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RESUM

Els senyals corticals del cervell permeten una visió més clara de l'activitat neuronal que l'estudi clàssic dels senyals superficials al crani i són de gran interès en l'estudi malalties neurodegeneratives, tals com l'Alzheimer. Amb els senyals adquirits sobre el còrtex prefrontal i somatosensorial de ratolins amb la mutació 3xTg-AD, s'han estudiat els estats espontanis UP i DOWN de les oscil·lacions lentes del cervell amb el subjecte anestesiat. En primer lloc, s'han aplicat tècniques de filtratge per eliminar interferències i deixar solament una banda d'interès, entre 0.1 i 200 Hz, en la qual s'han aplicat mesures d'anàlisi espectral. A continuació, s'ha utilitzat un nou filtratge entre 30 i 100 Hz, deixant només la banda espectral gamma associada a l'activitat UP i DOWN sobre la qual es calculen els paràmeters de connectivitat. D'entre els resultats obtinguts, s'han escollit aquel·lós paràmeters que presentaven una major significació estadística mitjançant proves T-Student i Wilcoxon. Finalment, amb aquests paràmeters s'han dissenyat dos procediments de classificació per anàlisi de discriminant lineal (Stepwise i Leave-one-out) que aconsegueixen diferenciar entre subjectes malalts d'Alzheimer i subjectes control amb un 88% i un 82% de precisió, respectivament.

RESUMEN

Las señales corticales del cerebro permiten una visión más clara de la actividad neuronal que el estudio clásico de las señales superficiales en el cráneo y son de gran interés en el estudio de enfermedades neurodegenerativas, como el Alzheimer. Con las señales adquiridas sobre el córtex prefrontal y somatosensorial de ratones con la mutación 3xTg-AD, se han estudiado los estados espontáneos UP y DOWN de las oscilaciones lentes del cerebro anestesiado. En primer lugar, se han aplicado técnicas de filtrado para eliminar interferencias y dejar solamente una banda de interés entre 0.1 y 200 Hz, en la cual se aplicaron medidas de análisis espectral. A continuación, se utilizó un nuevo filtrado entre 30 y 100 Hz, dejando únicamente la banda espectral gamma asociada a la actividad UP y DOWN sobre la cual se calculan los parámetros de conectividad. Entre los resultados obtenidos, se escogieron aquellos parámetros que presentaban una mayor significación estadística mediante las pruebas T-Student y Wilcoxon. Finalmente, con estos parámetros, se diseñaron dos procedimientos de clasificación por análisis de discriminante lineal (Stepwise y Leave-one-out) que consiguen diferenciar entre sujetos
enfermos de Alzheimer y sujetos control con un 88% y un 82% de precisión, respectivamente.

ABSTRACT

Cortical brain signals allow a clearer view of neuronal activity that the classical study of the surface signals on the scalp, and are of great interest for the study of neurodegenerative diseases, such as Alzheimer's Disease. With signals acquired on the prefrontal and somatosensory cortex of mice with the 3xTg-AD mutation, we have studied the spontaneous UP and DOWN states of the slow-wave oscillations of the brain from a sedated subject. First of all, we applied some filtering techniques to the signals in order to remove all interferences, leaving only a band of interest that ranges from 0.1 to 200Hz, on which we performed some spectral measurements. For the next step we used another filter between 30 and 100 Hz, leaving only the gamma spectral band associated to UP and DOWN activity, and proceeded to perform connectivity measurements. Amongst the obtained results, we selected those who showed the best statistical signification, calculated with T-Student and Wilcoxon tests. Finally, with the selected parameters we designed two classification procedures with linear discriminant analysis (Stepwise and Leave-one-out), which differentiate diseased subjects from control subjects with an 88% and 82% success rate, respectively.
CHAPTER 1:
INTRODUCTION

In the following pages we will discuss general concepts in order to help understand the study. We begin explaining how the nervous system of mice works, and the functions of the different parts of the cortex. Secondly, we will explain how the electroencephalographic signals are generated and what kind of signals we are studying. Finally, we will discuss Alzheimer’s Disease and how it affects the brain.

1.1 The nervous system of the mouse

1.1.1 Brief introduction to the nervous system

The nervous system is in charge of capturing and processing the signals and controlling all of the bodily functions. Anatomically speaking, it consists of two different structures that work together, the central nervous system and the peripheral nervous system.

The latter is a network of nerves that reach all the corners of the body and with two kinds of fibers, manage all the communication processes from the environment to the body. The afferent fibers transmit the sensitive impulses to the spinal cord and the brain while the efferent fibers do the exact opposite, transmitting the orders from the brain to the organs.

The central nervous system provides the higher functions, such as stimuli processing, behaviour control, memory, feelings or learning procedures. Two main structures form the central nervous system, the spinal cord and the encephallus.
The brain of the mouse is significantly less complex than human and ape brain, however it has proven helpful for scientists to study simpler brain models that can be adapted into more developed organisms. Mammals have most of their cortical fields in common, such as the visual, somatosensory or prefrontal cortex, and some connectivity patterns are also found in all species, such as pre-frontal parietal connections.

1.1.2 The cerebral cortex

The cerebral cortex is the outer layer of neural tissue of the brain, where the brain functions are located. It is mainly composed of gray matter (neuron bodies and axons without myelin sheaths), and distributed in layers (up to 6 layers have been found), which contain different distributions and make different connections with other regions. The regions of the cortex are grouped according their functionality:

- **Prefrontal cortex:** The higher brain functions are related to this area. This is the main unit of the brain, because it is also related to sensory and motor responses. Functions such as the temporary memory, the spatial orientation, the sequential behaviour (nest building, food gathering), behavioural flexibility and sexual behaviour are controlled by this area. Studies that are related to cognitive experience and behaviour often analyse the prefrontal cortex.
- **Somatosensory cortex:** This region has two different groups of functions, providing information on tactile stimuli while at the same time being capable of performing motor functions. This phenomenon is explained by the overlap of some areas of the somatosensory cortex with the prefrontal cortex. When injured, the reaction time of rats to tactile stimuli increases significantly, thus proving a relationship between the two regions. Recent hypotheses state that the motor cortex receives less sensory information when the somatosensory cortex is damaged, causing an impairment on the reaction movement in rats.

- **Motor Cortex:** Controls every process of voluntary movements, such as the selection of the movement, the spatial guidance which leads to a proper execution of the gestures made by the rat. It has been also proven that the activity of this region is not only related to movement but also related to other structures such as the glia and the other structures, such as the cerebellum region.

- **Visual cortex:** This region controls the visual interaction between the rat and the environment. A rat is a predator and a prey at the same time; therefore, an optimal functionality of the visual cortex is absolutely necessary for survival. The visual cortex is divided in several subregions such as the striate cortex (spatial contrast from the central field of view), the extrastriate visual cortex (stationary space, detection of movement) and temporal cortex (peripheral vision).

**Figure 2.** Cortices placement diagram. F/M stands for the prefrontal cortex, S1 for the somatosensory, A1 for the auditory and V1 for the visual. [UC San Diego, online]
- **Auditory cortex:** Several studies have proven this region to be severely underdeveloped in mice. The auditory cortex helps the rat to place the sounds in a specific space, but it is not necessarily. When damaged, the rat presents only a small impairment, and since the rat is not responsive to the perception of complex auditory signals patterns, it remains unclear the functional contribution of this area.

![Comparison between the mouse brain and the human brain.](image)

*Figure 3. Comparison between the mouse brain and the human brain.* [J.F. Cryan, 2005]

- **Posterior association cortex:** This area is related to the understanding of what the rat is experiencing, giving a meaning to experiences.

- **Associative insular cortex:** This area is still not completely understood, but recent studies link it with emotion, homeostasis and perception.
1.2 Cerebral biomedical signals, the EEG

1.2.1 Overview of the EEG: Definition, techniques, history.

Electroencephalography is the recording and analysis of the electric potentials generated by the brain and captured by electrodes on the scalp. The result is called an electroencephalogram (EEG), and it doesn’t display the sum of neuronal action potentials (due to its brevity and small amplitude), but the sum of the post synaptic potentials, which last longer and have a bigger amplitudes.

Frisch and Hitzig developed this technique in 1870, military doctors who observed movement when the brain was stimulated by galvanic current. Five years later, another physician, Caton, confirmed that the brain was capable of producing electrical activity. After almost 50 years of unsuccessful testing, the big leap forward for EEG came in 1924, when Hans Berger registered the oscillations on a 17 years old boy using needle electrodes and a string galvanometer. In 1934, Adrian and Matthews discovered the two first power band of the brain, the alpha and beta rhythms [Oxford Journals, online]

![Old EEG equipment and Emotiv System](image)

**Figure 4.** Old EEG equipment [Hospital de la Santa Creu i Sant Pau] and Emotiv System [Emotiv EPOC, online]

The following years led to an improvement on the quality of the recordings, helped by digital processing and electrode placement standardization, such as the 10-20 system. Modern EEG systems are advancing towards a more comfortable interface, with fewer wires (or even wireless), no need for electro-conductive gel and new materials for the electrodes.
The method used to record the brain signals in this study is the Intracranial EEG, which places the electrodes on the brain surface, after a surgical incision in the skull. This new procedure was developed in the early 1950s by Pendfield and Jasper, in order to treat patients with severe epilepsy, with the aim of identifying the epileptogenic zones, while the patients were under the effects of local anaesthesia. The major downside of this procedure is that it's very invasive, and requires a surgical procedure, but on the other hand it has several advantages over the conventional EEG [UPMC Brain Surgeons, online]:

- Can be performed before, after and during surgery
- Allows for direct electrical stimulation on the brain
- Great spatial resolution, because the recorded signal are closer to the source of origin, and not the sum of disperse potentials
- More resilience against external interferences.
- Protection against volume conduction, which can be defined as the transmission of electric or magnetic fields from a primary current source through biological tissue towards the sensors. Below 1000 Hz this effect can be neglected, but it must be taken into account when the sampling frequency is greater.

1.2.2 Neuronal connectivity and physiology of the brain signals

At the cellular level, the nervous system is composed by neurons (the central element), glial cells (polyvalent cells with support and protection functions) and vascular cells (in charge of the blood flow).

Neurons are in charge of the generation, transmission and codification of the brain signals via electrochemical gradients. Using electric stimulation on their plasmatic membrane, they are capable of receiving and stimulating different stimuli.

Morphologically speaking, neurons are composed by three differentiated parts:

- The soma contains the genetic information, and the rest of the cell vital systems.
- The axon transports the electrical impulse all the way to the next neuron or the target organ.
- The dendrites, on top of the soma, allow receiving electrical impulses. A neuron can have one or more dendrites, in order to coordinate several impulses at the same time, enhancing the comprehension of more complex stimuli.
The synapse is the bonding zone between neurons or a neuron and the effector cell. There are two sorts of synapses, chemical and electrical. The process is started by the axon, and it reaches the vesicular glans that contains the neurotransmitters. When the chemical messengers reach the post-synaptic indent, they produce changes on the electrical properties of the membrane. Once the neurotransmitter has achieved the desired effects, it deactivates itself. Depending on the sort of synapse, the neuron can be stimulated or inhibited, depending on the ionic flow through the membrane’s channels.
As for the electrical synapse, a neuron transmits the action potential to another via ionic flow, through the union channels between neurons. This phenomenon doesn’t involve chemical neurotransmitters and is faster than the other kind of synapse. The signals we measured are mostly generated by pyramidal neurons named after their geometry. Basal dendrites emerge from the base and the apical dendrites come out of the cell body.

1.2.3 The nervous impulse and the local field potentials

Impulses are generated due to changes on the electrical properties on the plasmatic membrane. When the neuron is not stimulated, its membrane separates the two regions (cytosol and extracellular environment) with an uneven concentration of Na\(^{+}\), K\(^{+}\) and Cl\(^{-}\). The outer part of the membrane is charged with a slight predominance of positive ions, while the inner part is mostly charged with negative ions. This potential gradient is called resting state.

![Action potential of the neuron during the synaptic process](image)

*Figure 7. Action potential of the neuron during the synaptic process [University of California Davis, online]*

The excitable cells are those who are able to change their rest state and generate a nervous impulse. This change on the membrane is called action potential (PA) and opens the sodium channels, generating an influx of Na\(^{+}\) directed towards the inner part of the membrane, causing a reduction on the trans-membrane potential (a phenomenon called depolarization) . At this moment, the action potential flows through the axon, reaching 15 mV of potential. A couple of milliseconds later, the
channels potential goes back to the rest state, due to the opening of the potassium and sodium channels, in a process called repolarization.

The local field potentials (LFP) are signals generated by the joined action of the currents flowing from multiple nearby neurons in a small volume of tissue. LFPs can only be recorded on deep layers of the brain cortex. Besides the acquisition mode, the main difference between these signals and conventional EEG is that the signal doesn’t have to propagate all over the brain in order to generate a recordable potential, other than that, LFPs and EEG display the same oscillation patterns during wake and sleep states. Electrodes must be placed in a space where the single action of a neuron doesn’t dominate other neurons. Once the signal is registered the usual processing involves bandpass filtering, with cutoff frequencies at 0.1 and 200 Hz, approximately. [A. Compte et al., 2008]

![Figure 8. Electrical and chemical synapse comparison](Tulane University Cellular Neuroscience department, online)

1.2.4 UP and DOWN states

When the brain is asleep it presents two differentiated states, called UP and DOWN states. This activity is completely spontaneous and appears in
rhythmic patterns, a slow one, appearing during the alternation between UP and DOWN states (<1 Hz) and faster rhythms in the beta band (10-30 Hz) and gamma band (30-100 Hz). [A. Compte et al., 2008]

High frequency oscillations in the UP states of cortical networks are associated to a variety of cognitive and perceptual processes indicating neuronal synchronization, and they can also be found in in-vitro experiments with cortical slices. In other words, the UP states are short periods where the brain "wakes up" and regains something similar to its woken activity, depolarizing the neurons and generating higher action potentials. [C. Wilson, 2008]

UP states appear as high amplitude plateaus with a duration that ranges from 0.4 to 1 second, opposite to the more stable and low potential DOWN states, also called "silent states" due to low cerebral activity are also relatively free of noise. Neurons regain their repolarized state thus generating lower action potentials.

