. We divided the signal into 8 time intervals of the same duration and frequency into 24 intervals. The frequency intervals are not divided uniformly; the higher the frequency gets, the wider the interval is (Figure 4.13). So, in the end, 192 t-tests had to be done to compare the dependency of two emotions. The outcomes of the t-tests over neighboring spectrogram segments are not independent because they are based on correlated data. Same as in the time domain, to manage the significance level under 5%, we have to set the significance level for each t-test as 0.05/all the t-tests done at one signal. So, in this case, it is $\frac{0.05}{192}$.

■ 4.5.3   The bootstrap

Bootstrap is a powerful statistical tool that has extensive applications. For example, it can be used for determining variances and standard errors of random variables from distributions that are not fully known. And among other uses, it can be used for hypothesis testing.
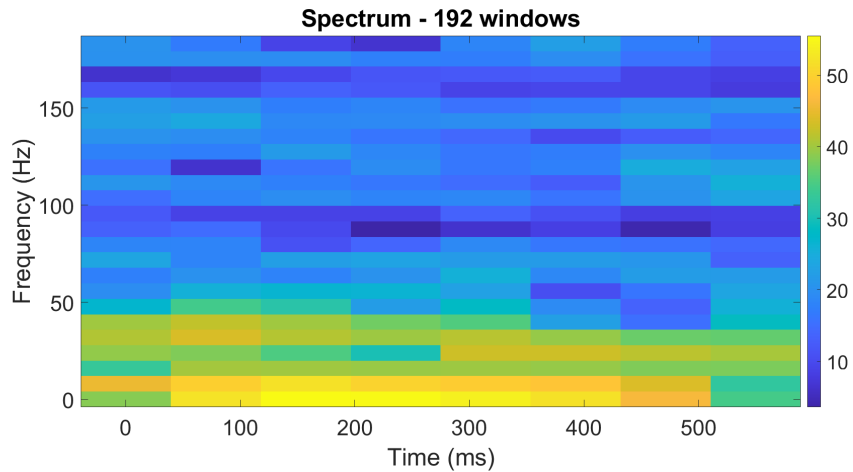
Figure 4.13: Example of reduced spectrogram

■ The bootstrap principle

The bootstrap is one of resampling methods, which means that its core principle is multiple repetitions of simulated replications with replacements.Or in different words, the simulation means that from all available data, it randomly selects the same amount of samples as the original has. The same sample can be selected several times, while other samples are not selected at all (Figure 4.14). And the process is repeated multiple times (for example, 1000 times).
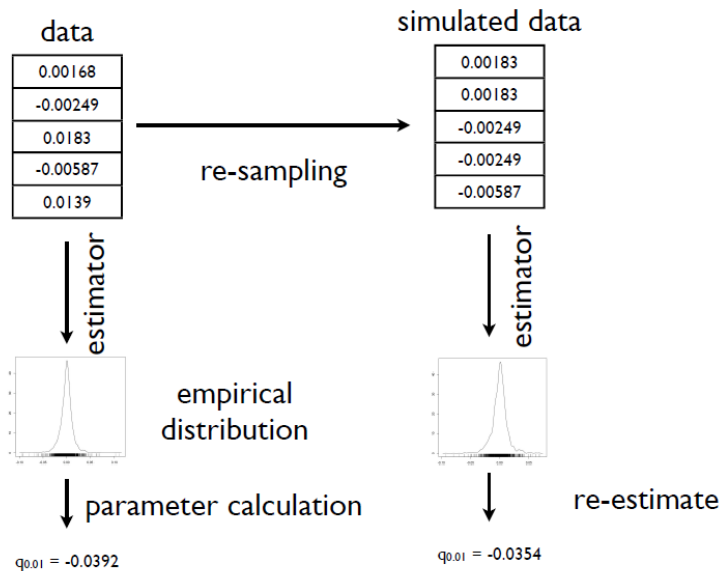
[30]

Figure 4.14: Schematic model of bootstrap resampling

The bootstrap principle for hypothesis testing. In bootstrap hypothesis testing, the null hypothesis is that there is no difference between the two tested groups. The alternative hypothesis is the opposite - the tested groups are different.

It is done in two steps. First, we assume the original data as it was measured. Some chosen statistic is computed from them. For example, if we have two groups of samples, the difference between the sample means of these groups can be computed as a statistic.

The second step is data stimulation. The new groups of samples with the same length as the original groups are created. However, the individual samples in the groups are chosen randomly with the replacements from all the samples (samples from both groups). These samples are called bootstrap samples $\tilde{x}$. We count the same statistic for each data simulation as with the origin data. For example, if the difference between the sample means of groups is used as a statistic for the original data, it is also used for the simulated data. This step is repeated multiple times, and we count the chosen statistic from each resampling. The number of times we repeated the data stimulation is labeled as B.

We need to calculate the p-values from the bootstrap samples for hypothesis testing. P-value can be computed as the percentage of the bootstrap samples which are bigger or equal to the statistic determined for the given data.

$$p = \frac{\#\{T(\tilde{x}_i) \geq T(x)\}}{B} \tag{4.9}$$

However, if the p-value is computed according to the formula 4.9, it can happen that the p-value will be zero, which is very unlikely to happen in reality. The p-value equal to zero is a problem because that gives us strong evidence for the alternative hypothesis, although the p-value could represent a noise. So considering this fact, it is better to count the p-value using formula 4.10, where the p-value cannot be zero and avoid this problem.

$$p = \frac{\#\{T(\tilde{x}_i) \geq T(x)\} + 1}{B + 1} \tag{4.10}$$

[31]

■ Implementing

The signal is tested as a whole using the bootstrap; it is not divided into parts like in the t-test. So there is no problem with the low significance level, and the significance level can be set directly to 5%. Before using bootstrap, we must decide which statistic we will use to evaluate the data.

Time domain. Several approaches can be used to compute the statistic in the time domain.

Maximum difference. It is probably the most straightforward approach. For example, consider that we will evaluate statistical dependecy of signals which occurs after neutral and happy images. The maximum difference between them is the biggest distance between the signal amplitudes of neutral and happy photos (Figure 4.15). This statistic is simple, but it is not robust to noise. If there is an artifact, it could give us wrong results. That is the reason why we did not use this method.
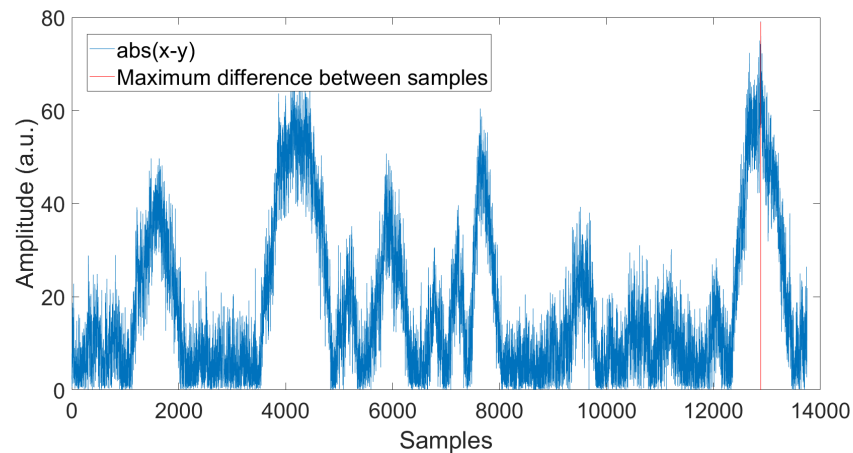
Figure 4.15: Example of maximum difference of the absolute difference of two signals

Maximum area under the curve. Another possible approach is not that straightforward, but it protects us from mistakes caused by noise. Again we consider evaluating the statistical dependence of the signals after happy and neutral images. So we have many signals of both types. First, we will compute an average signal, one for happy photos and one for neutral photos. After that, we subtract them from each other, so we get their difference. And we look at the maximum area between this curve and zero. However, the parts of the signals oscillating rapidly around zero are redundant and

can cause mistakes in the results, so we decimated the sampling frequency down by 100, so from 25 000 Hz to 250 Hz. After the decimation, we gained smoothed signal (Figure 4.16).

Counting the area under the curve is more robust to the artifacts because we use the sample mean of the signal. That is why we choose the maximum under the curve as the statistic.
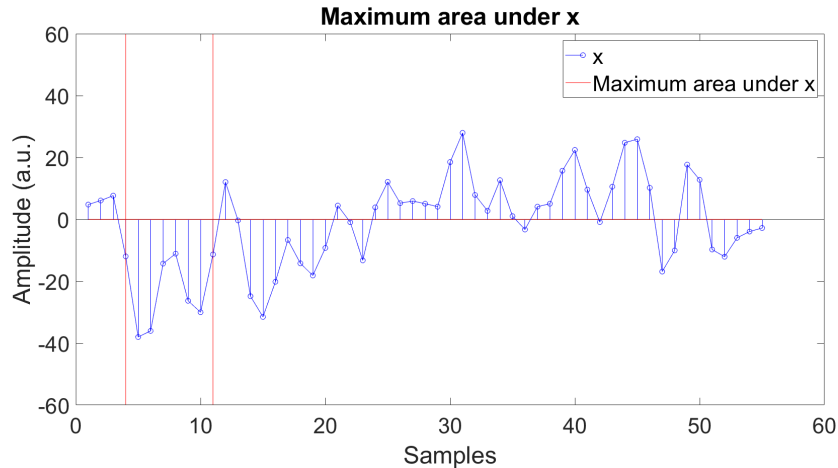
Figure 4.16: Example of maximum area under the signal

Frequency domain.   In the frequency domain, we used a similar approach as in the time domain to count the statistic. However, the spectral volume changes not just in time but also in frequency, it is moved to the 3D. So we comute the maximum volume under the area. We gain this area as a difference between the average spectrogram after one type of image and the average spectrogram after a different kind of image (Figure 4.17). The average spectrogram means that, firstly, the significant parts of the signal (550 ms after images of one type occur) are found in one recording. Secondly, the spectrogram is computed for all these signals. And lastly, the average spectrogram from all of these spectrograms is computed. It is more accurate than computing the average of the signals in the time domain and then converting it into the frequency domain.

■ 4.5.4   The chi-square test

The chi-square (χ^2) test examines if properties depend on each other (for example, if the hair color is related to the color of eyes). Usually, the data are in a contingency table. The contingency table shows the distribution of the
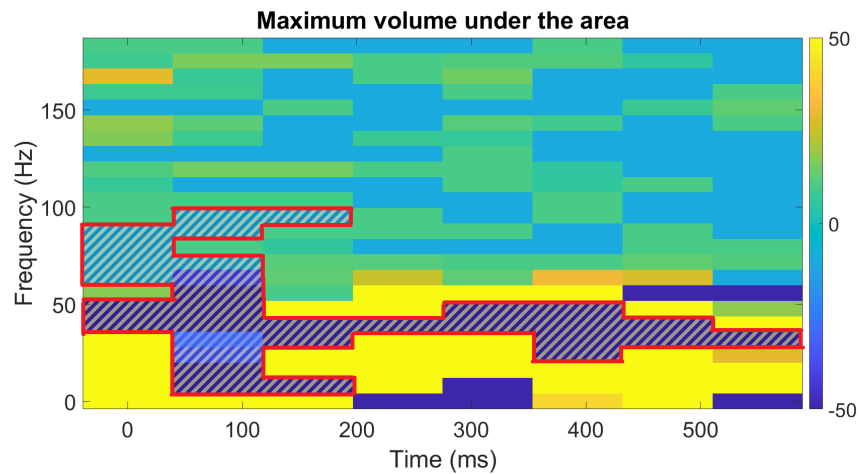
Figure 4.17: Example of maximum volume under the area - maxumum volume under the area is denoted with red.

observed results. The null hypothesis is that the values in different groups are the same, and their difference is caused by a chance. The alternative hypothesis is the opposite.

The chi-square test is based on comparing the observed values and the expected values if the groups were independent of each other. The most significant advantage of this approach is that it can be used even if the contingency table has more than two columns and rows. For using it, every value in the contingency table must be more than 5. [32]

◼ 4.5.5 The Fisher's Exact test

The Fisher's Exact test does not rely on assumptions about the distribution. Instead, it computes the probability of the observed data.

The Fisher's Exact test has similar usage as the Chi-square test, but in contrast to the Chi-square test, it does not require every value in the contingency table to be higher than 5 [32]. In this thesis, it is used only when the contingency table has two rows and two columns because the implementation of the Fisher Exact test in Matlab cannot work with bigger contingency tables.

Chapter 5

Results

In this chapter, we describe the results and how we got them. The first section describes preprocessing of the signals. And the second section shows the results of processing LFPx and MERx signals - in the time domain and then in the frequency domain.

5.1   Preprocessing

Before the recordings can be processed, we have to deal with interference and artifacts that could appear there. An artifact is a sample with significantly higher amplitude than the majority of the samples in the signal.

The first problem is an electromagnetic interference that affects the signal during the whole measurement. We use the comb filter to solve it (Figure 5.1). The comb filter is described in the section 4.2.2.

The second problem is that signals are mostly a mixture of the actual signals and artifacts. And biological signals are not an exception. In microelectrode recordings (MER), artifacts can affect almost 25% of the signal. Mainly, the artifacts are caused by some motion. [18] The motion artifacts arise as a result of, for example, a patient's speaking or slight movement of the electrodes in the brain. These artifacts are most apparent at the beginning and end of the recording, when most of the movement occurs.
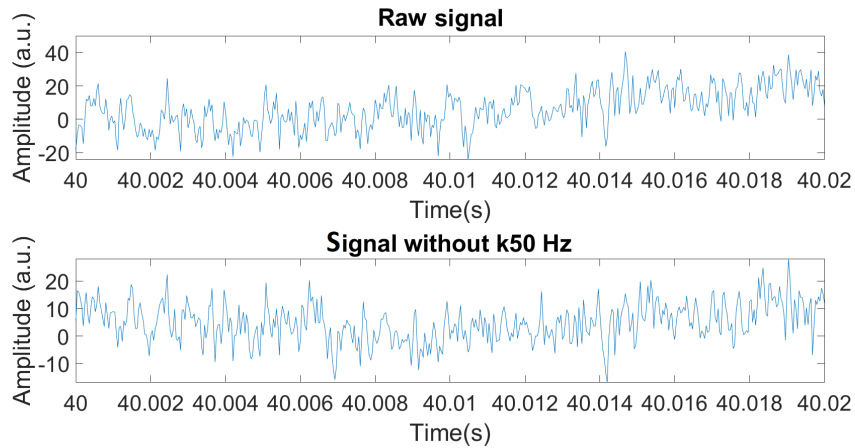
Figure 5.1: Example LFP signal before and after applying the comb filter

Before processing the signals, it is important to check if there were no artifacts. It was essential to remove parts of the signal where the artifacts were found because if we used them in the following calculations, the used statistics could give us wrong results. To prevent this, we plotted all the signals and manually checked them.