![Figure 9. UP and DOWN states found in our signals](image)

The most common methods to detect UP and DOWN states are based on all-points histogram subthresholding or the mean value of the Hilbert transform in the gamma band, later used in this study.
1.2.5 Interferences on the EEG signals

One of the main problems scientists face when recording EEG signals is that they have low amplitudes and that they exhibit low-frequency content, being prone to suffer external interferences greater than the biomedical signal itself. Depending on every subject and the level of cortical activity, the measurements can vary a lot; therefore it is important to pre-process and filter the interferences, so differences between subjects under the same conditions do not vary more than a 10% [M.R. Keshtkaran, 2014]. The most common sources of interferences are:

- **Capacitive and inductive interferences:** These interferences appear due to the inductive and capacitive properties on the elements that connect the patient and the recording system. This interference appears at the frequency of the electrical power line, 60Hz in the United States and 50 Hz in Europe.

- **Electrode-body surface contact interferences:** When the electrode touches the body, this can cause a distribution of the potential on the electrode surface. These interferences fluctuate at low frequencies (around 1Hz), and it's difficult to filter them because this frequency is normally an important part of the band of interest. The proper way to get rid of them is to make sure that the body surface and the electrode are adequately placed. Silver [Figure 10. Common histogram pattern for UP and down STATES [C.Wilson, 2008]}


[Image]
Chloride electrodes have a small polarization value, which helps avoiding contact interferences.

**Figure 11.** Common patterns of EEG artefacts [K. Adamczyk, 2014]

- **Interferences caused by other bioelectrical potentials:** Muscular movement, heartbeats, eye movement or breathing are other physiological events which may cause the apparition of electrical activity and may interfere with the recorded EEG.

- **Electrostatic charges:** These interferences are caused by the charges stored in the patient’s body, which flow to the ground. The effect of these charges appears as a slow oscillation, which causes a fluctuation of the baseline. In order to solve this problem, a high-pass filter between 0.05 Hz and 0.5 Hz is sufficient, but the filter must be carefully chosen because if the cut-off frequency is too high, it may affect the band of interest.

- **Reference electrode interferences:** In order to record a proper EEG, it is necessary to select an adequate reference that doesn’t
record any activity of interest, because in that case the activity won't be displayed in the recordings.

1.3 A general overview of Alzheimer's disease

1.3.1 What is Alzheimer's disease?

Alzheimer's Disease (AD) is a chronic neurodegenerative disease, whose main symptom is a dementia that worsens with time. Its symptoms may include behavioural changes, language problems, bladder incontinence and loss of other bodily functions, such as speech. This disease is the cause of 60-80% known cases of dementia, having a great impact in the family of affected people and in society.

This affection has no known cure, and its cause and apparition is not entirely understood by scientists. Diagnosing AD is a difficult task because patients suffering this disease in early stages have symptoms that can be confused with age associated memory loss. People over 60 years old are more prone to suffer Alzheimer’s even though it may also appear at the age of 40.

1.3.2 Causes

Alzheimer's Disease cause is unknown to scientists, and several factors have been proved to be influential for the development of the disease. Like other chronic conditions, experts believe that Alzheimer's develops as a complex result of multiple factors instead of just one main cause. However, there are two identified structures (plaques and tangles) that appear throughout the brain as AD spreads, which damage the neurons and end killing them.

Plaques appear when proteins called β-amyloid start to form clusters bonding with each other. These proteins originally come from the fatty tissue of the neuron. When they reach a certain size, these proteins block the synaptic connections of the neurons and damage them. As the cells begin to die, they also start to inflame, and it may lead to an activation of the immune system to devour such cells.
Tangles are curved strands of Tau protein, and they damage the neurons by destroying their vital supply system. In a healthy brain, Tau proteins remain straight, forming energy supply paths that get destroyed as the protein begins to form tangles, causing the neurons to die of lack of nutrients.

Scientists have identified two main risk factors for AD, age and genetics. As the age increases, so does the risk of developing the disease. After reaching 65 the risk doubles every five years, peaking at 85, when according to statistics, the risk of suffering Alzheimer's revolves around a 50%.

Over time, damage on the brain caused by AD is visible at a macroscopic scale. The cortex degradation affects the areas involved with thinking, memory and planning, the hippocampus shrinks, which makes the formation of new memories harder, and finally, ventricles full of fluids swell. All this factors combined give the AD brains its characteristic "spongy" look.
Genes also have a key role in the development of AD. There are two types of genes, which may cause the disease: risk genes and deterministic genes. Risk genes increase the probabilities of developing the disease, but it is not certain it will spread. There are four main genes of this type:

- **Amyloid precursor protein (APP):** It was discovered in 1987, being the first Alzheimer's gene to be tracked down. It's related to the apparition of amyloid plaques.

- **Presenilin-1 (PS-1) and Presenilin-2 (PS-2):** They are one of the most common causes of genetically transmitted AD.

- **Apolipoprotein E-4 (APOE4):** This gene has the biggest impact in terms of risk. It may appear in other forms, such as APOE-e2 or APOe-E3. Every person inherits some form of this gene from both parents, and the higher risk is carried by the APOE4 form. Those who have inherited APOE4 from both parents present the higher risk, being the cause of up to 25% of the total cases.

### 1.3.3 Symptoms

As a degenerative pathology, symptoms change and worsen over time. AD development can be split in 7 phases, but not all the patients suffer all of the symptoms and some stages may overlap [Alzheimer's Association, online]:

1. **Normal function:** No memory dysfunction can be identified during this phase.
2. **Very mild cognitive decline:** The patient may start noticing a few memory lapses, can still lead a completely normal life.
3. **Mild cognitive decline:** At this point, it starts to be noticeable that the patient's brain performance is decreasing. Doctors may be able to spot memory and concentration problems, such as difficulty to find objects, to come up with words or to interact with people.
4. **Moderate cognitive decline:** Recent events memories begin to fade and complex tasks difficulty is drastically increased.
5. **Moderately severe cognitive decline:** Important memories like the patient's own phone number or address, along with mathematical capabilities disappear.
6. **Severe cognitive decline:** Personality changes and help during daily activities are frequent at this stage. Patients are often
unable to have a proper sleep pattern, recognise familiar faces and may start to wander and become lost.

7. **Very severe cognitive decline:** In the final stage of this disease, patients lose the ability to respond to their environment, to carry on a conversation and, eventually, to control movement. They may still say words or phrases. At this stage, individuals need help with much of their daily personal care, including eating or using the toilet. They may also lose the ability to smile, to sit without support and to hold their heads up.

Life expectancy varies for each person with Alzheimer’s disease (AD). The average life expectancy after diagnosis is eight to 10 years. In some cases, however, it can be as short as three years or as long as 20 years [Alzheimer's Association, online].

### 1.3.4 Diagnose

To diagnose Alzheimer’s disease, doctors evaluate the signs and symptoms and conduct several tests. An accurate diagnosis of Alzheimer’s disease is an important first step to ensure the quality of the patient’s life. An early recognition is the best way to deal with the disease, but at the same time it's difficult sometimes to differentiate AD from age-related memory loss. During the early stages of AD a doctor would evaluate:

- The impairment of the patient’s cognitive skills.
- Behavioural/personality changes.
- Memory impairment.
- How dementia affects patient’s life.

Besides interviews to both patients and relatives, some other test are run:

- Thyroid examination in order to rule out vitamin B-12 deficiency.
- Neurological tests for other neurodegenerative diseases such as Parkinson's.
- Brain imaging tests such as MRI or CT scan to search for brain tumours, strokes or haemorrhages and at the same time state the level of cerebral degeneration.

In the current state of the art, scientists are investigating a number of disease markers and diagnostic tests, such as genes, disease-related proteins and imaging procedures, which may accurately and reliably indicate if the patient has AD and how much the disease has progressed. [Alzheimer's Association, online]

### 1.3.5 Treatment
As a multi-stage and multi-symptomatic disease, Alzheimer’s disease has several treatments which may work with a different degree of effectiveness, but for the moment they can't cure the disease, just slow the worsening of its symptoms.

For the memory loss there are two kinds of treatments depending on the stage of the disease. For the early and moderate stages, cholinesterase inhibitors are prescribed, in order to prevent the breakdown of acetylcholine, a neurotransmitter. It delays the worsening of symptoms for 6 to 12 months and it's generally well tolerated, with side effects such as nausea, loss of appetite and diarrhoea.

Current research is taking a whole new approach. Instead of dealing with the symptoms, scientists now also want to aim to the root of the problem, the formation of plaques and tangles and agree that the cure will be found when a medication that aims at all the different effects and causes of Alzheimer’s can be successfully developed. Researches have set 4 different targets for the drugs of the future:

- Beta Amyloid
- Tau protein
- Inflammation
- Insulin resistance

1.3.6 Statistics

As the following graphics show, dementia rates increase as in older population. Women have a higher prevalence of dementia. Two studies have been conducted at European level, EuroDem in 1999 and EuroCODE in 2006. As it can be seen, the prevalence rates in men stayed the same for almost every group of age, however, in women it has increased significantly. Researchers concluded that in this phenomenon is not due to an increase in AD patients, but due an underreporting on the females. Women have been found to be more prone to develop dementia as their age advances.
Figure 14. Prevalence of dementia on men, according to EuroDem and EuroCoDe studies. [Alzheimer Europe. Prevalence of dementia in Europe, 2006]

Figure 15. Prevalence of dementia on women, according to EuroDem and EuroCoDe studies. [Alzheimer Europe. Prevalence of dementia in Europe, 2006]
In 2006, a study conducted estimated that 7.3 million people in Europe were affected by Alzheimer’s disease, 2.3 million men and 5 million women.
CHAPTER 2: OBJECTIVES

2.1 General objectives

The main aim of this study is to assess the differences between control mice and mice with Alzheimer's disease using connectivity measurements, on two regions of the brain cortex (somatosensory and prefrontal), evaluating the magnitude of such differences and design a procedure to differentiate brains with Alzheimer’s from healthy brains.

2.2 Specific objectives

The following list states the specific objectives:

• Remove the interferences and correctly pre-process the signals.

• Detect of the UP and DOWN states automatically.

• Apply connectivity measurements and obtain the values on the gamma band.

• Select the most statistically significant variables.

• Design a procedure to differentiate signals from mice with Alzheimer's Disease from healthy mice.

• Study the differences between the somatosensory and prefrontal cortex and between healthy and diseased brains.

• Analyse the meaning of the obtained values.
Personally speaking, I expect this project to provide me a good insight on how biomedical engineers work during research with signals, allowing me to use the theoretical and practical concepts learnt during my studies, to improve my MATLAB programming skills and my comprehension of signal processing algorithms with both guided and autonomous work.
CHAPTER 3: METHODOLOGY

3.1 Description of the database.

Alzheimer’s appears normally after the age of 65 and because of that, it was necessary to create a murine model that resembled as much as possible the elderly people’s common symptom. A senescence-accelerated mouse (SAM) was achieved after adding senescence-prone inbred strains (SAMP) to their DNA. This newly introduced genetic information caused an early onset of age-related pathologies such as loss of activity, hair loss, amyloidosis, osteoporosis among others. The SAMP8 strain caused deficits in learning and memory, impaired immune response and immune circadian rhythms, and it was chosen as the best possible phenotype to work on an AD late-onset, brain model.

This newly developed mouse is based on early aging more than on the mutations themselves and their APP genes on the hippocampus don’t have any mutation that can be found on the human familial AD, however, cognitive decline is evident. Spatial learning and memory ability were tested using different kinds of mazes, and most of them showed a significantly increase on the time it took the mice to complete the trial. Other tests that showed good results were fear conditioning (used to measure hippocampal-dependent and associative memory by exposing the mice to aversive stimuli) and object recognition (used to test cortex-dependent declarative memory) [X.Cheng, 2014].

Besides the tests, the mice brain is also inspected, showing an increase in some signs associated to cerebral degeneration, such as neuron loss, gliosis, spongiosis, plaques and tangles.

The database we worked with is composed by 17 mice, 8 healthy controls and 9 diseased subjects. Each of the subjects provided two recordings, one from the somatosensory cortex and one from the prefrontal cortex.
3.2 Obtaining the signals

The signals were recorded at Sanchez-Vives laboratories, an organisation specialised in electrical activity spontaneously generated by the cortical network. This institution is affiliated to Institut d’Investigacions Biomèdiques Pi i Sunyer (IDIBAPS) which works on several fields of biomedical research, such as respiratory diseases, diabetes, among others.

Due to the invasive nature of the procedure, it is necessary sedate the mice before the insertion of the electrodes. The scientists perform a cut on the mice scalp and then proceed to deploy the electrodes on both hemispheres of the somatosensory or the prefrontal cortex and the leave the mice in asleep state, in order to record the spontaneous UP/DOWN states.

*Fig 16. A sedated mouse after implanting the electrode*

The chosen sensors to register the signal are made of tungsten, with a diameter of 50 µm and an impedance of 3MΩ. There are several advantages in using this type of electrodes such as high low frequency impedance, low high-frequency impedance, high signal-to-noise rate, ruggedness and stable recordings. This allows a production of electrodes with small tips which give a better isolation of the high frequencies of the gamma band. On the other hand, tungsten electrodes are noisy at very low frequencies, which make a high-pass filtering necessary prior to the analysis.

*Fig 17. Several tungsten electrodes for EEG recordings [Micromed, online]*
The signal was sampled with a CED (Cambridge Electronic Design) acquisition card and Spike software at a 20 KHz sampling rate. The multichannel system filters the signal with a bandpass filter digital system between 0.01 Hz and 2.5 KHz, and then proceeds to resample it to 5 kHz. Channel 1 is associated to the right hemisphere and channel 2 represents the left hemisphere. In order to be able to process the signals with MATLAB we developed a script which converts Spike’s .smr files into MATLAB’s .mat files.

3.3 Pre-processing of the signal

3.3.1 Power line interference removal

Power line interference may corrupt neural recording at 50 Hz and harmonic frequencies. Given that the band of interest of this study ranges from 0.1Hz to 200 Hz and also from 30 Hz to 100 Hz for the up/down states, it is absolutely necessary to eliminate such interference. A simple notch filter is not the appropriate solution for this case, because completely eliminating the 50 Hz frequency means removing information from the band of interest. In order to solve remove the right amount of the band, adaptive filtering was used.

![Block diagram of the adaptive filter](image)

**Figure 18.** Block diagram of the adaptive filter [M.R. Keshtkaran et al 2014]

Four steps comprise the algorithm used to remove the power line. First of all, an adaptive notch filter estimates the fundamental frequency of the interference. After obtaining the value using discrete time oscillators, the harmonics are found. Subsequently, through a recursive least squares algorithm, the estimated value without interference of the phase and amplitude of the signal is calculated. The last step is finally removing the interference data from the previous estimation.
Compared to other approaches, the method offers very good results, obtaining a high rejection rate on the output (SNR > 30dB), and a fast convergence time (<100 ms) because the algorithm doesn’t look for individual peaks, therefore is not necessary to analyse large sums of samples.
As the previous image shows, the power line interference is gone almost completely, but some of it still remains at 150 Hz. By looking at the amplitude (almost 3 decades smaller than the 10-30 Hz band), its influence on the signal can be considered negligible after filtering.

3.3.2 Selection of the band of interest

In order to study only the frequencies that contain relevant information it is necessary to establish the cutoff limits of the passband. There are several filters that can do such task, and amongst them the Chebyshev filter was chosen because of its steep roll-off causing a bigger attenuation at the beginning of the stopband than other filters, meaning that with a smaller order they can be more effective than, for instance, Butterworth filters. On the other hand, Chebyshev filters have a small ripple in their passband or in the stopband, according to the chosen type, which cause some minor modifications on the frequencies of interest.

Type I Chebyshev filters are the most common filters of their kind, and the ripple is found at the passband. The gain of the ripple is determined by the $\varepsilon$ factor, and normally ranges between ± 1 dB, thus establishing the Gain of the passband with the following equation:

$$G = \frac{1}{\sqrt{1 + \varepsilon^2}}$$

At the cutoff frequency, the attenuation increases, until it approaches the ripple value again.

*Figure 21. Comparison of the Chebyshev with other filters [Stack Overflow, online]*
Type II Chebyshev filters have the opposite effect on the signal, the produce ripple on the stopband, but have no effect on the stopband. However, one of the main disadvantages of the Type II is that the roll-off is not as steep as in Type I, and that it requires more physical components to be built. The gain on the stopband is determined by the following equation:

$$G = \frac{\varepsilon}{\sqrt{1 + \varepsilon^2}}$$

(2)

Given that Chebyshev filters feature an infinite impulse response, in order to maintain the group delay lineal it is necessary to apply them twice on the signal, with a temporal inversion, because otherwise the signal would suffer a nonlinear phase distortion.

Two kinds of passband filters have been used on the signals. The first of them has the cutoff frequencies at 0.1 and 200 Hz, and it's used for the spectral analysis. The second one has the cutoff frequencies at 30 and 100 Hz, and it's used for the connectivity measurements, in order to study the beta and gamma band, which displays clearly the up and down states of the brain.

![Power spectral density on the band of interest after filtering (30 - 100 Hz)](image)

**Figure 22.** Power spectral density on the band of interest after filtering (30 - 100 Hz)
Figure 23. Logarithmic power spectral density on the band of interest after filtering (30 - 100 Hz)

The filters used on the signals have been configured with the following characteristics:

- Type I
- 0.05 Hz roll-off
- 1 dB ripple on the passband
- 40 dB attenuation on the stopband

3.3.3 Segmentation of the UP/DOWN states

In order to properly separate de UP states from the DOWN states on the 30 to 100 Hz band, it is necessary to use a technique that allows obtaining the analytical signal, a complex-valued function without negative frequency components that facilitates the signal manipulation. The Hilbert transform is a linear operator that within the same domain, extends the function to the complex plane and adds information on the signal's phase. It's defined by the following equation:

$$\mathcal{H}[g(t)] = g(t) * \frac{1}{\pi t} = \frac{1}{\pi} \int_{-\infty}^{\infty} g(\tau) \frac{1}{\tau - t} d\tau = \frac{1}{\pi} \int_{-\infty}^{\infty} \frac{g(t - \tau)}{\tau} d\tau$$

The Hilbert transform is the convolution of $g(t)$ with the signal $1/nt$. It's the equivalent of a time-invariant filter that has an impulse response of
1/\pi t. One of the main problems of this operator is that it’s defined by an improper integral. In order to solve that problem, the Cauchy principal value ($\epsilon$) is applied on the integral limits in order to range the integral.

\[ \mathcal{H}[g(t)] = \frac{1}{\pi} \lim_{\epsilon \to 0^+} \left( \int_{t-1/\epsilon}^{t-\epsilon} \frac{g(\tau)}{t-\tau} d\tau + \int_{t+\epsilon}^{t+1/\epsilon} \frac{g(t-\tau)}{\tau} d\tau \right) \]

One of the main uses of this transform is to calculate the instantaneous phase and the envelope of the signal, which helps define the limits of the up and down states.

Once the envelope is calculated, thresholding with respect to the mean value of the envelope is used to separate both states. Those values which are above the threshold will be considered UP states and those values place under the mean value will be considered DOWN states. In order to filter short burst or oscillations, only epochs of 500 samples or more will be considered for the analysis. In connectivity measurements which require simultaneous channels, only the samples which can be found at the same UP or DOWN epoch in both channels will be considered for analysis.
3.4 EEG analysis techniques

Several variables have been calculated in order to obtain numerical information from the EEG signals. In these chapters, the measurements will be explained with thoroughly, in order to clarify which information is expected to be obtained from each one of them.

3.4.1 Cross-correlation

Cross-Correlation is one of the most classical and widespread measures of interdependence between two time series. Its principle is based on the measurement of similarity of the values of the two variables X and Y for a given time delay (τ), having the same or opposite sign. When a time series is normalized to have zero mean and unit variance, the correlation function is defined as [E. Pereda, 2005]:

\[ C_{xy}(\tau) = \frac{1}{N-\tau} \sum_{k=1}^{N-\tau} x(k + \tau) y(k) \]

(5)

Where N is the total number of samples. This normalized function ranges from -1 (total inverse correlation) to 1 (total direct correlation). As a general rule, when the value is higher than 0.7 the correlation index is
considered to be high. Normally, the correlation is measured for a range of delay values; obtaining the delay associated to the highest correlation value it’s possible to determine how apart in time are both signals. This function normally produces an index plot similar to the following:

![Normalized Cross-Correlation Plot](image)

**Figure 26.** Typical aspect of a normalized cross-correlation plot.  
[E. Pereda, 2005]

This procedure is used in several recent studies, and has several applications such as classifying epileptic seizures [Jerger et ál., 2005] and analyzing schizophrenia patients [Jalili and Knyazeva, 2011].

### 3.4.2 Approximate and Sample Entropy

The Approximate Entropy (ApEn) is a variable that allows calculating the conditional probability of similarity between a chosen data segment of a given duration and the next set of segments of the same duration. This parameter shows the complexity of a data set, the higher the entropy, the more complex the signals are.

\[
m = 2: \\
\hat{r}_{\text{max}} = (-0.02 + 0.23 \sqrt{sd_1/sd_2})/\sqrt{N/1000}.
\]

\[
m = 3: \\
\hat{r}_{\text{max}} = (-0.06 + 0.43 \sqrt{sd_1/sd_2})/\sqrt{N/1000}.
\]

\[
m = 4: \\
\hat{r}_{\text{max}} = (-0.11 + 0.65 \sqrt{sd_1/sd_2})/\sqrt{N/1000}.
\]

**Figure 27.** Signal-to-Noise ratio formulas for every embedded dimension. [S.Lu, 2008]
Two variables define the way ApEn is calculated, \( m \) and \( r \). The first one defines the embedding dimension, meaning the amount of samples that the segment to analyse contains, and it ranges between 2 and 4. The second one defines the signal tolerance to noise, and usually ranges between 0.1 and 0.2 times the standard deviation of the signal, but for brain signals this parameter is optimized to obtain the best entropy estimation as described in figure 27 [S.Lu, 2008]:

- **sd1**: Standard deviation of the signal differential, representing the short term variability.
- **sd2**: Standard deviation of the signal differential, representing the long term variability.
- **N**: Total number of points of the segment.

When the signal to ratio has been optimized, the ApEn index can be calculated with the following algorithm [S.Lu, 2008]:

1. Form vectors from \( X(1) \) to \( X(N-m+1) \) defined by:

\[
X(i) = [x(i), x(i+1), ... X(i+m-1)]
\]

\[
i = 1, N - m + 1
\]

\( (9) \)

2. The distance between vectors is defined as the maximum difference between their respective scalar components:

\[
d[X(i),X(j)] = \max_{k=0}^{m-1} |x(i+k) - x(j+k)|
\]

\( (10) \)

3. For each \( i \), from \( i=1, N - m +1 \):

\[
C^m_{(r)} = \frac{V^m(i)}{(N-m+1)}
\]

\( (11) \)

Being:

\[
V^m(i) = number \ of \ d[X(i),X(j)] \leq r
\]

\( (12) \)
4. The index $\phi$ for the embedded dimension $m$ is calculated as follows:

$$\phi^m(r) = \frac{1}{N - m + 1} \sum_{i=1}^{N - m + 1} \ln \left( \mathcal{C}^m_r(i) \right)$$

This procedure is repeated for every size of embedded dimension up to $m + 1$:

5. ApEn is calculated

$$\text{ApEn} (m, r, N) = \phi^m(r) - \phi^{m+1}(r)$$

SampEn (Sample entropy) is another modification on ApEn, which improves the resistance of the parameter to small data epochs and is easier to calculate. Other advantages of SampEn over ApEn are that it doesn’t compare the template vectors to itself, thus lowering the actual value of the calculation.

3.4.3 Mutual Information and area under the curve

Mutual Information evaluates, for a pair of given time series (X and Y), the amount of information of X can be known just by analysing Y. Mutual information calculating with the following formula:

$$M_{XY} = \sum_{i,j} p_{ij} \log \frac{p_{ij}}{p_i p_j}$$

With $p$ being the discrete probability distribution for each time series. If the closer the obtained index is to zero, the more independent are the time series, at the same time the parameter calculation can be modified so it attains its highest value at 1 (Wang et al. 2005). Mutual Information is symmetrical, meaning that changing the order or the X and Y crossing doesn’t provide information on the direction of the information. However by looking at the shape of the MI plot for a given $\tau$ delay, it can be estimated. The obtained plots have a look similar obtained to those obtained when calculating a cross-correlation,
In order to obtain an accurate estimation, MI information requires a large number of samples, analysed in small bins of the same size, otherwise many $p_{ij}=0$ may appear, resulting in an underestimation of the MI coefficient. More modern refinements on the method include an adaptive size of the embedded dimensions calculated by the $K$-nearest neighbour method. Another calculation obtained of the MI plot is the area under the curve as a measurement of linear and nonlinear couplings, and is calculated with the following formula:

$$\text{Area} = \frac{\sum_{i=-\text{Delay}+F_s}^{\text{Delay}+F_s} X(i) \times \text{Sampling Frequency}}{\text{Number of Samples}}$$

(16)

MI has been proved useful to measure stimuli-response relationships [Eckhorn and Popel 1974], to give a better view on synaptic connectivity [Yamada et al. 1993], as an index of synaptic efficiency [London et al., 2002], or more recently to study connectivity after drug administration [Alonso et al., 2010]

### 3.4.4 Phase-Lag Index

The Phase-Lag Index (PLI) measures how asymmetrically distributed the phases of two signals are. The phases of two signals are considered locked when their difference is constant. In order to calculate the phase synchronization, the instantaneous phase value is needed. The main aim of the PLI is to calculate the phase synchronization while at the same time reject the influence of common sources, such as reference electrodes [C.J. Stam 2007].
PLI values range from 0 (no coupling between phases) to 1 (perfect phase locking). Off the phase difference remains near to constant, the PLI will be closer to one. The PLI does not show which one of the signals is leading in phase.