■ 5.1.1 LFPx

We set a threshold of the amplitudes for LFP signals, which is already considered an artifact. We checked if there was an amplitude higher than the threshold in each selected part of the signal (550 ms after the tag occurred). If so, we did not include this part of the signal in the following processing.
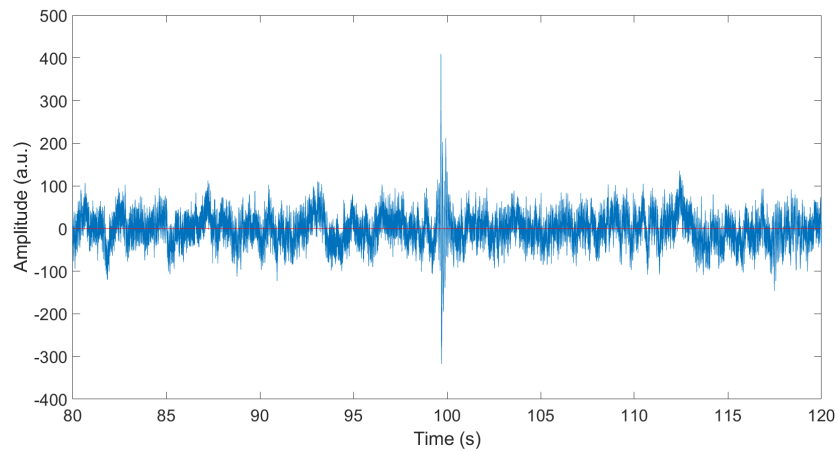
Figure 5.2: Example of LFP signal. An artifact occurs around 100 s mark

We also discovered that some of the signals were contaminated with low-frequency noise (Figure 5.3). This interference could be caused by the tremor of the people who undergo surgery because they are treated with Parkinson's disease. The measured voltage is relatively small, so the electrodes must be sensitive enough to catch the signals. Also, the wire between the microelectrode and the amplifier is not shielded, so the noise can be easily induced into the signal.
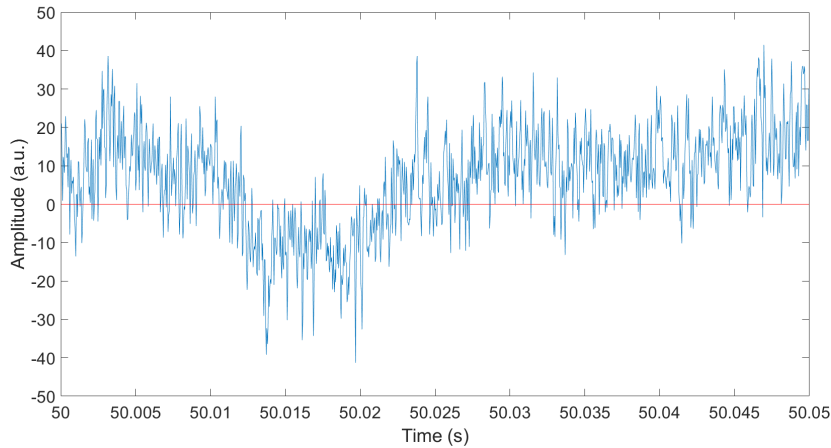
Figure 5.3: Example of LFP signal with low-frequency noise

To remove the low-frequency noise, we used a high-pass filter (Figure 5.4), which suppresses frequencies lower than 5 Hz (Figure 5.5). But neural activity, which we are looking for in the signals, could occur at these low frequencies so that we would lose it after filtering the signal. Because of this, we decided to do the statistics twice. First, we used the signal in the original state with the noise and periodicity. Second, we use the high-pass filter to eliminate all noise (but possibly lose important data).
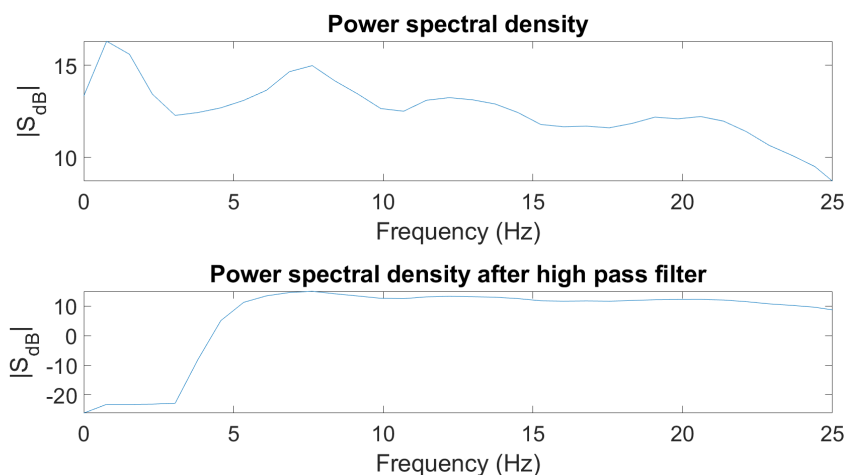
Figure 5.4: Power signal density of the signal before and after using the high pass filter on 5 Hz

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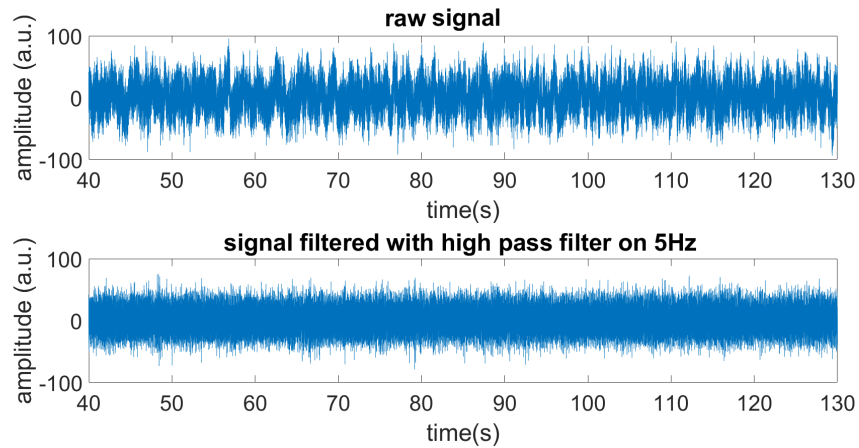
Figure 5.5: LFP signal before and after using the high pass filter on 5 Hz

■ 5.1.2 MERx

We did not manually set the threshold in MER signals. The times when artifacts occurred for every recording were part of the given data. If the part of the signal we were interested in (550 ms after the required tag occurs) is contaminated with the artifact, we remove it from further processing.

■ 5.2 Implementing the methods

The implemented methods were applied to four types of signals:

1. Unfiltered LFP signals - preprocessed LFPx signals

2. Filtered LFP signals - filtered preprocessed LFPx signals with the high pass filter on 5 Hz

3. MER signals with action potentials (AP) - preprocessed MERx signals

4. MER signals without action potentials (AP) - preprocessed MERx signals from which AP from close neurons were removed using the WaveClus algorithm

To examine if there are different reactions based on emotion, we did statistical tests for both time and frequency domains. We compared the

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signal parts of individual neurons after different types of images recorded by electrodes active in STN.
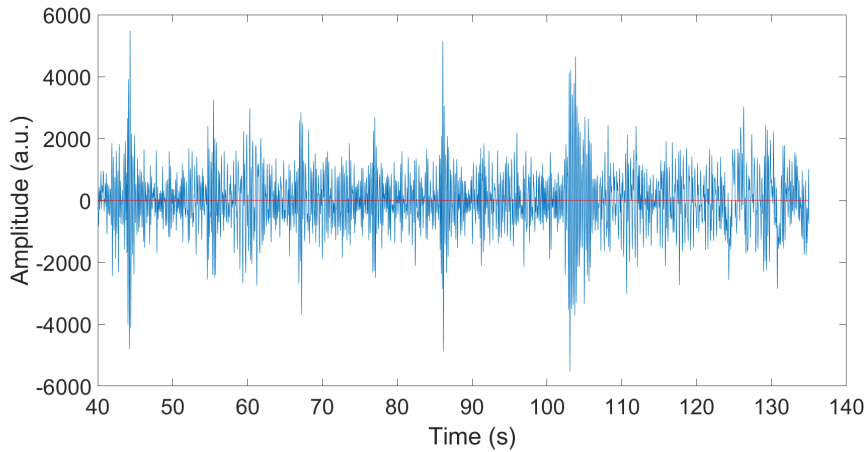
Figure 5.6: Example of MER signal with artifacts

The null hypothesis was that the signal after two different types of images came from the same distribution. This means that there were no spcific reaction to different emotions. And the alternative hypothesis was that the signals come from two different distributions. We were looking for statistical tests in which the p-value was lower than 5%, so we could reject the null hypothesis and assume that the signals are affected by watching the different images. These tests are denoted as significant.

To control the correctness of the statistical methods, we tried two of them - the t-test and the bootstrap. Both methods were used in the time and frequency domain.

Before using bootstrap, the sampling frequency of the signal was decimated. The decimation was done to smooth the signal, so the number of zero-crossing is reduced. Because of it, the area between the curve and zero corresponds to the trend of the signal. The sampling frequency was decreased a hundred times - from $25\,000\,\text{Hz}$ to $250\,\text{Hz}$. So instead of processing 13750 samples ($550\,\text{ms}$ after the image occurs), we processed only 138 samples. The decimation of the number of samples made computing of the bootstrap statistics significantly faster.

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5.2.1 Time domain

The t-test

We divided the signal into 55 intervals, and we used the t-test to compare the signal's amplitudes in each time interval.

The significance level was chosen to be 5%. But because we divided the signal into 55 intervals, to ensure that the significance value remained 5%, we had to set the significance level for each t-test to $\frac{0.05}{55} = 9.09 \cdot 10^{-4}$. So their sum is equal to 5%. Otherwise, the probability that at least one t-test from the total of 55 t-tets gives the type I error would be much higher than the required 5%. So we considered only p-values smaller than $9.09 \cdot 10^{-4}$. A more detailed description of the usage of the t-test in the time domain is in the section 4.5.2.

The results of the t-tests are in the figures 5.7, 5.8. The confidence interval for the signal was computed using the bootstrap. For each bootstrap sample (1000 repetitions), the mean of the signal was computed. After sorting the results, the lower limit was set to 2.5%, and the upper limit was set to 97.5%.

There were 51975 t-tests done (315 recordings $\cdot$ 3 types of combination of the images - NH, NF, FH $\cdot$ 55 p-values from each time interval).

T-tests with p-value lower than the level of significance:

- Unfiltered LFP: 22 t-tests (0.042%)
- Filtered LFP: 48 t-tests (0.092%)
- MER with AP: 16 t-tests (0.031%)
- MER without AP: 16 t-tests (0.031%)
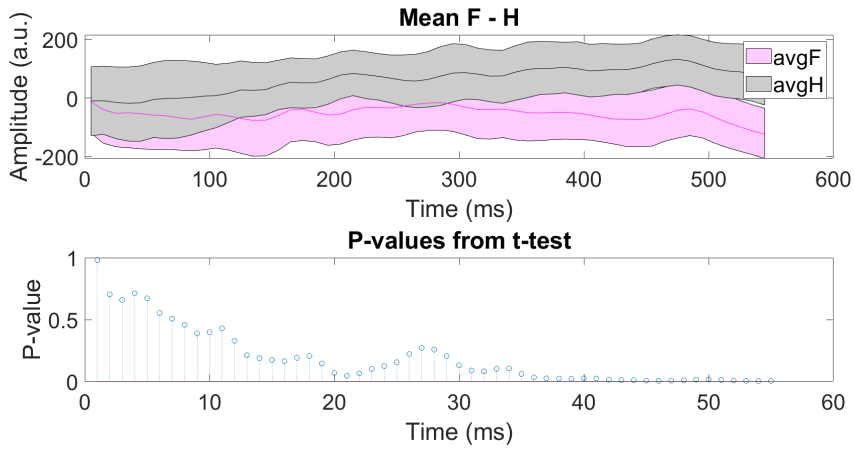
Figure 5.7: Example of the results from t-test on unfiltered LFP signals. The first figure shows an average signal from significant parts of the signal after fear and happy images occur and their confidence interval. And in the second figure, there are p-values for the individual time intervals.
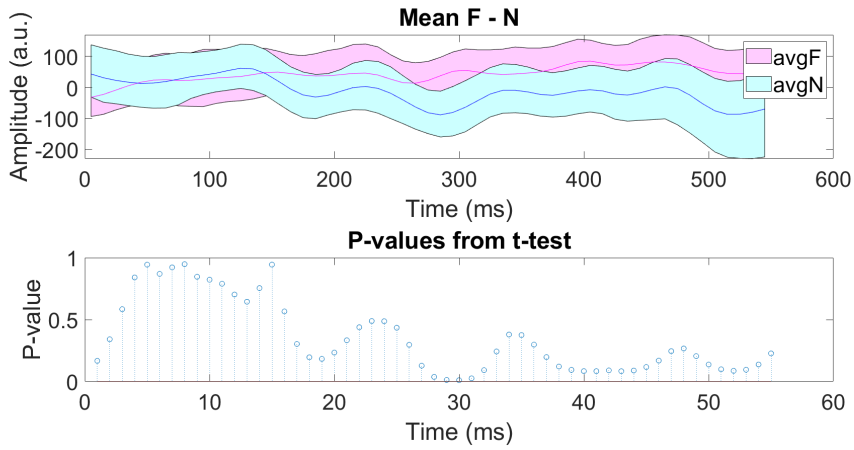
Figure 5.8: Example of the results from t-test on MER signal with AP. The first figure shows the average signal from significant parts of the signal after fear and nautral images occur and their confidence level. And in the second figure, there are p-values for the individual time intervals.

■ The bootstrap

In contrast to the t-test, for the bootstrap testing the signal was not divided into intervals, so we got just one p-value for each test. So the significance level remained 5%, and there was no problem with the low significance level as in t-tests. More information about implementing the bootstrap can be found in the section 4.5.3.

We also checked if the bootstrap results correspond to the t-test results. The p-values from the t-test should be low in the place where the maximum under the curve occurs (used bootstrap statistic). The time when the maximum of the area under the curve occurs is in the images 5.9 5.10 5.11 5.12 denoted with the red curve.

There were 945 bootstrap tests done (315 recordings · 3 types of combination of the images - NH, NF, FH).