A phase synchronization index can be defined as:

\[ |\Delta \phi_{m,n}| = |n\phi_1 - m\phi_2| \]  

(17)

In order to calculate the phase synchronization it is necessary to know the instant value of the phase. This value can be obtained by using the Hibbert transform, decomposing the time series in real and imaginary components:

\[ z(t) = x(t) + \mathbf{i}x = Ae^{i\phi(t)} \]  

(18)

The imaginary component \( \mathbf{x} \) is obtained integrating the following function

\[ \mathbf{x}(t) = \frac{1}{\pi} PV \int_{-\infty}^{\infty} \frac{x(\tau)}{t-\tau} d\tau \]  

(19)

Where PV refers to the Cauchy principal value, which is a number that prevents improper integrals from being undefined. Depending on the type of singularity, the assignation method varies. Once completed the integration, both instantaneous amplitude and phase can be calculated.

The PLI has been tested against common sources that could cause a miscalculation of the index, such as volume conduction and active reference electrode. This effect is obtained by discarding phase differences that centre on 0 mod n, by defining an asymmetry index for the distribution of the phases, when the distribution is centred around a phase difference of zero. When there’s no coupling between two time series, the distribution is flat, and when the distribution is not flat, the coupling exists.

The asymmetry of the phase distribution causes a different \( \Delta \phi \) for the intervals \([−n 0]\) and \([0 n]\). The assymetry implies a nonzero, phase difference, called 'lag', unaffected by the aforementioned artefacts.

Recent research has led to the development of an even more robust phase synchronization estimator, the Weighted Phase-Lag Index (WPLI). This index is more resistant to volume conduction, common sources and
small size of the samples bias. The novelty in the WPLI is the normalization in the denominator:

$$\phi = \frac{|E[|\Im\{X||\text{sgn}(\Im\{X\})|]|]}{|E[|\Im\{X|]|]|}$$

(20)

WPLI ponders the effect that the noise spectrum has on the estimation. The thickness of the coloured circles indicates the small relative weight of the frequencies nearby the noise sources have on the index's value, but the bigger relative weight of the frequencies as they move away from the noise source, whereas all frequencies have the same weight on the regular PLI [M.Vinck, 2011]

**Figure 29.** Representation of how the WPLI weights the value of $\phi$ for each frequency according to its distance to the noise. [M.Vinck, 2011]

### 3.4.5 Synchronization Likelihood

Generalized synchronization exists between two dynamical systems X and Y when Y responds to a variation of X, despite having a time series that doesn't resemble each other. Synchronization likelihood (SL) estimates the dynamical interdependences between all the channels, ranging from 0 (completely unsynchronized systems) to 1 (complete synchronized systems).
This index is based on the measurement of Euclidian distances between a pair set of points that belong to different channels, and if they are greater than a given critical measurement. In this case the system is considered to be unsynchronized, but if it's smaller, then some more calculations are applied to calculate the degree of synchronization.

The following parameters are used to calculate the S:

- **M**: Number of channels
- **k**: Indicates the number of the channel \( k = 1, \ldots, M \)
- **N**: Number of samples
- **i**: Indicates the number of the sample \( i = 1, \ldots, N \).
- **\( \varepsilon \)**: Critical Euclidean distance.
- **m**: Size of the embedded dimension.
- **I**: Size of the estimated lags.
- **\( \mathbf{X}_{k,i} \)**: Vector containing the samples.
- **\( \theta \)**: Heavyside step function.
- **\( w_1 \)**: Window for Theiler Correction.
- **\( w_2 \)**: Window to sharpen the time resolution.
- **\( p_{\text{ref}} \)**: Reference probability, ranging from 0 to 1.

The first step is used to calculate the probability of two embedded vectors of the same channel to be closer to each other than the critical distance \( \varepsilon \).

\[
P_{k,i}^\varepsilon = \frac{1}{2(w_1 - w_2)} \sum_{j=1}^{N} \theta(\varepsilon - |X_{k,i} - X_{k,j}|)
\]

(21)

After that, amongst all the vectors within the designed window that can be found under the critical distance are counted in every channel, obtaining a number between 0 and M, stored in the variable \( H_{k,i} \).

\[
H_{k,i} = \sum_{j=1}^{N} \theta(\varepsilon - |X_{k,i} - X_{k,j}|)
\]

(22)

The next step calculates the SL index for the embedded segment, defined as \( S_{k,i,j} \):

- If \( |X_{k,i} - X_{k,j}| < \varepsilon \) then \( S_{k,i,j} = \frac{H_{k,i,j} - 1}{M - 1} \)
- If \( |X_{k,i} - X_{k,j}| > \varepsilon \) then \( S_{k,i,j} = 0 \).
Averaging the $j$ dimension, the mean SL is obtained:

\[
S_{k,t} = \frac{1}{2(w_1 - w_2)} \sum_{j=1}^{N} S_{k,i,j}
\]

This technique was originally used to measure variations on the synchronization of the alpha band associated to eye-opening and gamma band decreases in Alzheimer's disease patients [Stam and van Dijk, 2001].

### 3.4.6 Power Spectral Density

This measurement describes the distribution of the amplitude of the signal for each frequency, stating how much they affect the characteristics of the signal. Calculating the real value of the power of the signal is not possible, because real signals are limited by finite time, are not fully stationary and are affected by different noise sources; therefore, the obtained value is estimation. By acknowledging the power of the signal other parameters can be calculated such as the mean and median frequency, which will be described further.

PSD is related to the Discrete Fourier's Transform (DFT) using Wiener-Khinchin's Theorem:

\[
S_{xx}(f) = FT(R_{xx}(\tau)) = \int_{-\infty}^{\infty} R_{xx}(\tau)e^{-2\pi if \tau} d\tau
\]

Where FT means Fourier Transform and $R_{xx}$ is the value of $x(t)$ autocorrelation function. Power is obtained integrating $S_{xx}$ for every frequency on the spectrum:

\[
P = \int_{-\infty}^{\infty} S_{xx}(f) df
\]

In order to obtain a smoother and averaged spectrum, which helps improving the performance of the estimators, a technique called Welch's Periodogram is used. Applying a Fast Fourier Transform (FFT) to different epochs of equal length and averaging the obtained results. The main downside of this method is that spectral resolution decreases and leakage increases. In order to solve the later problem, different types of windows can be applied. In this project we selected a Hanning window, a cosenoidal wave:
Figure 30. Normalized Hanning Window and its effect on lateral lobe attenuation.

The algorithm to calculate the periodogram works as it follows:

1. Splitting the signal in equal length epochs
2. Calculation of FFT for each epoch
3. Displacement of the window on the signal. If the FFT has $N$ points, the displacement ($D$) tends to be smaller than $N$, producing an overlap that helps keeping some of the spectral resolution.

$$x_i(n) = x(n + iD); \quad n = 0,1,...,N-1$$ \hspace{1cm} (26)

3.4.7 Mean and Median Frequencies

Mean Frequency (MNF) can be defined as the centre of gravity of the PSD and is calculated with the following expression:

$$MNF = \frac{\sum_{j=1}^{M} f_j P_j}{\sum_{j=1}^{M} P_j}$$ \hspace{1cm} (27)

Being $f_i$ the frequency and $P_j$ the value of the power for that frequency.
Median Frequency (MDF) is the frequency that divides the spectrum into two sub-regions of equal amplitude, having the properties stated in the following equation.

$$
\sum_{j=1}^{MDF} P_j = \sum_{j=MDF}^{M} P_j = \frac{1}{2} \sum_{j=1}^{M} P_j
$$

These two parameters are used frequently to describe the geometry of EMG spectrum [Phinyomark et al., 2010] and in stimulus-response parameterization for auditory response in EEG signals [Sharpe et al., 1997].

3.5 Representation of the obtained results

After obtaining the results, they must be displayed in a way that allows a clear view of the values. Amongst all the methods, we chose the notched boxplots, graphs that display the data using the quartiles of the values. Boxplots don't make assumptions on the distribution of the population, and allow us to see the dispersion and skewness of the data. Several information can be perceived just by looking at the lines of the notched boxplot, which makes it useful as a method to compare several datasets.

Figure 31. Parts of the notched boxplot [David's Statistics, online]

The following parts of the boxplot contain the most relevant information:
- **The box:** Contains the interquartile range (IQR) from the first quartile to the third quartile, where the 50% of the data is found.

- **The whiskers:** Are separated by 1.5 times the IQR from the third and first quartile, unless the first and third quartile are larger than such distance.

- **The line:** Indicates the median.

- **The notch:** Confidence interval. When the notches of two datasets don't overlap, there's a 95% chance that their medians are statistically different. It is very useful to assess if there are significant differences between two datasets.

![Boxplot Diagram](image)

*Figure 3. Boxplots are related to normal distributions [David's Statistics, online]*
3.6 Statistical tests

After obtaining all the values is necessary to analyse which of them contain relevant information. It is possible that some of the values follow some random pattern that is statistically unrelated which may lead us to a wrong interpretation of the results. In order to avoid this we chose, the T-Student and the Wilcoxon-rank-sum test.

3.6.1 T-Student

The T-test is a hypothesis test that assumes that the samples follow a Student distribution, reaching the highest probability at the mean value. It is very useful because of the central limit theorem, the symmetry and non-skewedness. While the normal distribution is used mostly for full populations, the Student distribution is optimized to work with small samples of the population. After the test, we obtain results that state if the samples follow a normal distribution or not, information of the mean of the samples group and the probability that the sample values are just random measurements.

Taking two sample groups (healthy and diseased mice) we can assess if the population means differ by pairing the samples and using non-paired samples to obtain extra information to increase the statistical power and reduce the effect of the confounders.

![Students T Distribution PDF](image)

**Figure 33.** Varying degrees of freedom affect the density [David’s Statistics, online]
The density function of the T-Student distribution is defined by the following equation:

\[ f(t) = \frac{1}{\sqrt{\nu}B\left(\frac{\nu}{2}, \frac{\nu}{2}\right)} \left(1 + \frac{t^2}{\nu}\right)^{-\frac{\nu+1}{2}} \]

(28)

Two parameters shape the function:

- **\( \nu \)**: Number of degrees of freedom, meaning the number of values which are free to vary without violating any constraint.
- **\( B \)**: Beta function, an Euler integral that has the following form:

\[ B(x, y) = \int_0^1 t^{x-1}(1 - t)^{y-1} dt \]

(29)

### 3.6.2 Wilcoxon-rank-sum test

This test is non-parametric, meaning that does not suppose that the samples follow a normal distribution. It is normally used as a counterpart to the T-Test because it has greater efficiency when working with non-normal distribution and has almost the same efficiency for normally distributed populations.

This test assumes that:

- All the observations from both groups are independent.
- The observations are ordinal, meaning that they have ranked values.
- Both group of samples follow the same distribution.
- The hypothesis of \( X > Y \), is not equal to 0.5

The value we need to obtain is \( U \), an unbiased estimator that used to compare the relationship between samples. This parameter has a known distribution function under the null hypothesis (supposing all values are unrelated). For a small number of samples, as in our case, the calculation of \( U \) is done by simply comparing the sample of one group to the rest of samples of the other group, and counting how many times it’s greater, in case of tie, 0.5 must be summed. Once the \( U \) value is obtained, in case the distribution is not normal, there are tables where the associated probability can be found.
For all the values statistical tests ran in our study, we set the statistical significance to 0.05.

### 3.7 Classification of the data

The last stage of the study is to design a procedure to know if with the most significant variables only is possible to determine if a mice has Alzheimer’s Disease or not. The mathematical operator that can separate two or more classes is called a classifier, and, amongst the several types of classifiers we chose the linear discriminant analysis (LDA).

#### 3.7.1 Linear discriminant analysis

LDA searches a linear combination of statistical characteristics that best describes the data and models the differences between the classes \( \omega_i \) of data. This method can’t measure interdependence, meaning that prior to the discriminant analysis; some work must be made in order to ensure that all the variables are independent.

Mathematically speaking, LDA takes a dataset with N dimensions, and tries to create a line which reduces the dimensionality, while at the same time preserving as much of the discriminatory information as possible.

![Figure 34. The line on the left separates the classes better than the line on the right [Ciemat, online]](image_url)

The samples are projected onto the following line:

\[
y = w^T x
\]

After that, the mean vectors for \( x \) and \( y \) are defined for each class:

\[
\mu_i = \frac{1}{N_i} \sum_{x \in \omega_i} x \quad \bar{\mu}_i = \frac{1}{N_i} \sum_{y \in \omega_i} y
\]
By substituting $x$ and $y$ for the equation of the projected line we obtain:

$$
\hat{\mu}_i = w^T \mu_i
$$

(32)

Working only with the mean value is not a good option, because it doesn't take into account the standard variation between classes. Fisher proposed to maximize the difference between means, by calculating the scatter within the same class, defined as the variance:

$$
s_i^2 = \sum_{y \in \omega_i} (y - \mu_i)^2
$$

(33)

The separability criterion function now takes the within-class scatter into account and it's defined as:

$$
J(w) = \frac{||\hat{\mu}_1 - \hat{\mu}_2||^2}{s_1^2 - s_2^2}
$$

(34)

![Figure 35. After Fisher's modification, the line also maximizes distance considering the scatter [Ciemat, online]](image)

In the feature space $X$, the scatter is defined as:

$$
S_i = \sum_{x \in \omega_i} w^T (x - \mu_i) (x - \mu_i)^T
$$

(35)

The sum of $x$ scatters, leads to obtaining the within-class scatter matrix $S_w$. The scatter of the $y$-projection can be expressed as a function of the scatter matrix in the space $x$:

$$
\hat{s}_i^2 = \sum_{y \in \omega_i} (y - \hat{\mu}_i)^2 = \sum_{x \in \omega_i} (w^T x - w^T \mu_i)^2 = \sum_{x \in \omega_i} w^T (x - \mu_i) (x - \mu_i)^T = w^T S_i w
$$

(36)
The quadratic sum of the scatters in the $y$-space is now related to the slope and the within-class scatter:

$$s_1^2 + s_2^2 = w^T S_w w$$  \hspace{1cm} (37)

Following the same procedure, the difference between projected means can be expressed in terms of the means in the original feature space and the between-class scatter, $S_B$.

$$(\bar{\mu}_1 - \bar{\mu}_2)^2 = w^T S_B w$$  \hspace{1cm} (38)

Now the criterion equation is expressed in terms of the scatter and the $w$ slope. Deriving it and solving the equation obtain the optimal $w$ value obtained, forming the line that better separates classes:

$$J(w) = \frac{w^T S_B w}{w^T S_w w}$$  \hspace{1cm} (39)

### 3.7.2 Leave-one-out

The cross validation is a procedure used in classifiers when the datasets are small, in order to increase the reliability of the results. Leave-one-out is one of the most common procedures of this kind, and it's principal characteristic is that it runs as many classification tests as there are samples.

The first sample is left apart, and the rest of the samples are used as training samples, in order to build the classifying model. Once the model is completed, the first sampled is classified and the results are noted. This procedure is repeated for every sample, until each one of them has been left apart once, finishing with $N$ classified samples and $N$ different training matrixes.

One of the main advantages of the Leave-One-Out procedure is that is very difficult to produce bias on the classifier. On the other hand, in order to obtain an optimal classification, the most significant parameters can't be chosen prior to the classification, because the optimal parameters can change with each new training matrix.
3.7.3 Stepwise regression

Stepwise regression is a procedure that builds a model by successively adding or removing variables, based on the values obtained during t-tests. It’s especially useful to work rapidly amongst large number of variables and select the best combination.

There are several approaches to the procedure; the one we used is called forward. It starts with variable that contains the best significant information, and it adds one variable at the time. For each step, the program computes the t-statistic and then squares it, obtaining a new coefficient called $F$, used to discard or select new variables. $F$-to-enter is the value that the model would have it the next variable would enter, while $F$-to-remove is the value that the model has with all the current variables. During the following step, the algorithm selects the variable with the highest $F$-to-enter and removes the variable with the smallest $F$-to-remove.

Speed is the major advantage of this procedure, on the other hand, stepwise regression doesn’t guarantee the development of a good model, it is necessary to assure that the variables included in the final selection have are logically related.

Figure 36. Stepwise regression flowchart [ Stockfusion, online]
3.7.4 Assessing the results of the classification

After obtaining the results of the classification, there are several parameters that allow us to assess the performance of the classifier:

- **Sensitivity**: Proportion of true positives over the total amount of positives.
  
  \[
  Sensitivity = \frac{TP}{TP + FN}
  \]  
  \( (40) \)

- **Specificity**: Proportion of true negatives over the total amount of negatives.
  
  \[
  Specificity = \frac{TN}{TN + FP}
  \]  
  \( (41) \)

- **Accuracy**: Proportion of correctly identified samples over the total amount of samples.
  
  \[
  Accuracy = \frac{TN + TP}{Total}
  \]  
  \( (42) \)

- **Positive-predictive value**: Proportion of true positives over the total amount of identified positives.
  
  \[
  PPV = \frac{TP}{TP + FP}
  \]  
  \( (43) \)

- **Negative-predictive value**: Proportion of true negative over the total amount of identified negatives.
  
  \[
  PPV = \frac{TN}{TN + FN}
  \]  
  \( (44) \)

- **Positive likelihood ratio**: Probability of a diseased subject testing positive divided by the probability of a control subject testing positive.
  
  \[
  PLR = \frac{Sensitivity}{1 - Specificity}
  \]  
  \( (45) \)
• **Negative likelihood ratio:** Probability of a diseased subject testing negative divided by the probability of a control subject testing negative.

\[
NLR = \frac{1 - Sensitivity}{Specificity}
\]  

(46)
CHAPTER 4: RESULTS
4.1 Results of the cross correlation analysis

The following plots display the most representative results for the obtained values:

**Figure 37.** Example of the results of the cross-correlation between channels for DOWN states obtained on the somatosensory cortex of a healthy mouse

**Figure 38.** Example of the results of the cross-correlation between channels for DOWN states obtained on the somatosensory cortex of a diseased mouse
Figure 39. Example of the results of the cross-correlation between channels for UP states obtained on the somatosensory cortex of a healthy mouse.

Figure 40. Example of the results of the cross-correlation between channels for UP states obtained on the somatosensory cortex of a diseased mouse.
There is a noticeable difference between UP and DOWN states, being the mean value of the later noticeably higher. However, UP states reach higher values, around 0.9, than DOWN states, whose highest values go around 0.7, and the same happens for the lowest values. Observing the DOWN plot it is visible that the values present more stability than UP states, which have more abrupt oscillations. Differences between control and diseased subjects are not noticeable.

**Figure 41.** Example of the results of the cross-correlation between channels for DOWN states obtained on the prefrontal cortex of a healthy mouse

**Figure 42.** Example of the results of the cross-correlation between channels for DOWN states obtained on the prefrontal cortex of a diseased mouse
Figure 43. Example of the results of the cross-correlation between channels for UP states obtained on the prefrontal cortex of a healthy mouse

Figure 44. Example of the results of the cross-correlation between channels for DOWN states obtained on the prefrontal cortex of a diseased mouse

The same patterns found on the somatosensory cortex, can also be found on the prefrontal cortex. The high peaks of the DOWN states reach values of 0.8. As for the UP states, there is a noticeable difference on the pattern; healthy mice show faster oscillations of correlation values than it does on the diseased mice. High peaks form plateaus, make look diseased subjects more stable than healthy ones.
The following tables show the mean values obtained for every recording, and a general average value as well.

**Table 1. Cross-correlation measurements results for control mice.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>1</td>
<td>0.3633</td>
<td>0.3292</td>
</tr>
<tr>
<td>2</td>
<td>0.2789</td>
<td>0.4076</td>
</tr>
<tr>
<td>3</td>
<td>0.2614</td>
<td>0.4098</td>
</tr>
<tr>
<td>4</td>
<td>0.2834</td>
<td>0.3967</td>
</tr>
<tr>
<td>5</td>
<td>0.3163</td>
<td>0.3155</td>
</tr>
<tr>
<td>6</td>
<td>0.246</td>
<td>0.2328</td>
</tr>
<tr>
<td>7</td>
<td>0.2369</td>
<td>0.3863</td>
</tr>
<tr>
<td>8</td>
<td>0.2178</td>
<td>0.3433</td>
</tr>
<tr>
<td>AVG.</td>
<td>0.2755</td>
<td>0.3527</td>
</tr>
</tbody>
</table>

**Table 2. Crossed correlation measurements results for mice with Alzheimer's disease**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
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<td>0.2795</td>
<td>0.4023</td>
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<tr>
<td>2</td>
<td>0.2419</td>
<td>0.4522</td>
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<tr>
<td>3</td>
<td>0.2076</td>
<td>0.3574</td>
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<tr>
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<td>0.3629</td>
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<td>0.3422</td>
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<td>0.2487</td>
</tr>
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<td>7</td>
<td>0.2157</td>
<td>0.3524</td>
</tr>
<tr>
<td>8</td>
<td>0.2234</td>
<td>0.3753</td>
</tr>
<tr>
<td>9</td>
<td>0.2432</td>
<td>0.596</td>
</tr>
<tr>
<td>AVG.</td>
<td>0.2595</td>
<td>0.3877</td>
</tr>
</tbody>
</table>

There is a noticeable difference in mean values between healthy and diseased states at the prefrontal cortex, but such difference is lower for the somatosensory cortex. All the values on the table shows that all DOWN states are significantly more correlated than UP states, the difference is greater on the prefrontal cortex for healthy mice and on the somatosensory cortex on diseased mice.
The following boxplots show the results for the quartile analysis:

**Figure 45.** Correlation boxplot of the somatosensory cortex.

**Figure 46.** Correlation boxplot of the prefrontal cortex.
The quartile analysis shows that for the obtained values of the DOWN states on the somatosensory cortex, the notch on both healthy and diseased mice overlap. Except for the two outliers, Alzheimer's values are more compact than those obtained on control mice. As for UP states, healthy and diseased mice follow the same distribution pattern, the mean, quartiles and whiskers all overlap or are very close.

On the prefrontal cortex, DOWN states present similar patterns of distribution. As for the UP states, the higher part of the notch of healthy mice overlaps only with the higher part of the notch of diseased mice. Another difference to consider is that Alzheimer's mice have an outlier in all measurement except for the UP states on the somatosensory cortex, while there are none on healthy mice.
4.2 Results of the approximate entropy analysis

The following plots display the most representative results for the obtained values:

**Figure 47.** Example of the approximate entropy values for DOWN states obtained on the somatosensory cortex of a healthy mouse.

**Figure 48.** Example of the approximate entropy values for DOWN states obtained on the somatosensory cortex of a mouse with Alzheimer's disease.
There are no visible differences between healthy and diseased mice in the mean value, however, especially for the DOWN states; high values descend less abruptly in diseased subjects than it does in control mice. DOWN mean values are significantly higher than UP mean values.
Figure 51. Example of the approximate entropy values for DOWN states obtained on the prefrontal cortex of a healthy mouse.

Figure 52. Example of the approximate entropy values for DOWN states obtained on the prefrontal cortex of a mouse with Alzheimer's disease.
Study of spontaneous cortical activity in transgenic mice with Alzheimer’s Disease

Figure 53. Example of the approximate entropy values for UP states obtained on the prefrontal cortex of a healthy mouse.

Figure 54. Example of the approximate entropy values for UP states obtained on the prefrontal cortex of a mouse with Alzheimer’s disease.

This time, besides the higher average values of the DOWN states, there are no visible differences between diseased and healthy subjects.

The following tables show the mean values obtained for every recording, and a general average value as well.
Table 3. Approximate entropy measurements results for control mice.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th></th>
<th></th>
<th>Prefrontal</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>DOWN</td>
<td>DIFF</td>
<td>UP</td>
<td>DOWN</td>
<td>DIFF</td>
</tr>
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<td>-0,1486</td>
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<td>0,3434</td>
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<td>0,2155</td>
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<td>-0,1229</td>
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<td>-0,1855</td>
</tr>
<tr>
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<td>0,3217</td>
<td>-0,0986</td>
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<td>0,3512</td>
<td>-0,1746</td>
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<td>-0,1090</td>
<td>0,1893</td>
<td>0,3486</td>
<td>-0,1594</td>
</tr>
</tbody>
</table>

Table 4. Approximate entropy measurements results for mice with Alzheimer's disease

<table>
<thead>
<tr>
<th>Subject</th>
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<th></th>
<th></th>
<th>Prefrontal</th>
<th></th>
<th></th>
</tr>
</thead>
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<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
<td>DIFF</td>
<td>UP</td>
<td>DOWN</td>
<td>DIFF</td>
</tr>
<tr>
<td>1</td>
<td>0,2203</td>
<td>0,3169</td>
<td>-0,0966</td>
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<td>-0,168</td>
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<td>0,3449</td>
<td>-0,1645</td>
</tr>
<tr>
<td>3</td>
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<td>0,3347</td>
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<td>0,358</td>
<td>-0,1686</td>
</tr>
<tr>
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<td>0,33655</td>
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</tr>
<tr>
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<td>0,3278</td>
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<td>0,1862</td>
<td>0,3531</td>
<td>-0,1669</td>
</tr>
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<td>0,3501</td>
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<td>0,3404</td>
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<td>0,3502</td>
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</tr>
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<td>9</td>
<td>0,2132</td>
<td>0,3388</td>
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<td>0,1993</td>
<td>0,3539</td>
<td>-0,1546</td>
</tr>
<tr>
<td>AVG.</td>
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<td>-0,10629</td>
<td>0,1886</td>
<td>0,3495</td>
<td>-0,1609</td>
</tr>
</tbody>
</table>

No significant differences can be found just by looking at the mean values. The distribution of the values is shown with the following boxplots.
Study of spontaneous cortical activity in transgenic mice with Alzheimer’s Disease

Figure 55. Approximate entropy boxplot of the somatosensory cortex.

Figure 56. Approximate entropy boxplot of the prefrontal cortex.

All of the boxplots have their notches overlapped, meaning that there are no significant differences on the quartiles distribution. Differences between UP and DOWN states are highly significant, though. Only the UP states on the somatosensory cortex have outliers.
4.3 Results of the sample entropy analysis

The following plots display the most representative results for the obtained values:

**Figure 57.** Example of the sample entropy values for DOWN states obtained on the somatosensory cortex of a healthy mouse.

**Figure 58.** Example of the sample entropy values for DOWN states obtained on the somatosensory cortex of a mouse with Alzheimer's disease.
Figure 59. Example of the sample entropy values for UP states obtained on the somatosensory cortex of a healthy mouse.

Figure 60. Example of the sample entropy values for UP states obtained on the somatosensory cortex of a mouse with Alzheimer’s disease.