Bootstrap tests with lower p-value than the level of significance:

- Unfiltered LFP: 39 tests (4.13%)
- Filtered LFP: 38 tests (4.02%)
- MER with AP: 29 tests (3.07%)
- MER without AP: 34 tests (3.60%)
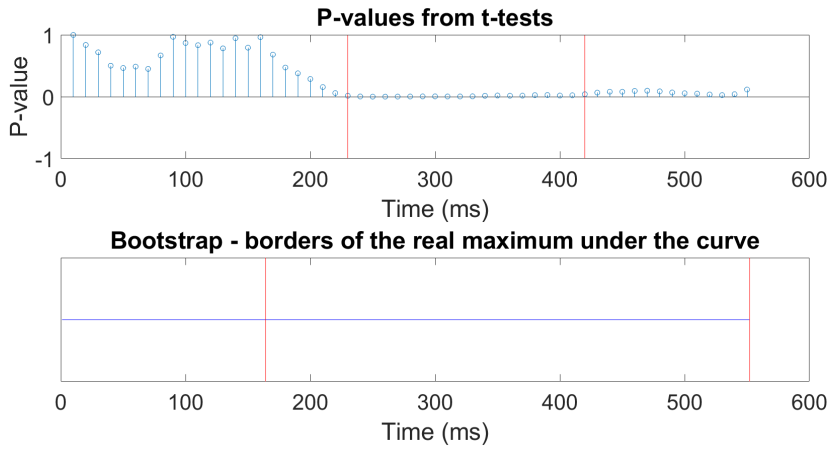
Figure 5.9: Example of verification of the bootstrap results with t-tests results in unfiltered LFP signals. Red curves in the t-test denote the p-values under 5% and red curves in the bootstrap denote the time when the maximum of the area under the curve occurs.
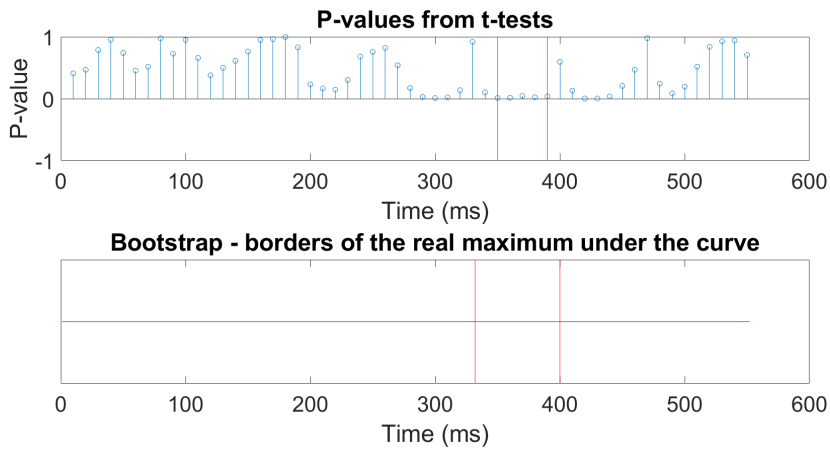
Figure 5.10: Example of verification of the bootstrap results with t-tests results in filtered LFP signals. Red curves in the t-test denote the p-values under 5% and red curves in the bootstrap denote the time when the maximum of the area under the curve occurs.
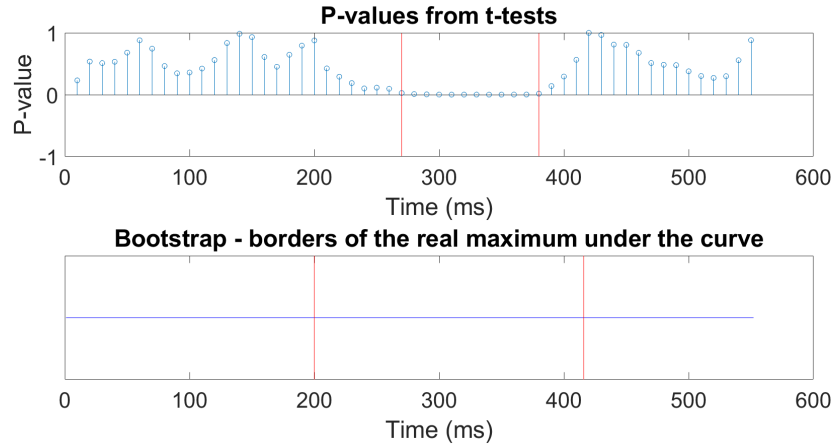
Figure 5.11: Example of verification of the bootstrap results with t-tests results in MER signals with AP. Red curves in the t-test denote the p-values under 5% and red curves in the bootstrap denote the time when the maximum of the area under the curve occurs.
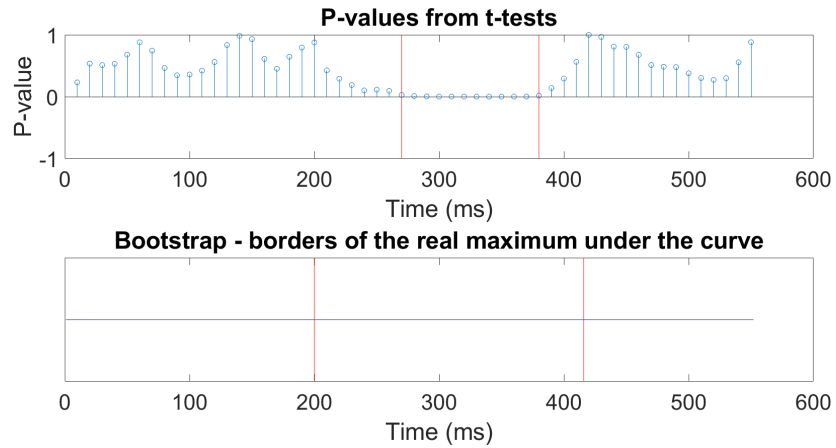
Figure 5.12: Example of verification of the bootstrap results with t-tests results in MER signals without AP. Red curves in the t-test denote the p-values under 5% and red curves in the bootstrap denote the time when the maximum of the area under the curve occurs.

Depth and hemisphere where the electrode is placed

We were also interested if there is some dependence on the depth or hemisphere where the recording electrode is placed. And because the t-test tested only signal intervals, not all signals, we used results from the bootstrap to examine this.

We created a histogram to compare if there were significantly more results that were significant at a specific depth of the recording, as you can see in the figure 5.13 5.14. To verify this dependency, we used the chi-square test at the 5% significance level (we used a downloaded Matlab function chi2cont). But for the chi-square test, all values in the contingency table have to be higher than five, so we had to merge some depths to manage it for significant neurons. The used contingency tables and the resulting p-value can be seen in the tables 5.1 5.2 5.3, 5.4. None of the chi-square tests reject the null hypothesis, so the occurrence of the significant tests does not depend on the depths of the electrodes.

Neurons	Depths of the electrodes					P-value
	(-8,-3>	(-3, -2>	(-2,-1>	(-1, 0>	(0,3>	
Insignificant	166	136	172	219	213	0.3902
Significant	5	5	11	12	6	

Table 5.1: Table of the chi-square test for unfiltered LFP signals in the time domain. Some depths have to be merged for usage of the chi-square test.

Neurons	Depths of the electrodes					P-value
	(-8,-3>	(-3, -2>	(-2,-1>	(-1, 0>	(0,3>	
Insignificant	160	135	178	224	210	0.4092
Significant	11	6	5	7	9	

Table 5.2: Table of the chi-square test for filtered LFP signals in the time domain. Some depths have to be merged for usage of the chi-square test.

Neurons	Depths of the electrodes				P-value
	(-8,-2>	(-2,-1>	(-1, 0>	(0,3>	
Insignificant	304	176	222	214	0.6582
Significant	8	7	9	5	

Table 5.3: Table of the chi-square test for MER signals with AP in the time domain. Some depths have to be merged for usage of the chi-square test.

Neurons	Depths of the electrodes					P-value
	(-8,-3>	(-3, -2>	(-2,-1>	(-1, 0>	(0,3>	
Insignificant	165	135	177	220	214	0.6940
Significant	6	6	6	11	5	

Table 5.4: Table of the chi-square test for MER signals without AP in the time domain. Some depths have to be merged for usage of the chi-square test.
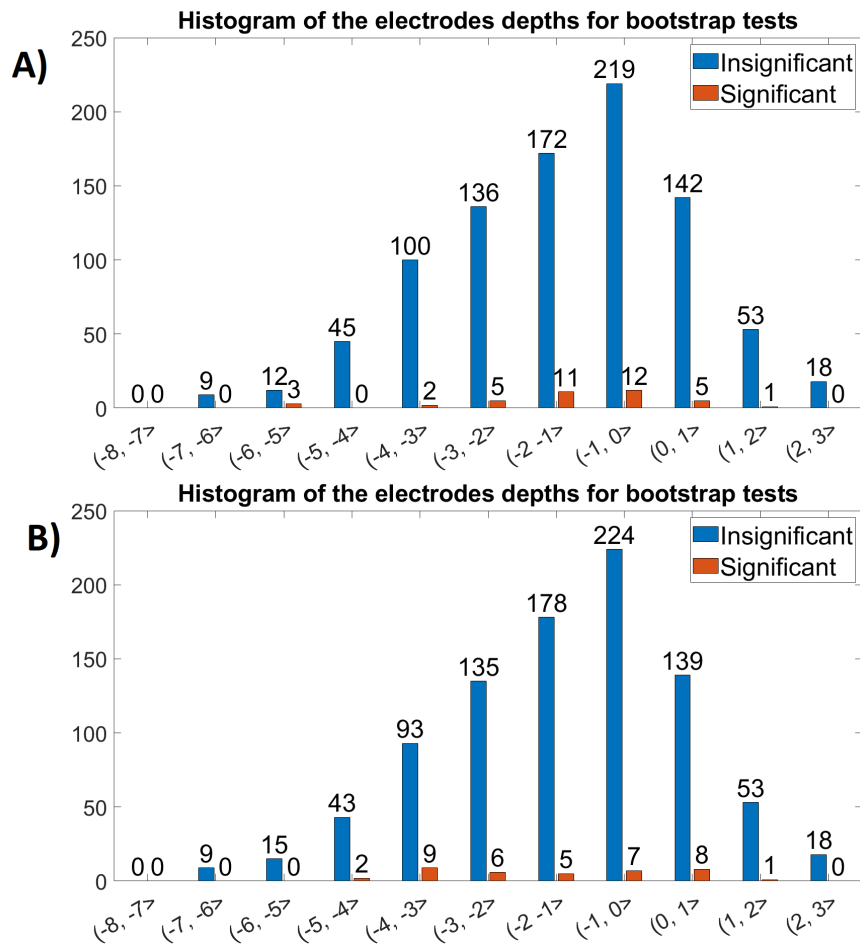
Figure 5.13: Histogram of the electrode depths for (A) unfiltered and (B) filtered LFP signals in the time domain. The depth is measured relative to the target in STN.

We also compare the number of results marked as significant in the left and right hemispheres, as you can see in the figure 5.15 5.16. We used the Fisher's Exact test to decide if significantly more significant tests are in one of the hemispheres. But at the 5% significance level, none of the Fisher's Exact tests reject the null hypothesis, so that means that there are no nonrandom associations between the left and the right hemisphere in none of the signal types. In the table 5.5, you can see the p-values for individual tests.
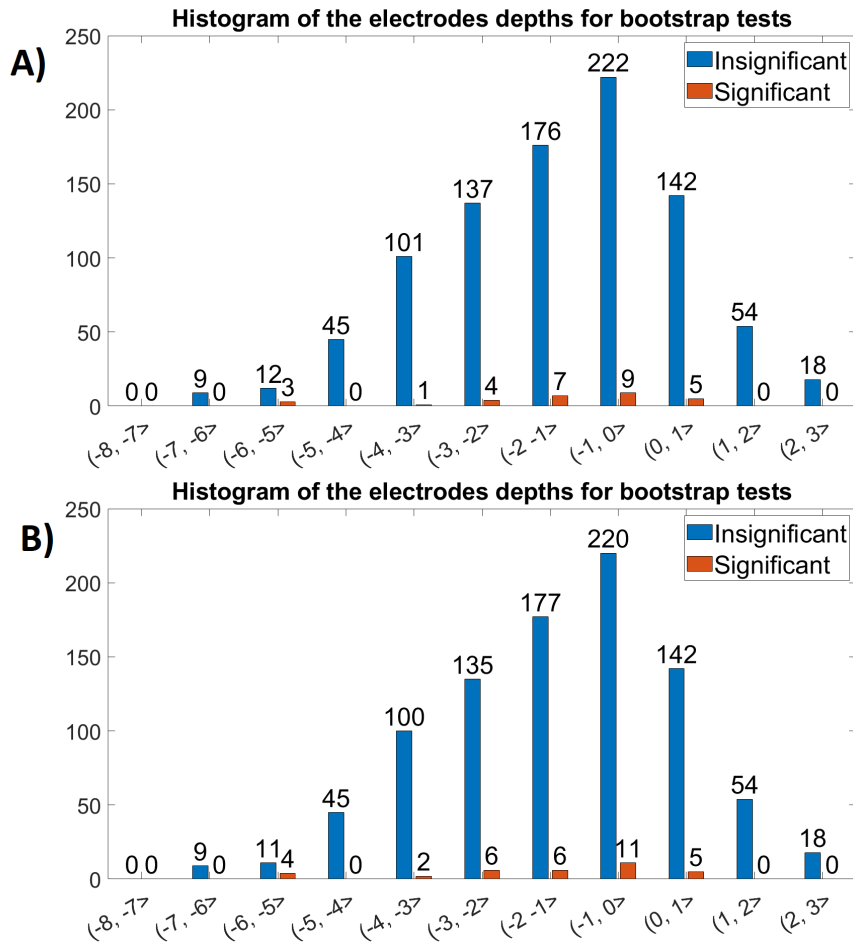
Figure 5.14: Histogram of the electrode depths for MER signals (A) with and (B) without action potentials in the time domain. The depth is measured relative to the target in STN.

Types of the signal	P-value	Decision
unfiltered LFP	1	fails to reject the null hypothesis
filtered LFP	0.0686	fails to reject the null hypothesis
MER with AP	0.8515	fails to reject the null hypothesis
MER without AP	1	fails to reject the null hypothesis

Table 5.5: The results of the Fisher's Exact tests for comparing the hemispheres in the time domain
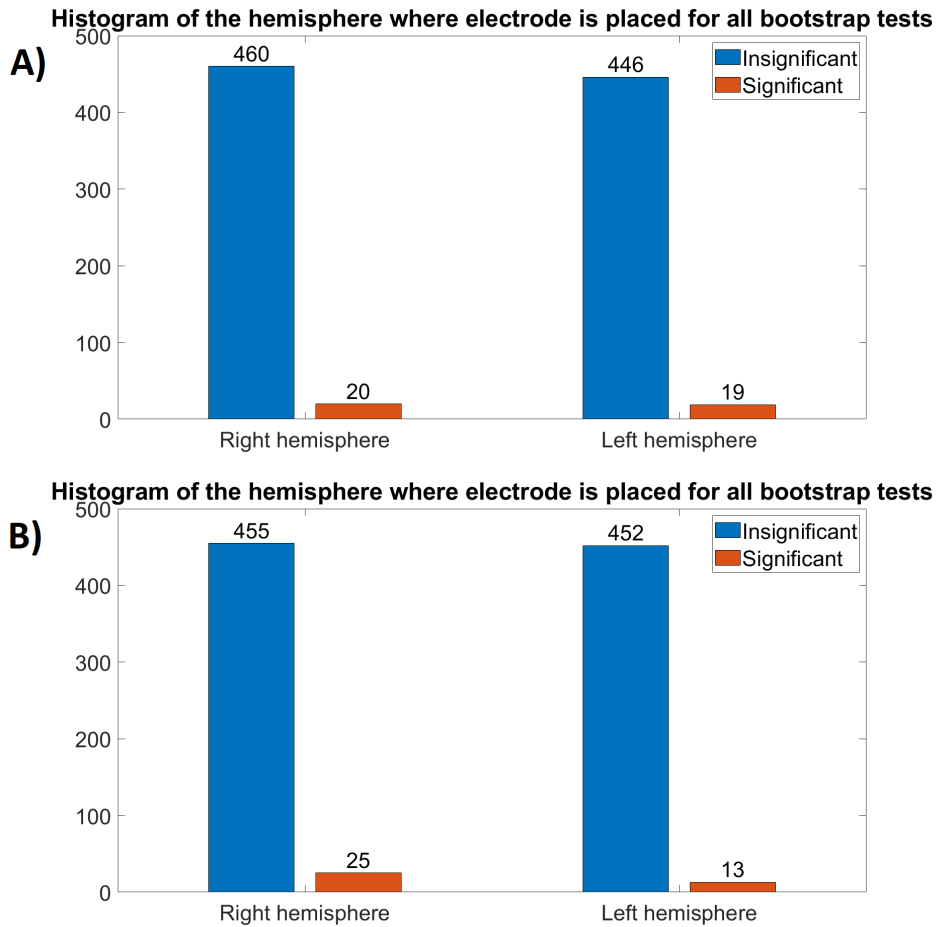
Figure 5.15: Histogram of the hemispheres for (A) unfiltered and (B) filtered LFP signals in the time domain

■ **Summary of the results in the time domain**

The significance level was set to 5% in every test, so we expected that 5% of the result would be the type I error. And because the number of total significant tests in all types of the signal was lower than 5%, it is possible that all of the results were just false positives (so the test found a specific reaction to the different emotions although nothing was there). But we are not able to decide if all significant tests were false positives.

We can divide the bootstrap results by the type of the test, which was denoted as significant. In the table 5.6, there is the percentage of the significant tests from the total of 315 tests for each type of test (neutral-happy, neural - fearful, fearful - happy).

Histogram of the hemisphere where electrode is placed for all bootstrap tests

A)

Histogram of the hemisphere where electrode is placed for all bootstrap tests

B)
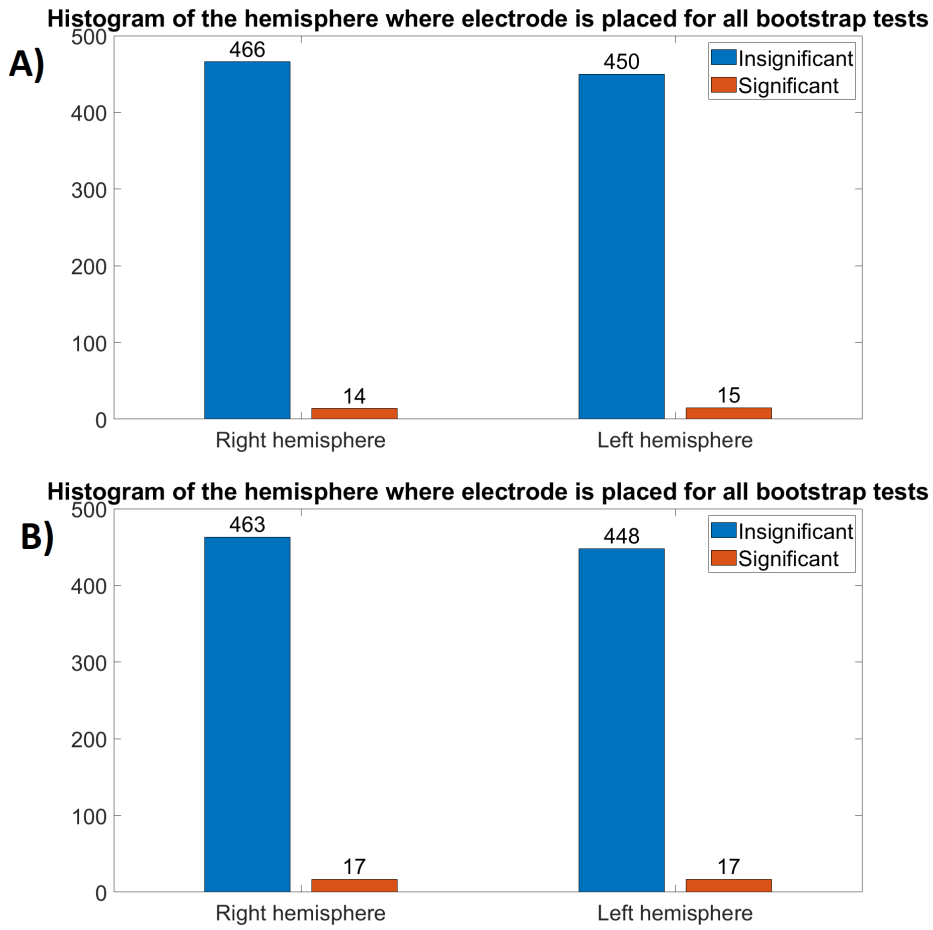
Figure 5.16: Histogram of the hemispheres for MER signals (A) with and (B) without action potentials in the time domain

Types of signals	Time domain		
	NH	**NF**	**FH**
unfiltered LFP	6.03%	3.17%	3.17%
filtered LFP	6.03%	4.13%	1.91%
MER with AP	3.17%	3.17%	2.86%
MER without AP	4.13%	2.85%	3.81%

Table 5.6: Table of significant bootstrap results for individual tests

As you can see in the verification results from the t-test and bootstrap for LFP signals (figure 5.9), the maximum area under the curve is much bigger than the area where the p-values from the t-test are under 5%. That is because the t-test used a more strict significance level than the bootstrap, so it has lower statistical strength.

In the filtered LFP signals, in contrast to unfiltered LFP signals, the area under the curve is smaller as we expected. It is not expected that the reaction to the images will be in the entire duration of the signal but just in some part of it.

In figures 5.17 5.18, the red area denotes the time where the difference between the curve (difference of the mean signals after different images) and the zero (bootstrap statistic) occurs in neurons where bootstrap tests have a lower p-value than 5%. In unfiltered LFP signal, there is a tendency that the maximum occurs more at the end of the signal and there are long intervals. In contrast, the intervals are more random and shorter in the filtered LFP signals. The filtration using a high pass filter can cause the loss of some important neuronal activity, or alternatively, the occurrence at the end of the interval in unfiltered LFP signal can be caused by noise. In MER signals, most of the red intervals are in the middle. In MER signals with AP, red intervals are mostly in the first half, but when action potentials from neurons close to the electrodes are removed, most of the intervals are in the second half. This could be caused by closer neurons that created the difference earlier, but when we removed them, the distant neurons affected it.

It does not seem that the number of significant tests depends on the depth or hemisphere where the recording electrode was placed. Only in the MER signals without AP is p-value under the 5% significance level so we concluded that in this specific case it depend on the depths of the electrode.

We were also interested in whether the reaction to the emotion can be seen in the different types of signals simultaneously. For example, if a test in filtered LFP signals was denoted as significant, the test done in the same position in unfiltered LFP signals would also be denoted as significant, and so on. We used the Fisher's Exact test at the 5% significance level to examine that. And only the bootstrap results were used because t-tests tested only signal intervals, not the whole signals. The results are in tables 5.7 5.8 5.9 5.10 5.11 5.12. However, the only p-value lower than 5% was in MER signals with and without AP. So those signals seem to be dependent.

Times of difference between the curve and zero for significanct neurons

Times of difference between the curve and zero for significanct neurons
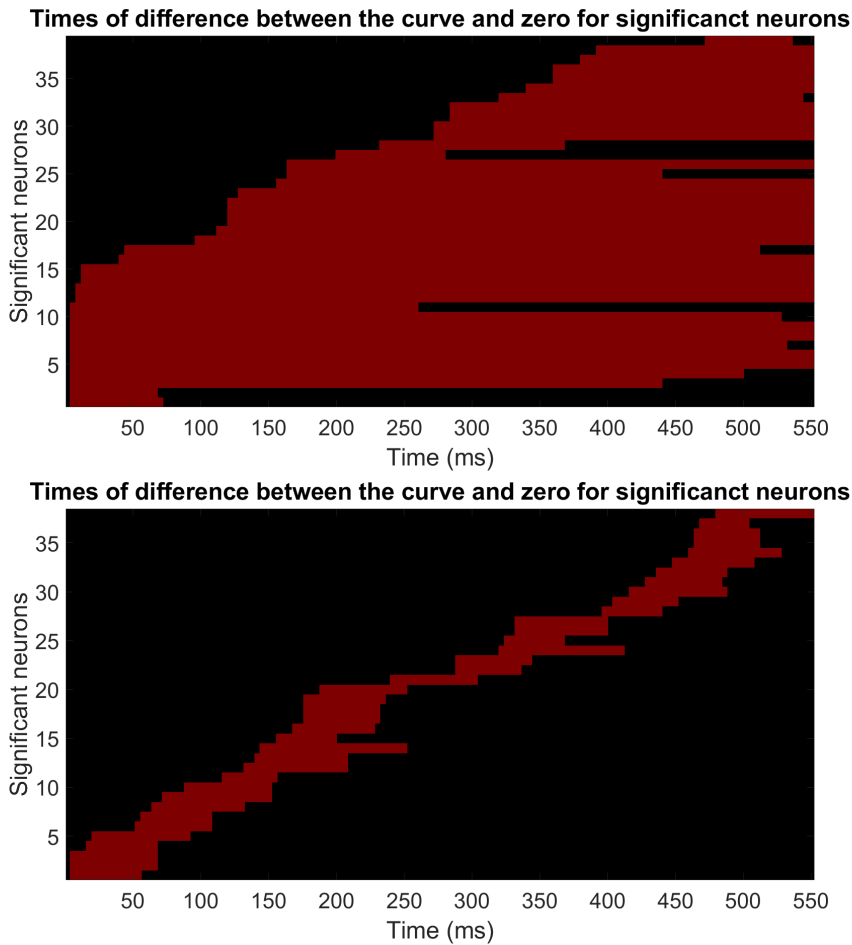
Figure 5.17: Schematic images of the time intervals where are the difference between the curve (difference of the mean signals after different images) and zero. In each row, red denotes time intervals, where there is the difference between the curve and zero. Each row corresponds to a single significant neuron. (A) unfiltered LFP signal, (B) filtered LFP signal

Another way to evaluate the results from bootstrap is to verify if there is a higher probability that if there was a specific neuronal activity on one type of emotion, there would also be a specific neuronal activity on the other two types of emotion. For example, if the test for happy and neutral images is significant, the test for happy and fearful or neutral and fearful images will be significant too. We used the Fisher's Exact test at the 5% significance level for examination. The results can be found in tables 5.13 5.14 5.15 5.16. There is a higher probability for the filtered LFP signal that if the neuron has a different neuronal activity for neutral and happy images, there will also be different neuronal activities for neutral and fearful or fearful and happy images. In the MER signal with AP, there is a higher probability that if the neuron has a different activity to a specific emotion, it will also have a different activity at least to one of the two other emotions. In the MER signal without AP, there is a higher probability that if the neuron has a different

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Times of difference between the curve and zero for significanct neurons

Times of difference between the curve and zero for significanct neurons
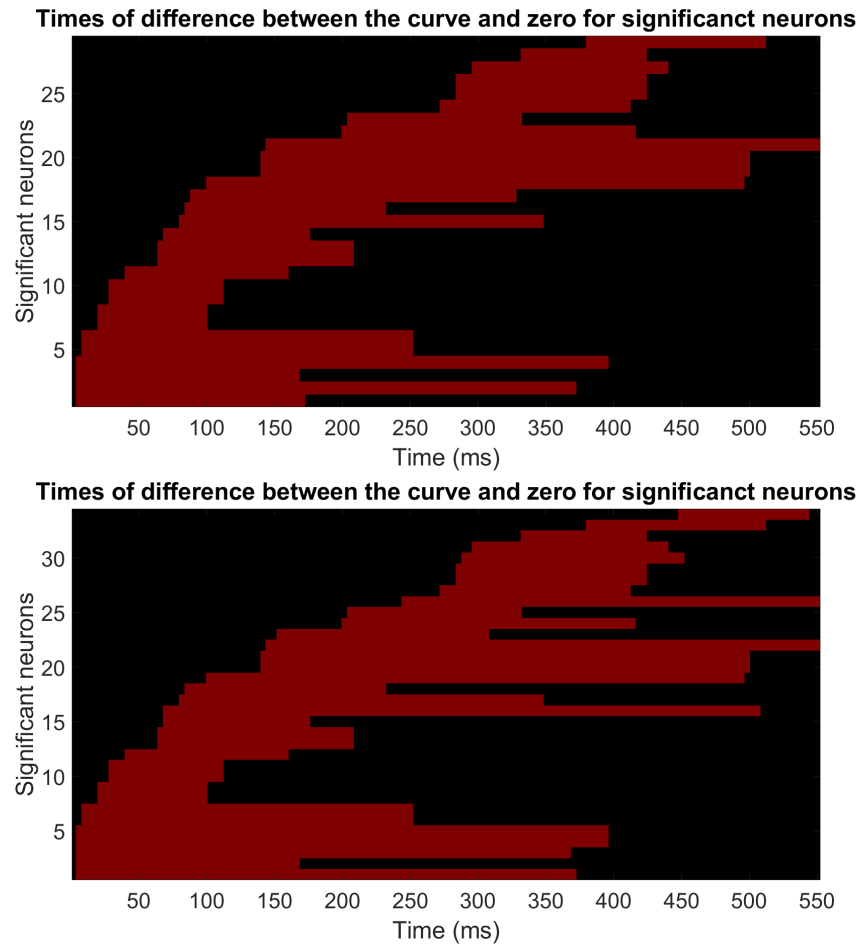
Figure 5.18: Schematic images of the time intervals where are the difference between the curve (difference of the mean signals after different images) and zero. In each row, red denotes time intervals, where there is the difference between the curve and zero. Each row corresponds to a single significant neuron. (A) MER signal with action potentials, (B) MER signal without action potentials

neuronal activity for neutral and fearful images, there will also be different neuronal activities for fearful and happy images or neutral and happy images.

Bootstrap results		LFP filtered		P-value
		Significant	**Insignificant**	
LFP unfiltered	**Significant**	3	36	0.2035
	Insignificant	35	871	

Table 5.7: LFP filtered x LFP unfiltered in the time domain

Bootstrap results		LFP filtered		P-value
		Significant	**Insignificant**	
MER with AP	**Significant**	0	29	0.6256
	Insignificant	38	878	

Table 5.8: LFP filtered x MER with AP in the time domain

Bootstrap results		LFP filtered		P-value
		Significant	**Insignificant**	
MER without AP	**Significant**	0	34	0.3939
	Insignificant	38	873	

Table 5.9: LFP filtered x MER without AP in the time domain

Bootstrap results		LFP unfiltered		P-value
		Significant	**Insignificant**	
MER with AP	**Significant**	0	29	0.6276
	Insignificant	39	877	

Table 5.10: LFP unfiltered x MER witht AP in the time domain

Bootstrap results		LFP unfiltered		P-value
		Significant	**Insignificant**	
MER without AP	**Significant**	0	34	0.3937
	Insignificant	39	872	

Table 5.11: LFP unfiltered x MER without AP in the time domain

Bootstrap results		MER with AP		P-value
		Significant	**Insignificant**	
MER without AP	**Significant**	27	7	$1.6 \cdot 10^{-43}$
	Insignificant	2	909	

Table 5.12: MER with AP x MER without AP in the time domain

Bootstrap results		NF		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	17	0.1187
	Insignificant	8	284	
		FH		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	16	0.0910
	Insignificant	7	283	
		NF		P-value
		Significant	**Insignificant**	
FH	**Significant**	1	9	0.2808
	Insignificant	9	294	

Table 5.13: NH x NF x FH - unfiltered LFP in the time domain

Bootstrap results		NF		P-value
		Significant	**Insignificant**	
NH	**Significant**	3	16	0.0382
	Insignificant	10	280	
		FH		P-value
		Significant	**Insignificant**	
NH	**Significant**	3	19	0.0185
	Insignificant	6	290	
		NF		P-value
		Significant	**Insignificant**	
FH	**Significant**	1	5	0.2263
	Insignificant	12	295	

Table 5.14: NH x NF x FH - filtered LFP in the time domain

Bootstrap results		NF		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	8	0.0365
	Insignificant	8	293	
		FH		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	8	0.0297
	Insignificant	7	294	
		NF		P-value
		Significant	**Insignificant**	
FH	**Significant**	2	7	0.0297
	Insignificant	8	294	

Table 5.15: NH x NF x FH - MER with AP in the time domain

Bootstrap results		NF		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	11	0.0493
	Insignificant	7	291	
		FH		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	11	0.0841
	Insignificant	10	288	
		NF		P-value
		Significant	**Insignificant**	
FH	**Significant**	2	10	0.0423
	Insignificant	7	292	

Table 5.16: NH x NF x FH - MER without AP in the time domain

■ 5.2.2 **Frequency domain**

The signals were divided into 8 time intervals and 24 frequency intervals in the frequency domain. The only exception is the filtered LFP signals. The first 5 Hz was filtered in the filtered LFP signals, so there is no reason to compare them. Because of that, the frequency intervals up to 5 Hz were removed from the spectrogram. So, in the end, for filtered LFP signals, we still had 8 time intervals but only 22 frequency intervals.