It is difficult to observe differences between UP and DOWN states, except for the fact that DOWN states seem to have less low peaks than UP states.
**Figure 61.** Example of the sample entropy values for DOWN states obtained on the prefrontal cortex of a healthy mouse.

**Figure 62.** Example of the sample entropy values for DOWN states obtained on the prefrontal cortex of a mouse with Alzheimer's disease.
Study of spontaneous cortical activity in transgenic mice with Alzheimer's Disease

Figure 63. Example of the sample entropy values for UP states obtained on the prefrontal cortex of a healthy mouse.

Figure 64. Example of the sample entropy values for UP states obtained on the prefrontal cortex of a mouse with Alzheimer's disease.

The main difference between healthy and diseased subjects is the number of low peaks. For DOWN states it's higher in diseased mice, but for UP states, is higher in healthy mice. Again, mean values for all recordings are very close and it's difficult to find a significant difference.
The following tables show the mean values obtained for every recording, and a general average value as well.

**Table 5.** Sample entropy measurements results for control mice.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>1</td>
<td>0,3052</td>
<td>0,3133</td>
</tr>
<tr>
<td>2</td>
<td>0,3636</td>
<td>0,3617</td>
</tr>
<tr>
<td>3</td>
<td>0,3753</td>
<td>0,3534</td>
</tr>
<tr>
<td>4</td>
<td>0,36</td>
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</tr>
<tr>
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<td>0,3637</td>
<td>0,3367</td>
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<tr>
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<td>0,353</td>
<td>0,3374</td>
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</tr>
<tr>
<td>8</td>
<td>0,3559</td>
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</tr>
<tr>
<td>AVG.</td>
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</tr>
</tbody>
</table>

**Table 6.** Sample entropy measurements results for mice with Alzheimer's disease

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
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<td>8</td>
<td>0,3856</td>
<td>0,3468</td>
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<tr>
<td>9</td>
<td>0,3559</td>
<td>0,3448</td>
</tr>
<tr>
<td>AVG.</td>
<td>0,3623</td>
<td>0,3426</td>
</tr>
</tbody>
</table>

The mean values are all very similar. For the UP states, they are slightly higher in healthy mice, however it's the opposite for DOWN states. The quartile analysis with the following boxplots will show the distribution of the values.
Figure 65. Sample entropy boxplot of the somatosensory cortex.

Figure 66. Sample entropy boxplot of the prefrontal cortex.
The somatosensory cortex shows no statistically significant differences, all of the notches overlap, and there's only one outlier on the UP state of a healthy mouse. On the other hand, the prefrontal cortex has a significant difference between the UP states of healthy and diseased mice, because the notches don't overlap. Besides that, the lower quartile of the healthy subject is higher than the median of the diseased subject. It is noteworthy how the diseased mice exhibit a greater range of values compared to healthy mice.
4.4 Results of the mutual information and the area under the curve analysis

The following plots display the most representative results for the obtained values:

**Figure 67.** Example of the mutual information values for DOWN states obtained on the somatosensory cortex of a healthy mouse.

**Figure 68.** Example of the mutual information values for DOWN states obtained on the somatosensory cortex of a mouse with Alzheimer’s disease.
Figure 69. Example of the mutual information values for UP states obtained on the somatosensory cortex of a healthy mouse.

Figure 70. Example of the mutual information values for DOWN states obtained on the somatosensory cortex of a mouse with Alzheimer's disease.

The displayed plots show that the main difference between UP and DOWN states is the height of the peaks. UP states reach higher levels than down states. There is a noticeable difference in the mean value of the UP states, being higher for the healthy subjects, and presenting more high peaks than the diseased subjects.
Figure 71. Example of the mutual information values for DOWN states obtained on the prefrontal cortex of a healthy mouse.

Figure 72. Example of the mutual information values for DOWN states obtained on the prefrontal cortex of a mouse with Alzheimer's disease.
Figure 73. Example of the mutual information values for UP states obtained on the prefrontal cortex of a healthy mouse.

Figure 74. Example of the mutual information values for UP states obtained on the prefrontal cortex of a mouse with Alzheimer's disease.

The main noticeable difference is that diseased subjects have a higher mean mutual information coefficient for the UP states, while for DOWN states this difference cannot be appreciated. The following table presents the obtained values for all the subjects and also the area under the curve values, derived of the mutual information calculation.
Table 7. Mutual information measurements results for control mice.

<table>
<thead>
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<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
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<td>DOWN</td>
</tr>
<tr>
<td><strong>UP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1</strong></td>
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Table 8. Mutual information measurements results for mice with Alzheimer's disease

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<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>DOWN</td>
</tr>
<tr>
<td><strong>UP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1</strong></td>
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<td><strong>5</strong></td>
<td>0.3808</td>
<td>0.2962</td>
</tr>
<tr>
<td><strong>6</strong></td>
<td>0.2657</td>
<td>0.2005</td>
</tr>
<tr>
<td><strong>7</strong></td>
<td>0.2699</td>
<td>0.2216</td>
</tr>
<tr>
<td><strong>8</strong></td>
<td>0.2558</td>
<td>0.2427</td>
</tr>
<tr>
<td><strong>9</strong></td>
<td>0.2692</td>
<td>0.4268</td>
</tr>
<tr>
<td><strong>AVG.</strong></td>
<td>0.2871</td>
<td>0.2801</td>
</tr>
</tbody>
</table>

The most noticeable difference is that mice with Alzheimer's disease have a higher index than healthy mice at the prefrontal cortex. There is also a visible difference on the DOWN states, again being higher at the prefrontal cortex for diseased mice. Regarding the somatosensory cortex, the differences are smaller than they are at the prefrontal cortex.
Table 9. Area under the curve measurements results for control mice.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area under the curve (bits·s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>1</td>
<td>6,7882</td>
<td>11,7279</td>
</tr>
<tr>
<td>2</td>
<td>6,5149</td>
<td>8,825</td>
</tr>
<tr>
<td>3</td>
<td>6,4314</td>
<td>9,2888</td>
</tr>
<tr>
<td>4</td>
<td>7,2054</td>
<td>10,9508</td>
</tr>
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<td>6,8411</td>
<td>8,3826</td>
</tr>
<tr>
<td>6</td>
<td>6,8379</td>
<td>7,8987</td>
</tr>
<tr>
<td>7</td>
<td>6,8809</td>
<td>9,7709</td>
</tr>
<tr>
<td>8</td>
<td>6,1528</td>
<td>7,9963</td>
</tr>
<tr>
<td>AVG.</td>
<td>6,7067</td>
<td>9,3551</td>
</tr>
</tbody>
</table>

Table 10. Area under the curve measurements results for mice with Alzheimer's disease.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area under the curve (bits·s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>1</td>
<td>6,4811</td>
<td>8,8762</td>
</tr>
<tr>
<td>2</td>
<td>7,6879</td>
<td>13,7791</td>
</tr>
<tr>
<td>3</td>
<td>7,1469</td>
<td>10,8798</td>
</tr>
<tr>
<td>4</td>
<td>7,5624</td>
<td>9,2283</td>
</tr>
<tr>
<td>5</td>
<td>7,2107</td>
<td>9,5449</td>
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<tr>
<td>6</td>
<td>7,3241</td>
<td>9,0633</td>
</tr>
<tr>
<td>7</td>
<td>7,42</td>
<td>11,3903</td>
</tr>
<tr>
<td>8</td>
<td>6,8707</td>
<td>11,1254</td>
</tr>
<tr>
<td>9</td>
<td>7,4918</td>
<td>18,3539</td>
</tr>
<tr>
<td>AVG.</td>
<td>7,2440</td>
<td>11,3601</td>
</tr>
</tbody>
</table>

The same differences have been found for the area under the curve; diseased mice have a higher index than healthy mice, for both UP and DOWN states. The difference between UP and DOWN states doesn't present noticeable differences except at the somatosensory cortex for control mice, where area is lower than for diseased mice.
The following boxplots show the results for the quartile analysis:

**Figure 75.** Mutual information boxplot of the somatosensory cortex.

**Figure 76.** Mutual information boxplot of the prefrontal cortex.
Figure 77. Area under the curve boxplot of the somatosensory cortex.

Figure 78. Area under the curve boxplot of the prefrontal cortex.
For the mutual information, boxplots show significant differences on both somatosensory and prefrontal cortices. The UP states of both cortices don't overlap their notches and whiskers and their medians are considerably apart. Outliers are only found on diseased subjects (not displayed on the prefrontal cortex due to visualisation issues).

The boxplots of the area under the curve show the same differences, up states of healthy and diseased mice do not show overlapping notches, indicating statistically significant differences. Again, outliers are only found in diseased mice. DOWN states overlap on both cortices, and do not show significant differences.
4.5 Results of the Weighted Phase Lag Index analysis

The following plots display the most representative results for the obtained values:

**Figure 79.** Example of the WPLI values for DOWN states obtained on the somatosensory cortex of a healthy mouse.

**Figure 80.** Example of the WPLI values for DOWN states obtained on the somatosensory cortex of a mouse with Alzheimer's disease.
UP and DOWN states do not present noticeable differences in their mean values between healthy and diseased subjects. UP states reach higher values of synchronization than DOWN states. One common pattern found on UP states is that they can have high values of phase synchronization which last just one epoch, followed by an abrupt fall to levels very close to zero, again staying just for one epoch, and then progressively increase to one mid-level synchronization plateau, which lasts longer, normally above the value of the smoothing line.
Figure 83. Example of the WPLI values for DOWN states obtained on the prefrontal cortex of a healthy mouse.

Figure 84. Example of the WPLI values for DOWN states obtained on the prefrontal cortex of a mouse with Alzheimer’s Disease.
**Figure 85.** Example of the WPLI values for UP states obtained on the prefrontal cortex of a healthy mouse.

**Figure 86.** Example of the WPLI values for UP states obtained on the prefrontal cortex of a mouse with Alzheimer's Disease.

DOWN states have a noticeably higher mean for mice with Alzheimer and have more high peaks than healthy subjects. Mice with Alzheimer's have also less abrupt falls and less low peaks than healthy mice. As for the UP states, it is noticeable that most of the high synchronization peaks come in pairs, with a high synchronization plateau inbetween. It can be said that mice with Alzheimer's appear to remain longer in high phase locking states than healthy mice.

The main difference between the somatosensory and prefrontal cortex is that the mean value is significantly higher in the later and that high synchronization levels appear to last longer as well. The following tables...
show the mean values obtained for every recording, and a general average value as well.

**Table 9.** Weighted Phase-Lag Index measurements results for control mice.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>1</td>
<td>0,3694</td>
<td>0,2693</td>
</tr>
<tr>
<td>2</td>
<td>0,2893</td>
<td>0,2564</td>
</tr>
<tr>
<td>3</td>
<td>0,2974</td>
<td>0,2349</td>
</tr>
<tr>
<td>4</td>
<td>0,3331</td>
<td>0,241</td>
</tr>
<tr>
<td>5</td>
<td>0,2809</td>
<td>0,2624</td>
</tr>
<tr>
<td>6</td>
<td>0,3281</td>
<td>0,2619</td>
</tr>
<tr>
<td>7</td>
<td>0,2729</td>
<td>0,2665</td>
</tr>
<tr>
<td>8</td>
<td>0,3248</td>
<td>0,2521</td>
</tr>
<tr>
<td>AVG.</td>
<td>0,3119</td>
<td>0,2555</td>
</tr>
</tbody>
</table>

**Table 10.** Weighted Phase-Lag Index measurements results for mice with Alzheimer's disease.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>1</td>
<td>0,2895</td>
<td>0,2429</td>
</tr>
<tr>
<td>2</td>
<td>0,2712</td>
<td>0,227</td>
</tr>
<tr>
<td>3</td>
<td>0,2976</td>
<td>0,234</td>
</tr>
<tr>
<td>4</td>
<td>0,454</td>
<td>0,3647</td>
</tr>
<tr>
<td>5</td>
<td>0,3492</td>
<td>0,2808</td>
</tr>
<tr>
<td>6</td>
<td>0,2862</td>
<td>0,2359</td>
</tr>
<tr>
<td>7</td>
<td>0,2921</td>
<td>0,2041</td>
</tr>
<tr>
<td>8</td>
<td>0,2614</td>
<td>0,2225</td>
</tr>
<tr>
<td>9</td>
<td>0,2577</td>
<td>0,2131</td>
</tr>
<tr>
<td>AVG.</td>
<td>0,3065</td>
<td>0,2472</td>
</tr>
</tbody>
</table>

The table shows that Alzheimer's disease mice have a higher difference in the synchronization between UP and DOWN states. The distribution of the values is shown with the following boxplots:
Study of spontaneous cortical activity in transgenic mice with Alzheimer’s Disease

Figure 87. WPLI boxplot of the somatosensory cortex.

Figure 88. WPLI boxplot of the prefrontal cortex.
The somatosensory cortex UP states show a median value of diseased mice reaches about the same height as the lower quartile of healthy mice, but the length of the whiskers is similar. DOWN states present significant differences, the only part that overlaps is the lower quartile of healthy mice with the higher quartile of the diseased ones.

As for the prefrontal cortex, there are significant differences for all states. The notches of DOWN states do not overlap, and for UP states only the median of the control mice and the lower quartile of disease mice do. All of the diseased states have outliers, while none of the healthy do.
4.6 Results of the synchronization likelihood analysis

The following plots display the most representative results for the obtained values:

**Figure 89.** Example of the synchronization likelihood for DOWN states obtained on the somatosensory cortex of a healthy mouse.

**Figure 90.** Example of the synchronization likelihood for DOWN states obtained on the somatosensory cortex of a mouse with Alzheimer's disease.
Figure 91. Example of the synchronization likelihood for UP states obtained on the somatosensory cortex of a healthy mouse

Figure 92. Example of the synchronization likelihood for UP states obtained on the somatosensory cortex of a mouse with Alzheimer’s disease

The mean value of the DOWN states is higher in diseased subjects than in control mice, however, UP states don’t present noticeable differences.
between groups. DOWN states have more peaks than UP states, but they are not as high as they are in the second group.

**Figure 93.** Example of the synchronization likelihood for DOWN states obtained on the prefrontal cortex of a healthy mouse

**Figure 94.** Example of the synchronization likelihood for DOWN states obtained on the prefrontal cortex of a mouse with Alzheimer's disease
For DOWN states, there are no differences in the mean value, however, the diseased subject presents more high peaks the control subject. UP states reach higher levels of synchronization than DOWN states. Comparing diseased and control subjects it can be seen that mice with AD
reach higher level of synchronization and have more amount of high peaks than mice with healthy brain.

The following table contains the obtained values for every recording.

**Table 11.** Synchronization likelihood measurements results for control mice.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>1</td>
<td>0,0875</td>
<td>0,0963</td>
</tr>
<tr>
<td>2</td>
<td>0,0767</td>
<td>0,0911</td>
</tr>
<tr>
<td>3</td>
<td>0,0788</td>
<td>0,0925</td>
</tr>
<tr>
<td>4</td>
<td>0,0866</td>
<td>0,0882</td>
</tr>
<tr>
<td>5</td>
<td>0,0752</td>
<td>0,0785</td>
</tr>
<tr>
<td>6</td>
<td>0,0663</td>
<td>0,0683</td>
</tr>
<tr>
<td>7</td>
<td>0,0781</td>
<td>0,0911</td>
</tr>
<tr>
<td>8</td>
<td>0,066</td>
<td>0,0805</td>
</tr>
<tr>
<td>AVG.</td>
<td>0,0769</td>
<td>0,0858125</td>
</tr>
</tbody>
</table>

**Table 12.** Synchronization likelihood measurements results for mice with Alzheimer's disease

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>1</td>
<td>0,0761</td>
<td>0,0876</td>
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<tr>
<td>2</td>
<td>0,0693</td>
<td>0,126</td>
</tr>
<tr>
<td>3</td>
<td>0,0948</td>
<td>0,1457</td>
</tr>
<tr>
<td>4</td>
<td>0,0926</td>
<td>0,0815</td>
</tr>
<tr>
<td>5</td>
<td>0,0715</td>
<td>0,0839</td>
</tr>
<tr>
<td>6</td>
<td>0,0694</td>
<td>0,0743</td>
</tr>
<tr>
<td>7</td>
<td>0,0781</td>
<td>0,0805</td>
</tr>
<tr>
<td>8</td>
<td>0,0734</td>
<td>0,0831</td>
</tr>
<tr>
<td>9</td>
<td>0,0861</td>
<td>0,1471</td>
</tr>
<tr>
<td>AVG.</td>
<td>0,07903</td>
<td>0,1011</td>
</tr>
</tbody>
</table>

Due to an outlier, the mean value of the UP states at the prefrontal cortex is significantly higher in diseased mice than it is in healthy mice. Generally speaking, DOWN states have higher values than UP states, except for the prefrontal cortex of diseased mice.
The following boxplots display the quartile analysis performed on every group.

**Figure 97.** Synchronization likelihood boxplot of the somatosensory cortex.

**Figure 98.** Synchronization likelihood boxplot of the somatosensory cortex.
The most significant differences are found at the prefrontal cortex, where the notches of the healthy and diseased mice are almost completely separated for UP states. In addition, the median of the diseased group is higher than the upper whisker of the healthy group. The rest of groups overlap their notches completely, evidencing no significant differences between groups.
4.7 Results of the spectral analysis

When analysing the spectra of the registers, it is expected to find a noticeable difference between up and down states, concretely a bump around the 10 Hz mark. Most of the information is contained in the low frequencies, peaking around 1 Hz, due to the slow UP/DOWN oscillation. We found that the 10 Hz peak is mostly found at recordings from the prefrontal cortex. The following table shows the percentage of the high frequency bumps found at UP recordings for both cortices and healthy and diseased mice:

<table>
<thead>
<tr>
<th>Table 13. High frequency bumps finding rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatosensory / Prefrontal</td>
</tr>
<tr>
<td>Healthy / Alzheimer's</td>
</tr>
<tr>
<td>0% / 11,1%</td>
</tr>
</tbody>
</table>

The displayed plots represent the most representative spectrum for each kind of recording.

![Spectrum of the somatosensory cortex for healthy mice](image)
Study of spontaneous cortical activity in transgenic mice with Alzheimer’s Disease

Figure 100. Spectrum of the somatosensory cortex for mice with Alzheimer’s disease.

Figure 101. Spectrum of the prefrontal cortex for healthy mice.
Figure 102. Spectrum of the prefrontal cortex for mice with Alzheimer's disease.

Besides the bump at 10 Hz, there is another protuberance surrounding the 25-75 Hz band, meaning that both beta and gamma bands are related to the UP states generation. The following tables show the mean and median frequencies obtained from the spectra:

Table 14. Mean frequency measurements results for control mice.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>1</td>
<td>15,6948</td>
<td>15,9127</td>
</tr>
<tr>
<td>2</td>
<td>12,8171</td>
<td>14,5196</td>
</tr>
<tr>
<td>3</td>
<td>13,5466</td>
<td>9,9593</td>
</tr>
<tr>
<td>4</td>
<td>10,92</td>
<td>8,1246</td>
</tr>
<tr>
<td>5</td>
<td>8,0705</td>
<td>6,8441</td>
</tr>
<tr>
<td>6</td>
<td>8,5796</td>
<td>7,4254</td>
</tr>
<tr>
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<td>7,0061</td>
<td>7,2812</td>
</tr>
<tr>
<td>8</td>
<td>8,2212</td>
<td>3,6457</td>
</tr>
<tr>
<td>AVG.</td>
<td>10,6070</td>
<td>9,2141</td>
</tr>
</tbody>
</table>
Table 15. Mean frequency measurements results for mice with Alzheimer’s disease.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
<td>DIFF</td>
<td>UP</td>
</tr>
<tr>
<td>1</td>
<td>12,7611</td>
<td>14,5658</td>
<td>-1,8047</td>
<td>18,3748</td>
</tr>
<tr>
<td>2</td>
<td>11,5767</td>
<td>9,6728</td>
<td>1,9039</td>
<td>13,8367</td>
</tr>
<tr>
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<td>11,1939</td>
<td>11,0741</td>
<td>0,1198</td>
<td>12,2587</td>
</tr>
<tr>
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<td>0,8066</td>
<td>14,6661</td>
</tr>
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<td>4,5653</td>
<td>3,14</td>
<td>1,4253</td>
<td>9,6651</td>
</tr>
<tr>
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<td>7,0158</td>
<td>6,8072</td>
<td>0,2086</td>
<td>14,9637</td>
</tr>
<tr>
<td>7</td>
<td>5,8916</td>
<td>3,0535</td>
<td>2,8381</td>
<td>11,6219</td>
</tr>
<tr>
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<td>7,8859</td>
<td>5,9677</td>
<td>1,9182</td>
<td>11,3059</td>
</tr>
<tr>
<td>9</td>
<td>5,5412</td>
<td>4,9525</td>
<td>0,5887</td>
<td>11,3427</td>
</tr>
<tr>
<td>AVG.</td>
<td>8,4643</td>
<td>7,5745</td>
<td>0,8894</td>
<td>13,1151</td>
</tr>
</tbody>
</table>

The mean frequency at the prefrontal cortex is significantly higher than at the somatosensory. Due to the frequency bumps, there is more power contained on high frequency bands. The values of DOWN states are all inferior to 10 Hz, due to the low activity on both beta and gamma bands. The mean frequency is very similar on both healthy and control subjects at the prefrontal cortex for the UP states. As for the somatosensory cortex, both UP and DOWN states have significantly higher values on healthy subjects.

Table 16. Median frequency measurements results for control mice.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>DOWN</td>
<td>DIFF</td>
<td>UP</td>
</tr>
<tr>
<td>1</td>
<td>9,0108</td>
<td>9,0172</td>
<td>-0,0064</td>
<td>4,365</td>
</tr>
<tr>
<td>2</td>
<td>6,7453</td>
<td>7,6188</td>
<td>-0,8735</td>
<td>19,7905</td>
</tr>
<tr>
<td>3</td>
<td>6,5473</td>
<td>4,0282</td>
<td>2,5191</td>
<td>7,7922</td>
</tr>
<tr>
<td>4</td>
<td>5,4664</td>
<td>3,4728</td>
<td>1,9936</td>
<td>9,5292</td>
</tr>
<tr>
<td>5</td>
<td>4,0985</td>
<td>3,2426</td>
<td>0,8559</td>
<td>9,91</td>
</tr>
<tr>
<td>6</td>
<td>4,2552</td>
<td>3,5908</td>
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<td>7,5743</td>
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<td>3,2689</td>
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<td>-0,047</td>
<td>5,1797</td>
</tr>
<tr>
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<td>3,5341</td>
<td>2,0473</td>
<td>1,4868</td>
<td>4,1098</td>
</tr>
<tr>
<td>AVG.</td>
<td>5,3658</td>
<td>4,5316</td>
<td>0,8342</td>
<td>8,5315</td>
</tr>
</tbody>
</table>
Table 17. Mean frequency measurements results for mice with Alzheimer’s disease.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
<th>Mean frequency values results (Somatosensory cortex)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>UP</td>
<td>DOWN</td>
<td>DIFF</td>
</tr>
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<td>6,6932</td>
<td>7,7427</td>
<td>-1,0495</td>
</tr>
<tr>
<td>2</td>
<td>6,078</td>
<td>4,1695</td>
<td>1,9085</td>
</tr>
<tr>
<td>3</td>
<td>6,6406</td>
<td>5,3942</td>
<td>1,2464</td>
</tr>
<tr>
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<td>5,7557</td>
<td>5,1563</td>
<td>0,5994</td>
</tr>
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<td>8,4896</td>
<td>5,8198</td>
<td>2,6698</td>
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<td>3,46</td>
<td>3,19</td>
<td>0,27</td>
</tr>
<tr>
<td>7</td>
<td>2,056</td>
<td>1,2588</td>
<td>0,7972</td>
</tr>
<tr>
<td>8</td>
<td>3,2217</td>
<td>2,3375</td>
<td>0,8842</td>
</tr>
<tr>
<td>9</td>
<td>2,6725</td>
<td>2,2589</td>
<td>0,4136</td>
</tr>
<tr>
<td>AVG.</td>
<td>5,0074</td>
<td>4,1475</td>
<td>0,8600</td>
</tr>
</tbody>
</table>

Again, the same pattern for UP states on the prefrontal cortex can be found due to the beta and gamma band activity. This time median frequency values are almost the same on healthy and diseased subjects on the somatosensory cortex.
Study of spontaneous cortical activity in transgenic mice with Alzheimer’s Disease

**Figure 104.** Mean frequencies boxplot of the somatosensory cortex

**Figure 105.** Median frequencies boxplot of the somatosensory cortex
The mean frequencies do not show significant differences for any of the recordings, and most of the notches overlap and the whiskers have approximately the same length. In addition, there are no outliers in any of the groups. As for the medians, no significant differences can be found on the somatosensory cortex, except for the size of the boxes between healthy and diseased states at the prefrontal cortex. In spite of this fact, the notches of most of the plots overlap, meaning that there are no significant differences between groups.

**Figure 106. Median frequencies boxplot of the prefrontal cortex**
4.8 Results of the statistical analysis

The following tables display the results obtained after running the paired t-tests and the Wilcoxon tests and those considered statistically significant (p<0.05) have been highlighted using boldface.