■ **The t-test**

The t-test in the frequency and time domain are very similar. But here, we did not compare the signals' amplitudes but the power spectral density of their spectrum. The spectrum was divided into 24 frequency intervals · 8 time intervals = 192 intervals. The only exception is the filtered LFP signals, with only 22 frequency intervals, so their spectrum was divided into 176 intervals.

As well as in the t-test in the time domain, the significance level was set to 5%. But because we divided the signal into many intervals, to ensure that the significance level remained 5%, we had to set the significance level for each t-test to $\frac{0.05}{192} = 2.6 \cdot 10^{-4}$. So their sum is equal to 5%. Otherwise, the probability that at least one t-test from all t-tets gives the type I error would be much higher than the required 5%. So we considered only p-values smaller than $2.6 \cdot 10^{-4}$. The filtered LFP signals have a lower number of intervals, so here is the significance level equal to $\frac{0.05}{176} = 2.84 \cdot 10^{-4}$. A more detailed description of the usage of the t-test in the frequency domain is in the section 4.5.2

There were 181440 t-tests done (315 recordings · 3 types of combination of the images - NH, NF, FH · 192 p-values from each time interval) for unfiltered LFP and MER with and without AP. For filtered LFP 166320 t-tests were done. (315 recordings · 3 types of combination of the images - NH, NF, FH · 176 p-values from each time interval)

T-tests with lower p-value than level of significance:

- Unfiltered LFP: 22 t-tests (0.012%)

- Filtered LFP: 19 t-tests (0.010%)

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- MER with AP: 19 t-tests (0.010%)

- MER without AP: 19 t-tests (0.010%)

■ The bootstrap

With the usage of bootstrap, the problem with a low level of significance disappears, so the significance level remains at 5%. More about the usage of bootstrap on our data you can find in section 4.5.3

As well as in the time domain, we checked if the bootstrap results corresponded to the results of the t-tests. The p-value from the t-test should be low in intervals where is the maximum volume under the area (bootstrap statistic). The maximum real volume under the area is denoted with the blue color in figures 5.19 5.20 5.21.

There were 945 bootstrap tests done (315 recordings · 3 types of combination of the images - NH, NF, FH).

Bootstrap tests with lower p-value than level of significance:

- Unfiltered LFP: 45 tests (4.76%)

- Filtered LFP: 51 tests (5.4%)

- MER with AP: 16 tests (1.69%)

- MER without AP: 18 tests (1.90%)
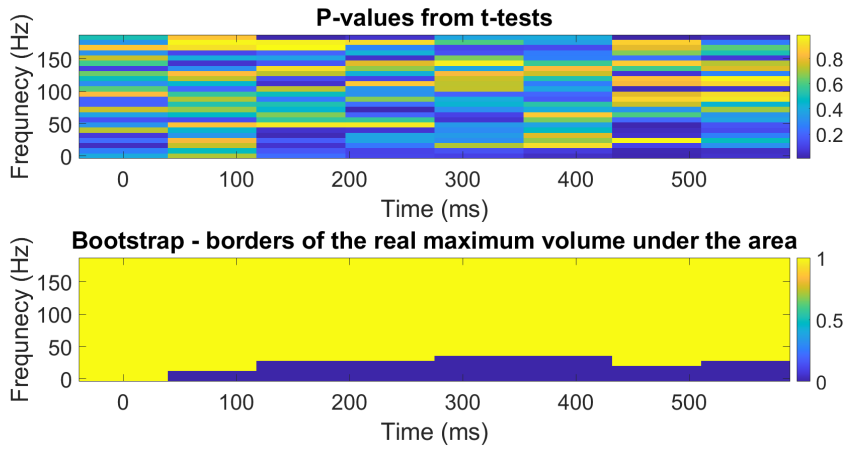
Figure 5.19: Example of verification the bootstrap results with t-tests results in unfiltered LFP signals. In the first figure, there is the p-value from t-tests for each time and frequency interval. In the second figure, the intervals where we can find the maximum volume under the area is denoted with the blue color.
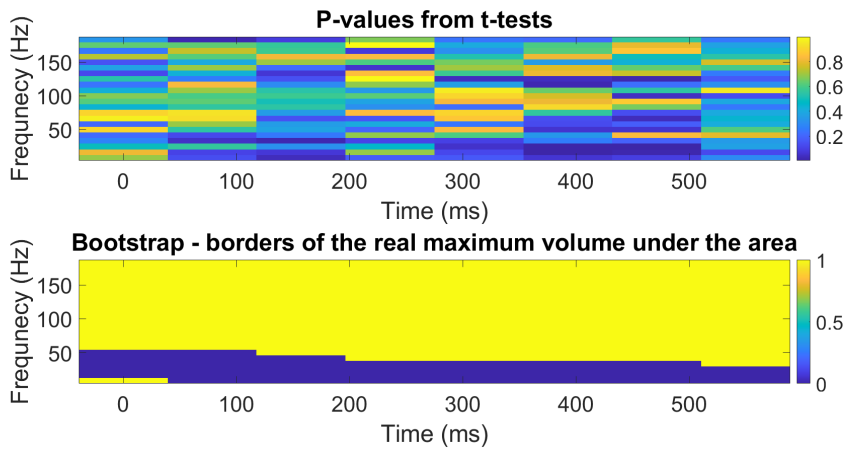
Figure 5.20: Example of verification the bootstrap results with t-tests results in filtered LFP signals. In the first figure, there is the p-value from t-tests for each time and frequency interval. In the second figure, the intervals where we can find the maximum volume under the area is denoted with the blue color

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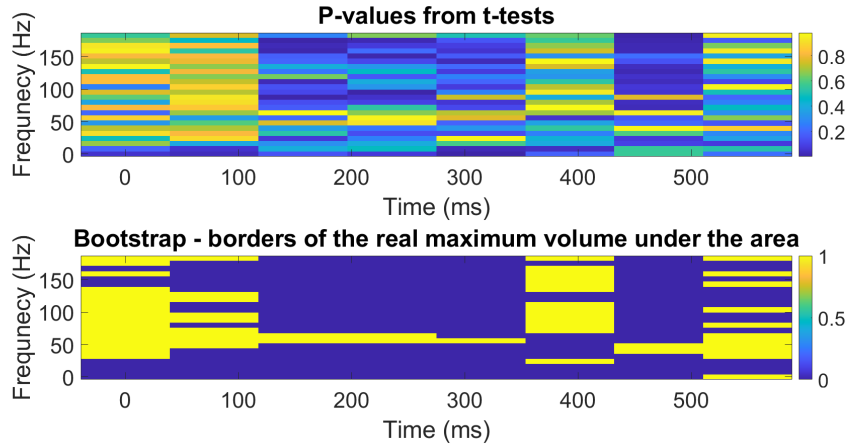
Figure 5.21: Example of verification the bootstrap results with t-tests results in MER signals with action potentials. In the first figure, there is the p-value from t-tests for each time and frequency interval. In the second figure, the intervals where we can find the maximum volume under the area is denoted with the blue color

■ Depth and hemisphere where the electrode is placed

Like in the time domain, we were also interested in whether there is a dependence on the depths or hemisphere where the recording electrodes are placed. To examine this, we used the same approach as in the time domain. The histograms of the depths and the hemispheres of the recording electrodes can be found in figures 5.22 5.23 and 5.24 5.25.

We used the chi-square test to evaluate the dependency on the electrode depths, and again, because of its restriction (every value in the contingency table must be five or higher), we had to merge some depths. But in this case, the p-value for each signal type was higher than 5%. You can find the used contingency tables and their p-values in the tables 5.17 5.18 5.20.

Neurons	Depths of the electrodes						P-value
	(-8,-3>	(-3, -2>	(-2,-1>	(-1, 0>	(0,1>	(1, 3>	
Insignificant	163	136	174	223	137	67	0.6329
Significant	8	5	9	8	10	5	

Table 5.17: Table of the chi-square test for unfiltered LFP signals in the frequency domain. Some depths have to be merged for usage of the chi-square test

Neurons	Depths of the electrodes						P-value
	(-8,-4>	(- 4, -3>	(-3, -2>	(-2,-1>	(-1, 0>	(0,3>	
Insignificant	64	95	130	178	215	212	0.1472
Significant	5	7	11	5	16	7	

Table 5.18: Table of the chi-square test for filtered LFP signals in the frequency domain. Some depths have to be merged for usage of the chi-square test

Neurons	Depths of the electrodes		P-value
	(-8,-1>	(-1, 3>	
Insignificant	48	442	0.8475
Significant	8	8	

Table 5.19: Table of the chi-square test for MER signals with AP in the frequency domain. Some depths have to be merged for usage of the chi-square test

The evaluation of the dependence on the hemisphere where the electrode is placed was done with the help of Fisher's exact test. Nevertheless, similar to the time domain, all Fisher's Exact tests fail to reject the null hypothesis at the significance level of 5%. This means that there are no nonrandom associations between the left and the right hemisphere in any of the signal types. The results of the Fisher's exact test are in the table 5.21.

■ Summary of the results in the frequency domain

Processing the signals in the frequency domain brought similar results as processing the signals in the time domain. The significance level was 5%, and therefore, the expected number of false-positive results was 5% of all the tests. In none of the tests was the number of the significant tests significantly higher than 5%, so it is possible that all of them were false positives. Unfortunately, we are not able to decide it.

The bootstrap results can be divided by the type of the test, which was denoted as significant. In the table 5.22 is the percentage of the significant tests from the total of 315 tests for each type of test (neutral-happy, neural - fearful, fearful - happy).

We noticed that the maximum volume under the area is often in lower frequencies and also from the very beginning of the signal. That is strange because the brain needs some time to transport the information. It is impossible to react to the image immediately after seeing it.

Neurons	Depths of the electrodes		P-value
	(-8,-1>	(-1, 3>	
Insignificant	485	442	0.7854
Significant	10	8	

Table 5.20: Table of the chi-square test for MER signals without AP in the frequency domain. Some depths have to be merged for usage of the chi-square test
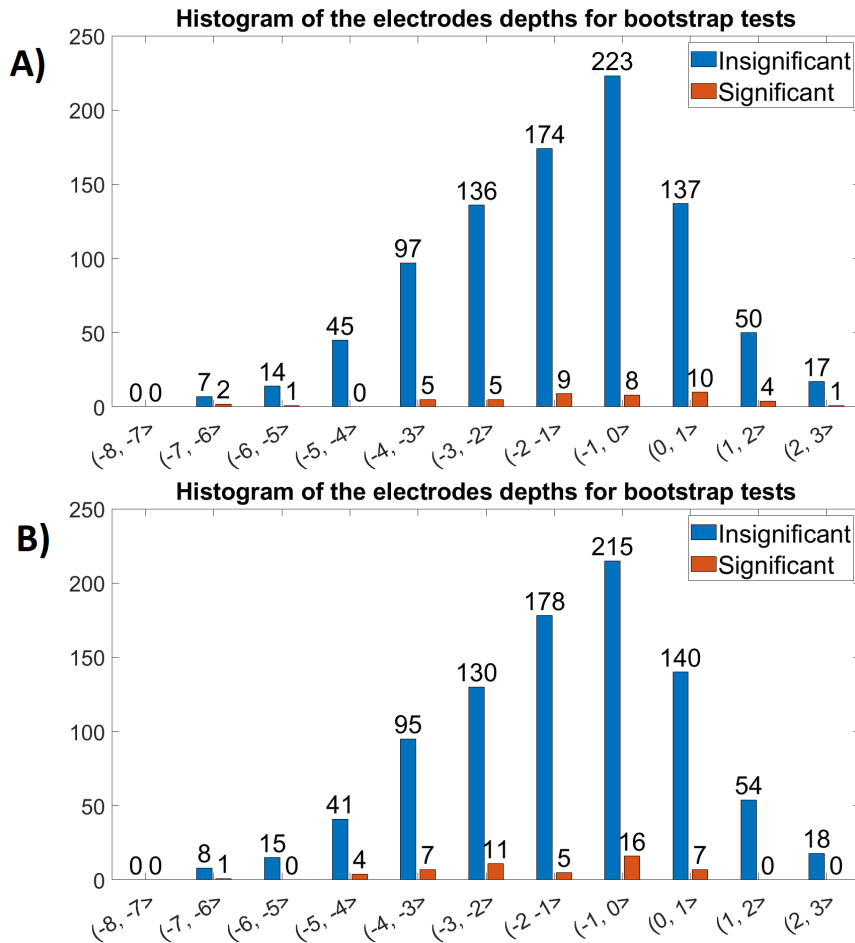
Figure 5.22: Histogram of the electrode depths for (A) unfiltered and (B) filtered LFP signals in the frequency domain. The depth is measured relative to the target in STN.

Types of the signal	P-value	Decision
unfiltered LFP	0.7617	fails to reject the null hypothesis
filtered LFP	0.3137	fails to reject the null hypothesis
MER with AP	0.1347	fails to reject the null hypothesis
MER without AP	0.3481	fails to reject the null hypothesis

Table 5.21: Table of the results of the Fisher's Exact tests for comparing the hemispheres in the frequency domain
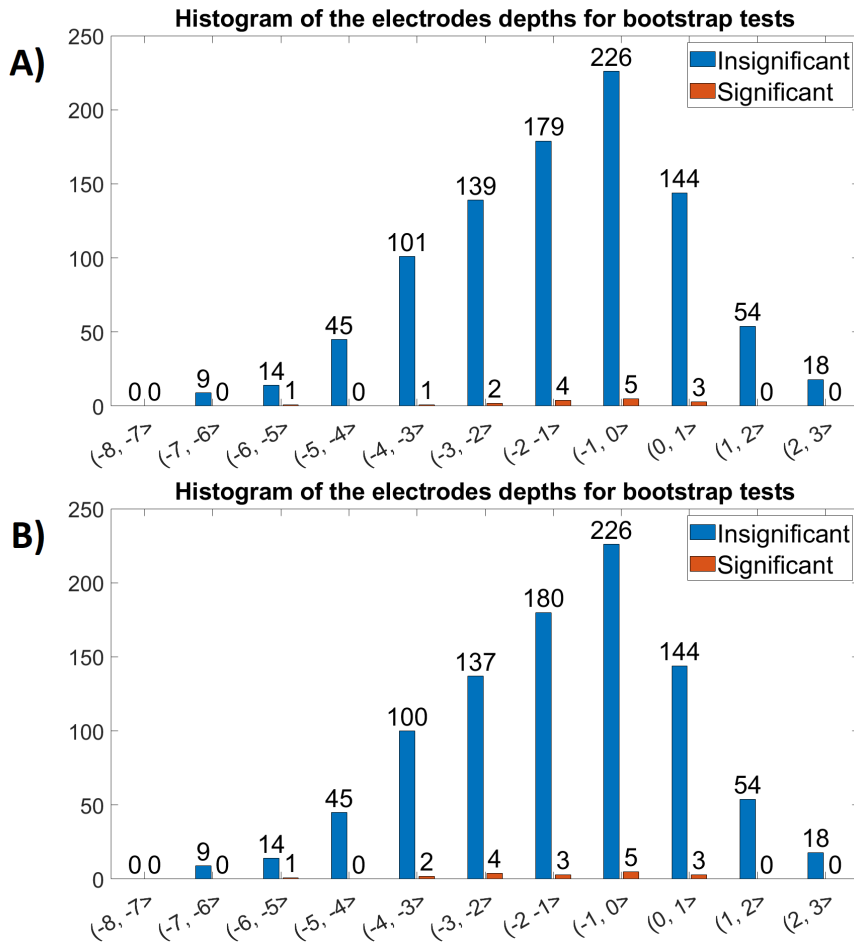
Figure 5.23: Histogram of the electrode depths for MER signals (A) with and (B) without action potentials in the frequency domain. The depth is measured relative to the target in STN.

Types of signals	Frequency domain		
	NH	**NF**	**FH**
unfiltered LFP	4.76%	3.81%	5.71%
filtered LFP	4.13%	6.03%	6.03%
MER with AP	1.56%	2.22%	1.27%
MER without AP	2.22%	1.90%	1.59%

Table 5.22: Table of significant bootstrap results for individual tests

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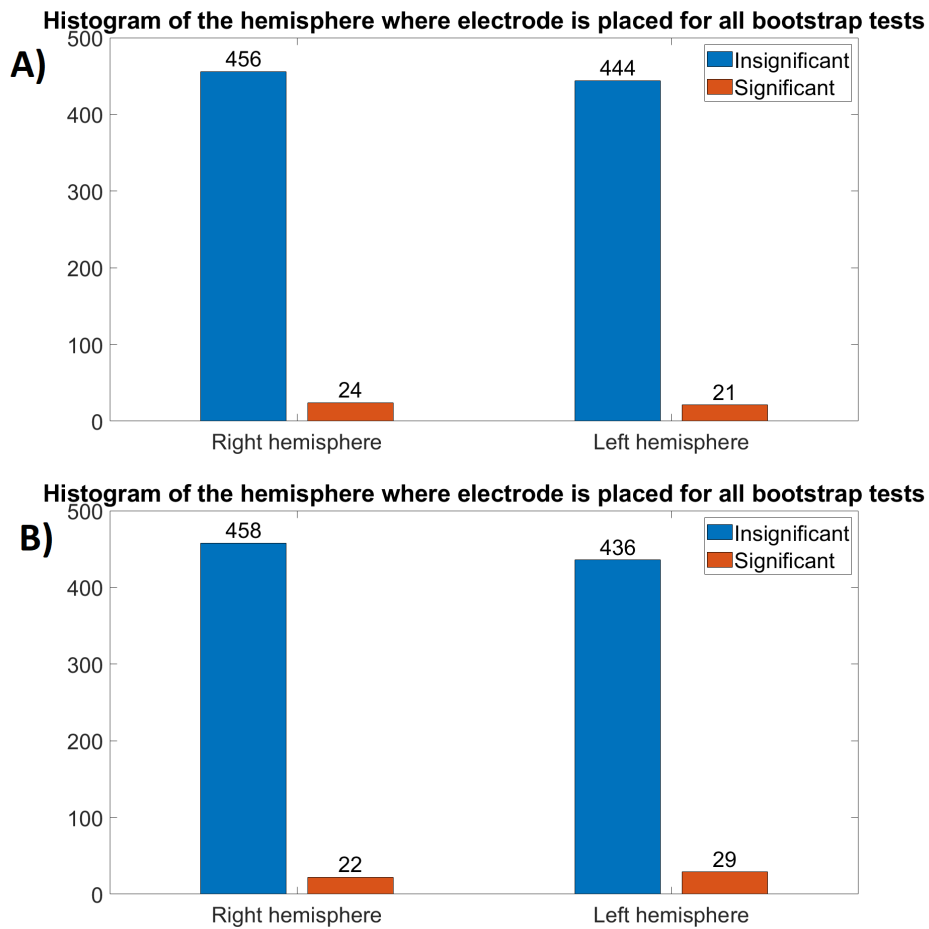
Figure 5.24: Histogram of the hemisphere for (A) unfiltered and (B) filtered LFP signals in the frequency domain

In the figures 5.26 5.27 5.28 5.29 , color denotes the time and frequency intervals where the difference between the area and the zero (bootstrap statistic) occurs in neurons where bootstrap tests have a lower p-value than 5%. Time intervals in the LFP signals are distributed randomly. There are just a few time intervals where the maximum volume under the area occurs in most of the frequencies. In MER signals with AP, the time intervals where the maximum volume under the area occurs in most of the frequencies appears randomly. For MER signals without AP, there were no significant results. In all types of signals, the frequency intervals where the maximum volume under the area occurs in most of the time intervals appear in the lower frequencies. They are not so common in the higher frequencies.

From the tables 5.17 5.18 5.20 5.21, it is clear that the number of significant tests does not depend on the depths and hemisphere where the recording electrode is placed.
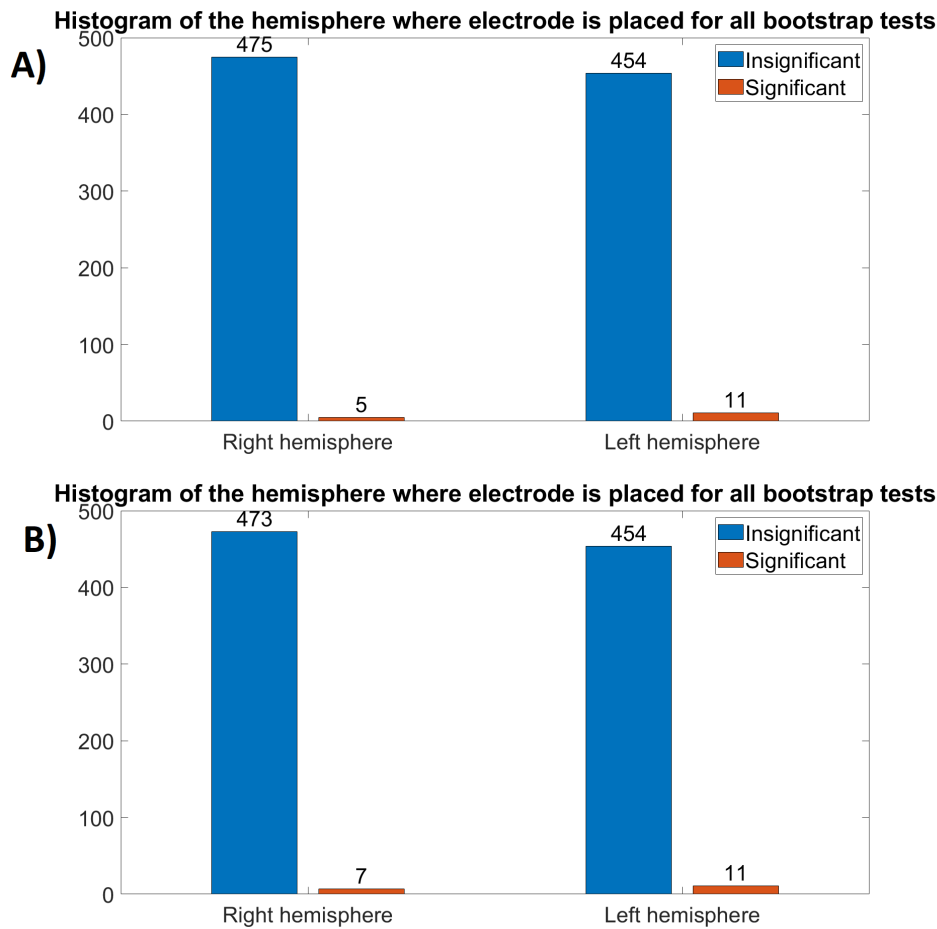
Figure 5.25: Histogram of the hemisphere for MER signals (A) with and (B) without action potentials in the frequency domain

The next approach to evaluate the results from the bootstrap (the bootstrap tests the whole signals, not just intervals as the t-test) is to examine if the reaction to the emotion can be seen in the different types of signals simultaneously. For example, if a test in filtered LFP signals was denoted as significant, the test done for the same position in unfiltered LFP signals would also be significant, and so on. We used the Fisher's Exact test at the 5% significance level to examine that. The results are in tables 5.23 5.24 5.25 5.26 5.27 5.28. However, the only p-value lower than 5% was in MER signals with and without AP. So those signals seem to be dependent.

Another approach to evaluate the results from bootstrap is to see if there is a higher probability that if there was a specific neuronal activity on one type of emotion, there would also be a specific neuronal activity on the other two types of emotion. For example, if the test for happy and neutral images is significant, the test for happy and fearful or neutral and fearful images will be significant too. We used the Fisher's Exact test at the 5% significance

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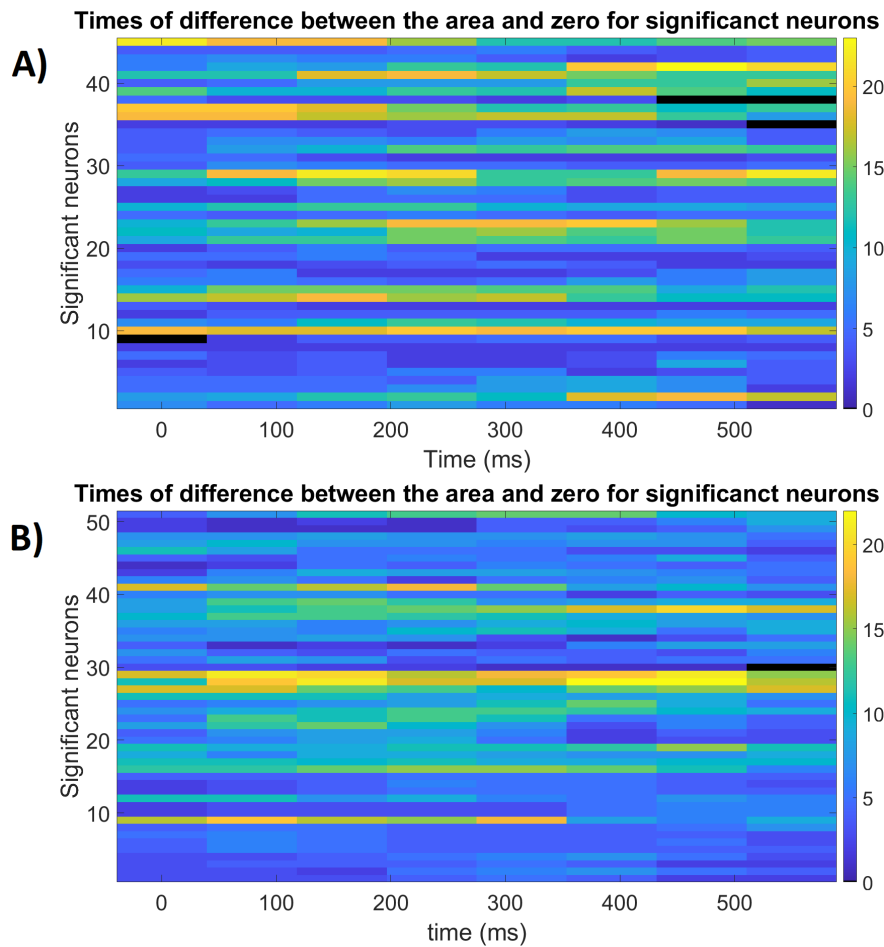
Figure 5.26: Schematic image of the time intervals where there are the difference between the area and zero. In each row, there are time intervals where there is a difference between the area and zero. Each row represents a single significant neuron. The number of significant frequency interval in each time interval is denoted with the color.
(A) unfiltered LFP signal, (B) filtered LFP signal

level for examination. The results can be found in tables 5.29 5.30 5.31 5.32. There is a higher chance for the MER signals with and without AP that if the neuron reacts differently to the neutral and happy images, it will also react differently to neutral and fearful or fearful and happy images. We also found a dependence between fearful and happy images and neutral and fearful images in the unfiltered LFP signals.
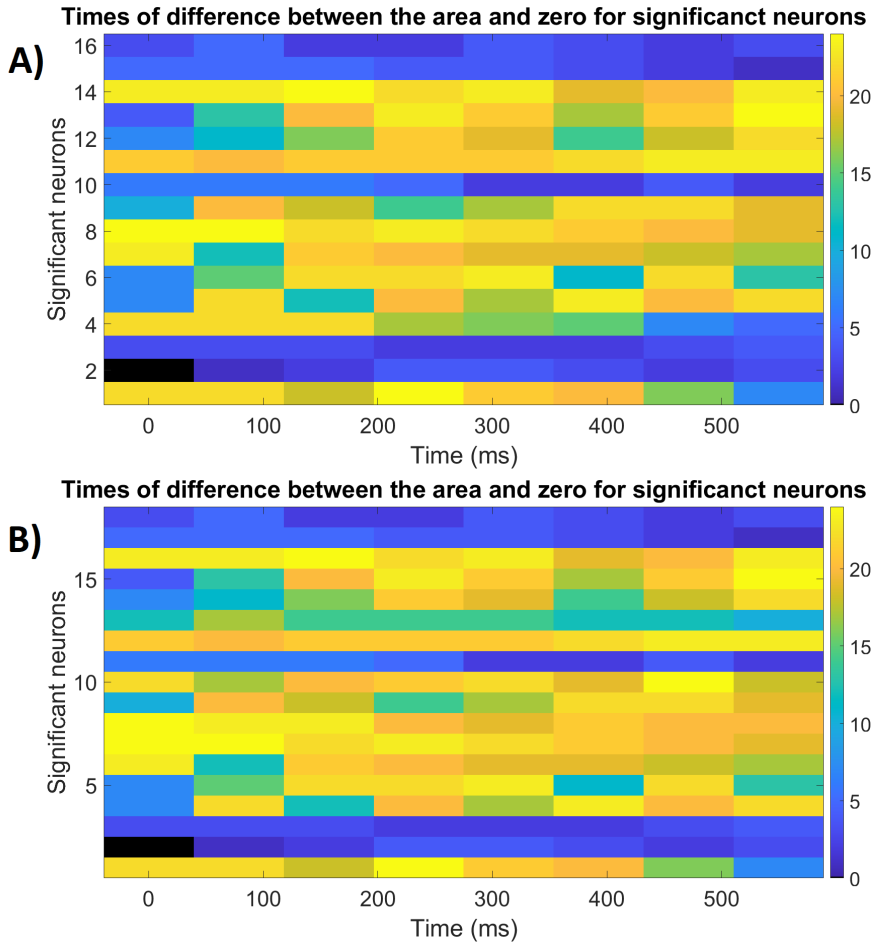
Figure 5.27: Schematic image of the time intervals where there are the difference between the area and zero. In each row, there are time intervals where there is a difference between the area and zero. Each row represents a single significant neuron. The number of significant frequency interval in each time interval is denoted with the color.
(A) MER signal with action potentials, (B) MER signal without action potentials