Table 18. Results of the paired t-tests

<table>
<thead>
<tr>
<th>Variable</th>
<th>Somatosensory</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Prefrontal</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
<td></td>
<td></td>
<td></td>
<td>UP</td>
<td>DOWN</td>
<td></td>
</tr>
<tr>
<td>SampEn</td>
<td>0.6473</td>
<td>0.9097</td>
<td></td>
<td></td>
<td></td>
<td><strong>0.0045</strong></td>
<td><strong>0.0293</strong></td>
<td></td>
</tr>
<tr>
<td>ApEn</td>
<td>0.3384</td>
<td>0.5008</td>
<td></td>
<td></td>
<td></td>
<td>0.8885</td>
<td>0.8248</td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td><strong>0.0068</strong></td>
<td>0.1089</td>
<td>0.0749</td>
<td>0.099</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>0.4552</td>
<td>0.3857</td>
<td>0.2218</td>
<td>0.6245</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F Mean</td>
<td>0.1677</td>
<td>0.4083</td>
<td>0.8247</td>
<td>0.1569</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F Med</td>
<td>0.7307</td>
<td>0.73</td>
<td>0.9793</td>
<td>0.1917</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td><strong>0.05</strong></td>
<td>0.9603</td>
<td>0.2098</td>
<td>0.359</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL</td>
<td>0.6325</td>
<td>0.1847</td>
<td>0.2899</td>
<td>0.353</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WPLI</td>
<td>0.826</td>
<td>0.6478</td>
<td>0.0893</td>
<td><strong>0.0175</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Only 5 variables exhibit significant statistical differences between healthy and diseased mice. Regarding the somatosensory cortex, the area under the curve and the mutual information for UP states; and with respect the prefrontal cortex the sample entropy for both UP and DOWN states and the DOWN states of the weighted phase lag index.
**Table 19. Results of the Wilcoxon tests**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Somatosensory</th>
<th>Prefrontal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UP</td>
<td>DOWN</td>
</tr>
<tr>
<td>SampEn</td>
<td>0.6918</td>
<td>0.7614</td>
</tr>
<tr>
<td>ApEn</td>
<td>0.8148</td>
<td>0.4234</td>
</tr>
<tr>
<td>Area</td>
<td><strong>0.0079</strong></td>
<td>0.1139</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.3213</td>
<td>0.6058</td>
</tr>
<tr>
<td>F Mean</td>
<td>0.1672</td>
<td>0.3704</td>
</tr>
<tr>
<td>F Med</td>
<td>0.6058</td>
<td>0.8884</td>
</tr>
<tr>
<td>MI</td>
<td><strong>0.0274</strong></td>
<td>0.6058</td>
</tr>
<tr>
<td>SL</td>
<td>0.9079</td>
<td>0.7616</td>
</tr>
<tr>
<td>WPLI</td>
<td>0.4234</td>
<td>0.1139</td>
</tr>
</tbody>
</table>

Compared to t-test, this nonparametric method has evidenced more statistically significant differences. Regarding the somatosensory cortex, only the area and the mutual information for both states. However, for the prefrontal cortex several variables show significant results: the sample entropy for both states, the area under the curve for both states, the mutual information for UP states and the weighted phase lag index for both states.

After obtaining the results for both tests, there is strong evidence that it’s it can’t be assumed that the variables follow a normal distribution. In order to have more variables to work with, we will use those who have been found statistically significant after running the Wilcoxon test.
4.9 Results of the classification

4.9.1 Stepwise procedure

SPSS was able to find the optimal set of variables in four steps. The selected variables were:

- Area under the curve, somatosensory cortex, UP state.
- Area under the curve, prefrontal cortex, UP state.
- Mutual information, somatosensory cortex, UP state.
- Sample entropy, prefrontal cortex, UP state.

After analyzing the 17 subjects, the following results were obtained, defining Alzheimer's brain as negative and control mice as positive:

- 9 true negatives
- 6 true positives
- 2 false negatives
- 0 false positives

With those results, the following statistical parameters were calculated:

- **Sensitivity**: 0.75
- **Specificity**: 1
- **Accuracy**: 0.8824
- **Positive-predictive value**: 1
- **Negative-predictive value**: 0.8182
- **Positive likelihood ratio**: $\infty$
- **Negative likelihood ratio**: 0.25

4.9.2 Leave-one-out procedure

Several combinations of variables were tested in order to find the best set. The best obtained result was 83% accuracy, and several combination parameters reached this value. The number of variables that achieve the best results ranges between two and three. Given that most of the combinations have the same results, the following sets of variables have been labeled as "Set 1", so the data can be displayed in a table:

- Sample entropy of the prefrontal cortex and the area under the curve of the prefrontal cortex.
- Sample entropy of the prefrontal cortex and the area under the curve of the somatosensory cortex.
- Area under the curve of the prefrontal cortex and the area under the curve of the somatosensory cortex.
- Area under the curve of the somatosensory cortex and the mutual information of the somatosensory cortex.
- Area under the curve of the somatosensory cortex and weighted phase lag index of the prefrontal cortex.

While the variables area under the curve of the somatosensory cortex and the mutual information of the prefrontal cortex have been labeled as "Set 2".

Table 20. Results classification for the Leave-one-out procedure

<table>
<thead>
<tr>
<th>SET</th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Sens</th>
<th>Spec</th>
<th>Acc</th>
<th>PPV</th>
<th>NPV</th>
<th>PLR</th>
<th>NLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>0.875</td>
<td>0.778</td>
<td>0.824</td>
<td>0.778</td>
<td>0.875</td>
<td>3.938</td>
<td>0.161</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>0.750</td>
<td>0.889</td>
<td>0.824</td>
<td>0.857</td>
<td>0.889</td>
<td>6.750</td>
<td>0.281</td>
</tr>
</tbody>
</table>

Stepwise model has a better specificity and the leave-one out procedure has a better sensitivity, meaning that the results of both tests can complement each other.
CHAPTER 5: DISCUSSION

In the following pages we will discuss the obtained results during the calculation, statistical analysis and finally the classification.

Our starting hypothesis is that a healthy brain is a very-complex functioning organ and that makes it difficult to model, due to the high numbers of connected neurons that generate continuously high-speed electrical impulses. As Alzheimer's Disease progresses, an increasing number of neurons starts losing its functionality, thus reducing the number of electrical impulses. The brain becomes simpler, easier to study, analyse and parameterise, appearing to be more connected. It's also fitting that most of the differences between healthy and diseased subjects have been found in the prefrontal cortex, due to its association to cognitive and memory procedures, while the somatosensory is mostly associated to sensory and movement procedures and it's affected only during the latest states of the disease.

The Hilbert transform approach has worked well separating UP from DOWN states in the gamma band, which has also showed that it contains enough relevant information to help us differentiate between healthy and diseased subjects, even when discarding the analysis of the beta band. Histograms didn't show enough differences between UP and DOWN states to separate them correctly.

UP states have been proven to be more useful to analyses than DOWN states, because during UP states the brain activity resembles more an awake and functioning brand, while DOWN states are mostly silent states containing noise. The parameters that have shown more differences between healthy and diseased mice are:

- Mutual information
- Area under the curve
- Weighted phase lag index
- Sample entropy
Three connectivity measurements and one complexity measurement that fit our starting hypothesis. Mutual information, area under the curve and weighted phase lag index values are significantly higher in diseased subjects, and sample entropy is lower in diseased brain, all of them non-linear, fitting the complexity of the brain processes. None of the linear parameters have been proved useful for our study. It is important to notice that some connectivity measurements such as the weighted phase lag index or the synchronization likelihood are fairly new and require more testing to assess their reliability.

Sample entropy is described as an improvement over approximate entropy due to its adaptation to small numbers of samples, as in our case, and it has been proved to be true because sample entropy has the best results in both t-test and Wilcoxon tests, while on the other hand, approximate entropy doesn't come close to pass any of the tests.

Both classification procedures have shown good results, with more than an 80% success rate for each one. Linear discriminant analysis has proved to be a good classifier for our cases. Stepwise procedure works perfectly detecting non-diseased mice, but only has a 75% success rate identifying diseased mice, with an overall accuracy of 88,8%. Leave-one out procedure is very good at detecting diseased subject (88,8%) but is not as effective detecting control mice (77,8%) with an overall accuracy of 82,3%. We consider that the fact that one procedure is better at detecting healthy mice while the other is better at detecting diseased mice is good, because both tests can be crossed to improve the reliability of the obtained results.

After testing with several combinations of variables we believe that the best models are sample entropy combined with the area under the curve or the mutual information, the complexity measurement and a connectivity measurement. The area under the curve is a measurement dependent on the values obtained during the calculation of the mutual information, and when put together with the sample entropy, the classification results don't improve, due to the redundancy of their information and adds bias to the model. Because of those reasons we consider it's better no to use them together when using the leave-one-out procedure.

The Wilcoxon test has the best results finding the most statistically significant values. The paired t-test would fail to detect the significance of the area under the curve, one of the most important parameters for the classifier, while the Wilcoxon test states that after the sample entropy is the second most important variable for our analysis. Mutual information and the area under the curve help improve the specificity of the classifier. Sample entropy alone doesn't succeed finding a threshold to separate
healthy and diseased subjects, thus the presence of the connectivity measurements is necessary to improve the robustness of the model. The fact that we are working with a number of variables, smaller than the half of the population proves that we have found a robust model without biasing the classifier.

Despite the good results, we still have a lot of work ahead of us in order to improve our model. First of all it would be optimal to have a bigger database, so we would have enough samples to separate training and classification populations, and we wouldn't need to rely on the leave-one-out procedure, that normally has smaller accuracy than other approaches. With more samples, we could also try to implement more complex classifiers, such as Supporting Vector Machine or $k$-nearest-neighbours. In addition, recording signals of fully awake mice would add information which would give a better view on the dynamics of the brain with Alzheimer’s disease.

Another possibility for future studies would be studying in-depth individual UP states, in order to try to find an explanation to the abrupt oscillations from high synchronization peaks to low synchronization states, including the beta band as well to assess if it contains relevant information which can improve the obtained results.
CHAPTER 6: BUDGET

The budget is divided in three parts:

- **Materials**: Includes equipment and software licenses.
- **Personnel**: Includes the total hours spent on the project for both student and director.
- **Global budget**: Summary of the total costs

### 6.1 Materials

The following equations are used to calculate the variable cost of the used materials:

\[
Variable \ cost = \frac{Fixed \ cost}{Durability \cdot Annual \ work \ hours}
\]

(47)

The annual working hours are calculated as it follows:

\[
Annual \ work \ hours = \left(\frac{21.5 \ working \ days}{1 \ working \ month}\right) \cdot \left(\frac{6 \ hours}{1 \ working \ day}\right) \cdot \left(\frac{4 \ working \ months}{1 \ year}\right)
\]

(48)

For a total of 516 annual working hours.

The total cost for every license and hardware is calculated multiplying the variable cost (€/h) times the time spent (h). The cost of the calculus software licences (MATLAB R2014b and IBM SPSS) is calculated using the price for one year.
Table 21. Equipment and materials costs

<table>
<thead>
<tr>
<th>Item</th>
<th>Units</th>
<th>Fixed cost (€/unit)</th>
<th>Overall durability (years)</th>
<th>Variable cost (€/h)</th>
<th>Dedicated time (h)</th>
<th>Total amount (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacBook Air 11”</td>
<td>1</td>
<td>721</td>
<td>5</td>
<td>0,2795</td>
<td>480</td>
<td>134,16</td>
</tr>
<tr>
<td>Matlab Student R2014b</td>
<td>1</td>
<td>69</td>
<td>1</td>
<td>0,1337</td>
<td>480</td>
<td>64,18</td>
</tr>
<tr>
<td>IBM SPSS Statistics 20</td>
<td>1</td>
<td>2530</td>
<td>1</td>
<td>4,9031</td>
<td>480</td>
<td>2353,49</td>
</tr>
<tr>
<td>Office Home &amp; Student 2013</td>
<td>1</td>
<td>119</td>
<td>3</td>
<td>0,0769</td>
<td>480</td>
<td>36,90</td>
</tr>
</tbody>
</table>

Total (IVA included): 2588,73 €

Table 22. Office costs

<table>
<thead>
<tr>
<th>Item</th>
<th>Units</th>
<th>Monthly (€/month)</th>
<th>Months</th>
<th>Total amount (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electricity bill</td>
<td>1</td>
<td>80</td>
<td>4,5</td>
<td>360</td>
</tr>
<tr>
<td>Office rental</td>
<td>1</td>
<td>300</td>
<td>4,5</td>
<td>1350</td>
</tr>
</tbody>
</table>

Total (IVA included): 1710,00 €

6.2 Personnel budget

The following table states the amount of hours spent on every task, with a separate cost for the student and the thesis director

Table 23. Cost of the student's tasks

<table>
<thead>
<tr>
<th>Task</th>
<th>Cost (€/hour)</th>
<th>Hours</th>
<th>Total amount (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administration of the project</td>
<td>10</td>
<td>5</td>
<td>50,00</td>
</tr>
<tr>
<td>Project planning</td>
<td>20</td>
<td>25</td>
<td>500,00</td>
</tr>
<tr>
<td>Documentation and research</td>
<td>20</td>
<td>50</td>
<td>1000,00</td>
</tr>
<tr>
<td>Scripting</td>
<td>20</td>
<td>200</td>
<td>4000,00</td>
</tr>
<tr>
<td>Redaction</td>
<td>20</td>
<td>400</td>
<td>4000,00</td>
</tr>
<tr>
<td>Meetings with the director</td>
<td>20</td>
<td>50</td>
<td>1000,00</td>
</tr>
</tbody>
</table>

Total: 10550,00 €

Total + 21% IVA: 12765,50 €
### Table 24. Cost of the director's tasks

<table>
<thead>
<tr>
<th>Task</th>
<th>Cost (€/hour)</th>
<th>Hours</th>
<th>Total amount (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meeting with the students</td>
<td>35</td>
<td>50</td>
<td>1750,00</td>
</tr>
<tr>
<td>Project guidance</td>
<td>35</td>
<td>25</td>
<td>875,00</td>
</tr>
</tbody>
</table>

**Total:** 2625,00 €

**Total + 21% IVA:** 3176,25 €

### 6.3 Global budget

Total costs of the project:

### Table 25. Global budget

<table>
<thead>
<tr>
<th>ITEM</th>
<th>Total amount (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment costs</td>
<td>2588,73</td>
</tr>
<tr>
<td>Office costs</td>
<td>1710,00</td>
</tr>
<tr>
<td>Student tasks</td>
<td>12765,50</td>
</tr>
<tr>
<td>Director tasks</td>
<td>3176,25</td>
</tr>
</tbody>
</table>

**Total (IVA included):** 20240,48 €
CHAPTER 7:

BIBLIOGRAPHY

7.1. Bibliographical references


7.2. Consultation bibliography


David’s Statistics. Notched Box Plots. https://sites.google.com/site/davidsstatistics/home/notched-box-plots