Frequencies of difference between the curve and zero for significanct neurons
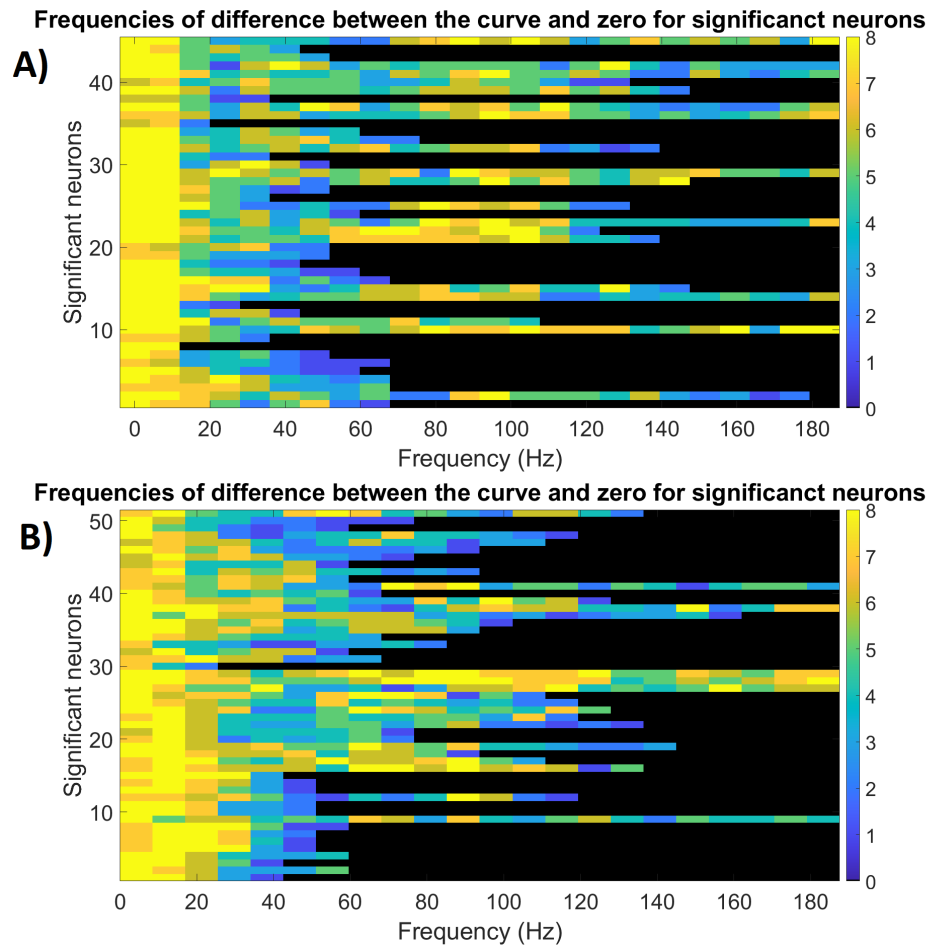
Figure 5.28: Schematic image of the frequency intervals where there are the difference between the area and zero. In each row, there are frequency intervals where there is a difference between the area and zero. Each row represents a single significant neuron. The number of significant time interval in each frequency interval is denoted with the color.
(A) unfiltered LFP signal, (B) filtered LFP signal

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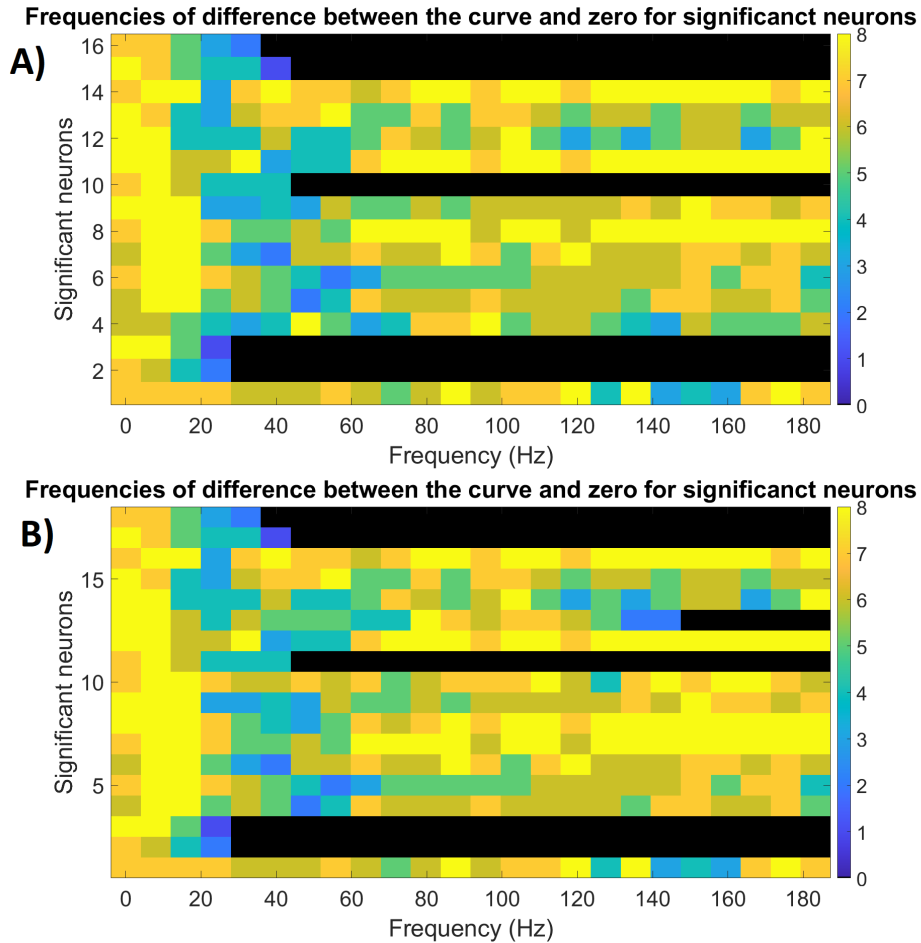
Figure 5.29: Schematic image of the frequency intervals where there are the difference between the area and zero. In each row, there are frequency intervals where there is a difference between the area and zero. Each row represents a single significant neuron. The number of significant time interval in each frequency interval is denoted with the color.
(A) MER signal with action potentials, (B) MER signal without action potentials

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Bootstrap results		LFP filtered		P-value
		Significant	**Insignificant**	
LFP unfiltered	**Significant**	5	40	0.0888
	Insignificant	46	854	

Table 5.23: LFP filtered x LFP unfiltered in the frequency domain

Bootstrap results		LFP filtered		P-value
		Significant	**Insignificant**	
MER with AP	**Significant**	1	15	0.5914
	Insignificant	50	879	

Table 5.24: LFP filtered x MER with AP in the frequency domain

Bootstrap results		LFP filtered		P-value
		Significant	**Insignificant**	
MER without AP	**Significant**	1	17	1
	Insignificant	50	877	

Table 5.25: LFP filtered x MER without AP in the frequency domain

Bootstrap results		LFP unfiltered		P-value
		Significant	**Insignificant**	
MER with AP	**Significant**	2	14	0.1745
	Insignificant	43	886	

Table 5.26: LFP unfiltered x MER witht AP in the frequency domain

Bootstrap results		LFP unfiltered		P-value
		Significant	**Insignificant**	
MER without AP	**Significant**	2	16	0.2098
	Insignificant	43	884	

Table 5.27: LFP unfiltered x MER without AP in the frequency domain

Bootstrap results		MER with AP		P-value
		Significant	**Insignificant**	
MER without AP	**Significant**	15	3	$4.45 \cdot 10^{-29}$
	Insignificant	1	926	

Table 5.28: MER with AP x MER without AP in the frequency domain

Bootstrap results		NF		P-value
		Significant	**Insignificant**	
NH	**Significant**	1	14	0.4513
	Insignificant	11	287	
		FH		P-value
		Significant	**Insignificant**	
NH	**Significant**	1	10	0.4132
	Insignificant	13	277	
		NF		P-value
		Significant	**Insignificant**	
FH	**Significant**	3	15	0.0263
	Insignificant	9	282	

Table 5.29: NH x NF x FH - unfiltered LFP in the frequency domain

Bootstrap results		NF		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	11	0.1844
	Insignificant	17	281	
		FH		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	11	0.1844
	Insignificant	17	281	
		NF		P-value
		Significant	**Insignificant**	
FH	**Significant**	3	16	0.1018
	Insignificant	16	274	

Table 5.30: NH x NF x FH - filtered LFP in the frequency domain

Bootstrap results		NF		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	3	0.0042
	Insignificant	5	301	
		FH		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	5	0.0060
	Insignificant	4	306	
		NF		P-value
		Significant	**Insignificant**	
FH	**Significant**	1	3	0.0869
	Insignificant	6	303	

Table 5.31: NH x NF x FH - MER with AP in the frequency domian

Bootstrap results		NF		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	5	0.0063
	Insignificant	4	300	
		FH		P-value
		Significant	**Insignificant**	
NH	**Significant**	2	7	0.0140
	Insignificant	5	303	
		NF		P-value
		Significant	**Insignificant**	
FH	**Significant**	1	4	0.0928
	Insignificant	5	303	

Table 5.32: NH x NF x FH - MER without AP in the frequency domian

Chapter **6**

Conclusion

Patients with Parkinson's disease undergo deep brain stimulation hoping that it alleviates the motor symptoms of this disease. But some patients have changes in emotions, although the electrodes for DBS are placed in the subthalamus nucleus (motoric centrum). To study this phenomenon, patients fulfill the emotional task during the implantation of the electrodes for DBS. This thesis aimed to examine if the neuronal activity in STN was specific to different emotions - happiness, neutral, and fear.

The results were gained by processing 105 records from 22 people diagnosed with Parkinson's disease. Several recordings in different depths of the electrodes in the brain were done for each patient. The results from t-tests were used just to verify the bootstrap results because the t-test tests signal parts while the bootstrap tests the signals as a whole.

Four types of signals were tested:

1. Unfiltered LFP signals - preprocessed signals of local field potentials

2. Filtered LFP signals - filtered preprocessed signals of local field potentials with the high pass filter on $5\,\mathrm{Hz}$

3. MER signals with action potentials (AP) - preprocessed signals from microelectrode recordings

4. MER signals without action potentials (AP) - preprocessed signals from microelectrode recordings from which AP from close neurons were removed using the WaveClus algorithm

We first evaluated if there is a specific neuronal activity by comparing the results from bootstrap tests of signals after neutral and happy (NH), neutral and fearful (NF), and fearful and happy (FH) images. Because the significance level was set to 5%, it is expected that 5% of the results are false positives. In the time and frequency domain, there were just a few significant tests, and some of them, even all, could be false positives. But because we can not decide if the significant results are false positives or true positives, we should consider them all significant.

We also compared the number of all significant bootstrap tests in different depths of the brain where the electrodes were placed. However, none of the p-values for the chi-square test was higher than the significance level (5%), so no dependency of the significant neurons on the depth of recording electrodes was detected.

We evaluated the number of all significant bootstrap tests in the left and the right hemispheres to see which hemisphere is more involved in emotional processing. Nevertheless, from the results of Fisher's Exact tests, the number of significant tests does not depend on the hemisphere in the time domain and not even in the frequency domain.

We examined if the reaction to the emotion can be seen in different types of signals simultaneously. For example, if a test in filtered LFP signals were significant, the test done for the same position in unfiltered LFP signals would also be denoted as significant, and so on. The only dependency was found in MER signals with and without AP in both the time and the frequency domain. So it seems that removing the action potentials of the close neurons doesn't affect the results much. This could be caused by the fact that the neurons close to the electrodes do not affect emotional processing, but the activity of the distant neurons affects it so much that they are apparent even if there are still action potentials from close neurons in the signal. Another possible explanation is that the activity of both close and distant neurons is connected with emotion processing.

Lastly, we evaluated if there is a higher probability that whether there were a specific neuronal activity on one type of emotion, there would also be a specific neuronal activity on the other two types of emotion. The results show some dependency for the filtered LFP signals in the time domain, for MER signals with and without AP in both the time and the frequency domain, and for unfiltered LFP signals in the frequency domain. Because the test was significant for neutral and happy images and also, for example, for neutral and fearful images, it is not likely that these results are caused by chance or that they are false positivities. So these signals might be candidates for real emotional response. But to confirm that, more research is needed.

While processing the bootstrap results, we discovered that the maximum area under the curve or the maximum volume under the area often begins immediately after the picture is shown. And that is impossible because the brain can not process the environment so quickly. Some time is needed to transmit the information through neurons. This could happen because there might have been some artifacts left which could affect the results, or also, there could be some problem with the recorded data.

To conclude our results, there is no significant proof that STN affects the processing of different emotions. On the other hand, it also did not bring any significant evidence that STN did not affect it because it is important to remember that our recordings come from DBS. DBS is used only for patients who can not be treated with drugs, so usually in an advanced phase of Parkinson's disease, where STN does not perform its function. So the results could differ for healthy people. Another factor that could affect the results is that the patients stopped taking the medicaments just a day before the surgery, so they could still affect the brain, especially when the patients took them for a longer period.

Our results support the hypothesis that the involvement of STN does not depend on the type of emotion but that STN has a more general function in emotional processing. One of the possibilities is that STN decides on the relevance of the information from the environment. And based on it, it modifies performed movements. Electrodes placed in STN for deep brain stimulation could affect relevance detection and therefore cause changes in patients' emotions. [13] Nevertheless, more research is required to verify this theory. Especially, data from neurologically healthy people should be gained.

Appendix A

Bibliography

[1] Radovan Hudák and David Kachlík. *Memorix anatomie*. Triton, Praha, 1. vydání edition, c2013.

[2] Ivan Dylevský. *Základy funkční anatomie*. Poznání, Olomouc, 2011.

[3] Per Brodal. *The Central Nervous system*. Oxford, fourth edition edition, 2010.

[4] Clement Hamani, Jean A. Saint-Cyr, Justin Fraser, Michael Kaplitt, and Andres M. Lozano. The subthalamic nucleus in the context of movement disorders. *Brain*, 127(1):4–20, 01 2004.

[5] N. O. of Communications and P Liasion. *Parkinosn's disease: Hope trhough research*. NIH Publication, n0. 20-ns-139 edition, 2020.

[6] Martin Jakobs, Anton Fomenko, Andres M. Lozano, and Karl Kiening. Cellular, molecular, and clinical mechanisms of action of deep brain stimulation a systematic review on established indications and outlook on future developments. *EMBO Molecular Medicine*, 11, 2019.

[7] Filippo Agnesi, Matthew D. Johnson, and Jerrold L. Vitek. Chapter 4 - deep brain stimulation: how does it work? In Andres M. Lozano and Mark Hallett, editors, *Brain Stimulation*, volume 116 of *Handbook of Clinical Neurology*, pages 39–54. Elsevier, 2013.

[8] Joachim K. Krauss, Nir Lipsman, Tipu Aziz, Alexandre Boutet, Peter Brown, Jin Woo Chang, Benjamin Davidson, Warren M. Grill, Marwan I Hariz, Andreas Hoen, Michael Schulder, Antonios Mammis, Peter A. Tass, Jens Volkmann, Lozano, and Andres M. Technology of deep brain stimulation: current status and future directions. *Nature Reviews Neurology*, 17, 2021.

[9] Jiří Wild. Automatic processing of neuronal single-cell recordings from deep structures of humain brain, 2008.

[10] M.A.J. Lourens, H.G.E. Meijer, M.F. Contarino, P. van den Munckhof, P.R. Schuurman, S.A. van Gils, and L.J. Bour. Functional neuronal activity and connectivity within the subthalamic nucleus in parkinson's disease. *Clinical Neurophysiology*, 124(5):967–981, 2013.

[11] Tereza Serranová, Robert Jech, Petr Dušek, Tomáš Sieger, Filip Ruzicka, Dušan Urgošík, and Evžen Růžička. Subthalamic nucleus stimulation affects incentive salience attribution in parkinson's disease. *Movement Disorders*, 26, 2011.

[12] Anne Buot, Marie-Laure Welter, Carine Karachi, Jean-Baptiste Pochon, Eric Bardinet, Jérôme Yelnik, and Luc Mallet. Processing of emotional information in the human subthalamic nucleus. *Journal of neurology, neurosurgery, and psychiatry*, 84, 10 2012.

[13] Julie Péron, Sascha Frühholz, Marc Vérin, and Didier Grandjean. Subthalamic nucleus: A key structure for emotional component synchronization in humans. *Neuroscience and biobehavioral reviews*, 37, 01 2013.

[14] Gyorgy Buzsáki, Costas Anastassiou, and Christof Koch. The origin of extracellular fields and currents—eeg, ecog, lfp and spikes. *Nature reviews. Neuroscience*, 13:407–20, 05 2012.

[15] Š. Polanský. *Processing of Electroencephalograms*. Master thesis, Brno University of Technology, 2011.

[16] Wikipedia. Eeg recording. `https://commons.wikimedia.org/wiki/File:EEG_recording.jpg`, 2001-. Accessed: 2022-04-20.

[17] Itzhak Fried, Ueli Rutishauser, Moran Cerf, and Gabriel Kreiman. *Single Neuron Studies of the Human Brain*. The MIT Press, 2014.

[18] Eduard Bakštein, Tomáš Sieger, Jiří Wild, Daniel Novák, Jakub Schneider, Pavel Vostatek, Dušan Urgošík, and Robert Jech. Methods for automatic detection of artifacts in microelectrode recordings. *Journal of Neuroscience Methods*, 290:39–51, 2017.

[19] Joseph P. Hornak. The basics of mri, c1996-2020.

[20] Changkyun Im and Jong-Mo Seo. A review of electrodes for the electrical brain signal recording. *Biomedical Engineering Letters*, 6:104–112, 08 2016.

[21] Ph.D. Steven W. Smith. The scientist and engineer's guide to digital signal processing, c1997-2011.

[22] Alan V. Oppenheim, Ronald W. Schafer, and John R. Bruck. *Discrete-Time Signal Processing*. Prentice Hall, New Jersey, second edition edition, 1999.

[23] Václav Hlaváč and Miloš Sedláček. *Zpracování Signálů a Obrazů*. Vyda-vatelství ČVUT, Praha, second edition edition, 2002.

[24] Wikimedia Foundation. Filter bandform diagrams. `https://en.wikipedia.org/wiki/File:Bandform_template.svg`, 2001-. Accessed: 2022-04-30.

[25] Sajal K. Paul, Chandan Kumar Choubey, and Gaurav Tiwari. Low power analog comb filter for biomedical applications. *Analog Integr. Circuits Signal Process.*, 97(2), 2018.

[26] Wikipedia. Aliasedspectrum. `https://commons.wikimedia.org/wiki/File:AliasedSpectrum.png`, 2001-. Accessed: 2022-04-30.

[27] R. Quian Quiroga, Z. Nadasdy, and Y. Ben-Shaul. Unsupervised Spike Detection and Sorting with Wavelets and Superparamagnetic Clustering. *Neural Computation*, 16(8):1661–1687, 08 2004.

[28] Mirko Navara. *Pravděpodobnost a matematická statistika*. Nakladatelství ČVUT, Praha, 2007.

[29] Larry Wasserman. *All of statistics : a concise course in statistical inference.* Springer, 2010.

[30] Cosma Rohilla Shalizi. Advanced data analysis from an elementary point of view. 2012.

[31] Mgr. Tomáš Sieger. Processing and statistical analysis of single-neuron recordings, 2013.

[32] Michel Hersen Alan S. Bellack. Comprehensive clinical psychology. 3, 2000.

Appendix B

Sofware documentation

This appendix contains documentation of the used Matlab function. Matlab functions can be downloaded from: `https://gitlab.fel.cvut.cz/kutovmag/analysis_dbs_recordings`. Matlab version R2020a was used for implementing the methods. In the given data, the signals were marked differently - MEx = macro electrode recordings (LFP), UExd = microelectrode recordings (MER).

▉ B.1 Preprocessing

In preprocessing the data were loaded from .d files, filtered with the comb filter and then divided into matrixes of the signals parts which occur 550 ms after required emotion tag - H (happy), N (neutral), F (fearful). If any of the significant parts contained an artifact - that part was not used.

comb_filter.m is a Matlab function that suppresses the 50 Hz and its multiplies with the use of the comb filter. Arguments and returns of this function are described in its documentation.

Loading.m is a Matlab file that loads the data from .d files and filters them with the comb filter. *comb_filter.m* is used for filtering. Filtered data are saved as origin_name.d_preprocessed.mat files into the save_file. It is used a list_modified2.txt file with the names of the recordings. The function

does not have arguments or returns.

high_pass_filter.m is a Matlab function that filters the signal with FIR high pass filter on the frequency f_0. Arguments and returns of this function are described in its documentation.

artefact_cuts.m is a Matlab function that, from a given signal, cuts the parts of the signal which occur after the required tags. The cut part of the signal has a given length $fs \cdot time$.

Arguments:

- **x** - KxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns.

- **tags** - tags read from a .d file

- **tagsToCut** - name (or a cell array of names) of tags (their 2-character abbreviations) to cut.

- **time** - time interval which we want to cut after the tag occurs (int)

- **quantity** - number of tags that occurs in the signal

- **filter** - if the data were filtered with the function filtfilt - the first and last second of the signal cannot be used for forwarding processing

- **treshold** - minimum amplitude of the signal, which is denoted as an artifact

Returns:

- **y** - LxM matrix with the cuted parts of the signal where M is $fs(default) \cdot time$ and L is quantity minus the number of the signal parts occured after the required tags which cannot be used because there is an artefact

artefact_cuts_uexd.m is a Matlab function that from the given signal cuts the parts of the signal which occur after the required tags, the cut part of the signal has a given length $fs \cdot time$. It is a modification of *artefact_cuts.m* for a different way of detecting artifacts.

Arguments:

- **x** - KxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns.

- **tags** - tags read from a .d file

- **tagsToCut** - name (or a cell array of names) of tags (their 2-character abbreviations) to cut.

- **time** - time interval which we want to cut after the tag occurs (int)

- **quantity** - number of tags that occurs in the signal

- **artifact** - Mx2 matrix of indexes of the found artifact in the signal. The first column is for the beginning. The second is for the end of the artifact.

- **fs** - sampling frequency

Returns:

- **y** - LxM matrix with the cut parts of the signal where M is $fs \cdot time$ and L is quantity - artefact_count

- **artefact_count** - the number of the signal parts that occurred after the required tags but cannot be used because there is an artifact

tags_count.m is a Matlab function that counts the number of required tags. Arguments and returns of this function are described in its documentation.

decimation.m is a Matlab function that decimates the sampling frequency 100 times. Arguments and returns of this function are described in its documentation.

▊ B.2 Removing action potentials

fill_artefact.m is a Matlab function that removes action potentials of all the neurons from the original signal. But it preserves the length of the signal (fills back the artifacts). Arguments and returns of this function are described in its documentation.

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UExd_STN_noart is a Matlab file that cuts the artifacts and shorten the signal from MER signals from STN. Data without artifacts are saved as origin_name.mat files into the save_file.

It uses text files with those information:

- list_modified2.txt - names of the .d files with the data

- fs.mat - the sampling frequency for data

- STN_neurons.mat - matrix, in the first column is the order of the recordings which were identified as in STN, and in the second column is the number of the electrode which was active in STN (recorded in STN)

- UExd_artefact.txt - list of the .csv files with the besginning and the end of the time where the artifact occurs.

demoWC_STN is a Matlab file that runs the WaveClus algorithm and saves the results.

UExd_STN_noAP is a Matlab file that removes actions potentials from all of the neurons from the MER signals. Because the action potentials were computed from MER signals without artifacts, it is modified to the original length (with the use of the function *fill_artefact.m*) of the signal so it can be processed same as the MER signals with action potentials. The information about the action potentials are loaded from the output of the WaveClus algorithm saved in the time_file. MER signals with artifacts and without action potentials are saved into the .mat files in the path - save_file.

It uses text files with those information:

- list_modified2.txt - names of the .d files with the data

- fs.mat - the sampling frequency for data

- STN_neurons.mat - matrix, in the first column is the order of the recordings which were identified as in STN, and in the second column is the number of the electrode which was active in STN (recorded in STN)

- UExd_artefact.txt - list of the .csv files with the beginning and the end of the time where the artifact occurs.

◼ B.3   Time domain

In this section a description of an implementation of statistical tests modified for the time domain can be found.

max_area.m is a Matlab function that returns the maximal area between the given curve and zero. Arguments and returns of this function are described in its documentation.

interval_ttests.m is a Matlab function that does a t-test of x and y in the time domain at the 5% significance level.

Arguments:

- **X** - KxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns

- **Y** - KxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns

- **b** - number of the done t-tests - length of the avgX and avgY

Returns:

- **p_value** - array length b of the p_values from done t-tests

- **intervals** - indexes of the test where the p_value is lower than the required error rate - alpha (0.05/b)

average.m is a Matlab function that cuts the signal into the required number of time intervals, and the average number of the interval represents each interval. Arguments and returns of this function are described in its documentation.

bootstrap_statistic.m is a Matlab function that does the bootstrap - generates random indexes of the signal with replacement with the function bootstrp and then for each bootstrap sample counts the statistics - max area under the difference of the mean of the signals.

Arguments:

- **x** - KxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns

- **y** - LxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns

- **real_maximum** - statistics computed on the origin data

- **B** - number of bootstrap samples we want

Returns:

- **p_value** - int, result of the bootstrap statistic

▉ B.4   Frequency domain

In this section a description of an implementation of statistical tests modified for the frequency domain can be found.

get_spectrogram.m is a Matlab function that returns a spectrogram of the signal, but in the required size to the frequency 200 Hz, the size of the final spectrogram is set in the code. Arguments and returns of this function are described in its documentation. STN

spectrogram_mean is a Matlab function that computes the mean of the spectrograms from all of the signals in the matrix X with the use of the function *get_spectrogram.m.*

Arguments:

- **X** - KxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns

- **fs** - sampling frequency

Returns:

- **spect_mean** - 24x8 matrix (set default) of the mean from spectrograms from all of the signals, the spectrum is just up to 200 Hz and the size of the frequency intervals is modified into default size in function *get_spectrogram.m*

- **F_200** - array of origin distribution of frequencies up to 200 Hz for the spectrogram

real_max_volume.m is a Matlab function that computes the maximum volume under the area x for the spectrum. It also prints where the maximum can be found.

Arguments:

- **x** - KxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns

- **num_time** - number of time intervals in the spectrogram

- **num_freq** - number of frequency intervals in the spectrogram

Returns:

- **maximum** - maximum volume under the area x

- **table_interval** - num_freq x num_time matrix, indexes of the position where the maximum volume can be found are denoted with 0 othervise it is 1

max_volume is a Matlab function that returns the maximum volume under the area y and on which indexes we can find it - can find volume above zero or under zero (determined by the parameter sgn). The volume is computed in 4 directions - it has to be connected with the other squares of the same sign on the left, right, top, or bottom to be taken into consideration. For both signs, we look for positive values, so in case we look for a volume under the zero, we have to change the sign in all signal samples. Arguments and returns of this function are described in its documentation.

interval_ttests_spectrum.m is a Matlab function that does t-tests of x and y in the frequency domain with an error rate of 5% in all t-tests.

Arguments:

- **X** - KxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns

- **Y** - KxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns

- **b** - number of the done t-test - length of the Spect_x and Spect_y

- **fs** - sampling frequency

Returns:

- **p_value** - array of the p_values of X and Y in spectral domain

- **intervals** - indexes of the test where the p_value is lower than the required error rate - alpha (0.05/b)

count_spectrum.m is a Matlab function that computes spectrogram from the individual signals and reshapes them into the required size (1x(24x8)). Arguments and returns of this function are described in its documentation.

bootstrap_statistic_spectrum.m is a Matlab function that does the bootstrap - generates random indexes of the signal with replacement with the function bootstrp and then for each bootstrap sample counts the statistics - max volume under the difference of the mean spectrogram of the signals.

Arguments:

- **x** - KxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns

- **y** - LxN matrix of signals (recorded in individual channels); signals come in K rows, samples in N columns

- **real_maximum** - statistic counted on the origin data

- **B** - number of bootstrap samples we want

Returns:

- **p_value** - int, result of the bootstrap statistic

■ B.5   Usage of the implemented functions

STN.m is a Matlab file that runs both hypothesis testing (bootstrap and t-tests) for the LFP signal in STN in the time domain.

It uses text files with information:

- list_modified2.txt - names of the .d files with the data
- fs.mat - the sampling frequency for data
- STN_neurons.mat - matrix, in the first column is the order of the recordings which were identified as in STN, and in the second column is the number of the electrode which was active in STN (recorded in STN)
- LFP_artefacts.txt - txt file of the name of the signal and for its every electrode is set the minimum amplitude for the artifact (how big the sample has to have amplitude to be considered an artifact)

Data are loaded from .mat files (only electrodes active in STN) and are already preprocessed. The results (p-values for t-tests and bootstrap, borders of the maximum area between the curve and zero - difference of two signals and zero) are saved into the .mat files in the path - save_file.

STN_spec.m is a Matlab file that runs both hypothesis testing (bootstrap and t-test) for the LFP signal in STN in the frequency domain.

It uses text files with information:

- list_modified2.txt - names of the .d files with the data

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- fs.mat - the sampling frequency for data

- STN_neurons.mat - matrix, in the first column is the order of the recordings which were identified as in STN, and in the second column is the number of the electrode which was active in STN (recorded in STN)

- LFP_artefacts.txt - txt file of the name of the signal and for its every electrode is set the minimum amplitude for the artifact (how big the sample has to have amplitude to be considered an artifact)

Data are loaded from .mat files (only electrodes active in STN) and are already preprocessed. The results (p-values for t-tests and bootstrap, borders of the maximum volume between the area and zero - difference of two signals and zero) are saved into the .mat files in the path - save_file.

STN_UExd.m is a Matlab file that runs both hypothesis testing (bootstrap and t-test) for the MER signal in STN in the time domain.

It uses text files with information:

- list_modified2.txt - names of the .d files with the data

- fs.mat - the sampling frequency for data

- STN_neurons.mat - matrix, in the first column is the order of the recordings which were identified as in STN, and in the second column is the number of the electrode which was active in STN (recorded in STN)

- UExd_artefact.txt - list of the .csv files with the beginning and the end of the time where the artifact occurs.

Data are loaded from .mat files (only electrodes active in STN) and are already preprocessed. The results (p-values for t-tests and bootstrap, borders of the maximum area between the curve and zero - difference of two signals and zero) are saved into the .mat files in the path - save_file.

STN_UExd_spec.m is a Matlab file that runs both hypothesis testing (bootstrap and t-test) for the MER signal in STN in the frequency domain.

It uses text files with information:

- list_modified2.txt - names of the .d files with the data

- fs.mat - the sampling frequency for data

- STN_neurons.mat - matrix, in the first column is the order of the recordings which were identified as in STN, and in the second column is the number of the electrode which was active in STN (recorded in STN)

- UExd_artefact.txt - list of the .csv files with the beginning and the end of the time where the artifact occurs.

Data are loaded from .mat files (only electrodes active in STN) and are already preprocessed. The results (p-values for t-tests and bootstrap, borders of the maximum volume between the area and zero - difference of two signals and zero) are saved into the .mat files in the path - save_file.