UNIVERSITAT DE BARCELONA

Final Degree Project

Biomedical Engineering Degree

Analysis of slow waves in the cerebral cortex during aging

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Abstract

Slow oscillations, which are the default mode of the cortex and the main oscillation in slow-wave sleep, may be affected by aging. In this project, aging effect on the properties of the cortical slow oscillation is studied in 3, 7, 15 and 20 months of age sedated mice with Slowpy, a specialized pipeline for slow oscillations.

Results show that aging affects the duration of Ups and Downs, as they slightly decrease with age, as well as the is an increase of the firing rate of the Up states with age. These effects can be explained by several biological and physiological reasons, such as changes in neuronal excitability and network connectivity. However, the effects of aging in functional connectivity remain unclear with this project, as this relationship has not been directly addressed in this study.

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1. Introduction

The aim of this report is, mainly, to write down each of the elements and steps that have been done in this Final Degree Project. In this first section, the main lines of the project will be defined, as well as its objectives, methodology, limitations, scope and span.

1.1. Motivation

In the last twenty years, brain oscillations have been studied as a relevant topic in neuroscience, as they are critical for neural communication and cognitive functions. Also, they are often target for therapeutic interventions. In particular, brain slow oscillations are studied in the field of slow wave sleep, although they are also associated to states of anesthesia and even of brain lesions. Moreover, they play a crucial role in various sleep-related brain processes like replay and memory consolidation.

Then, this project aims at taking a closer look at these oscillations, and study their relationship with aging, adding a new contribution on the research done in this field.

1.2. Objectives

The aim of this project is to study how age affects the properties of the cortical slow oscillation in 3, 7, 15 and 20 months of age sedated mice. This study will be performed using a previously developed pipeline. Another objective is to provide some new functionality to it, as during the execution of this project there will be total access to its code.

1.3. Methodology

This project will be executed under the direction of Mavi Sánchez Vives, MD, PhD in Neurosciences, who has been ICREA Research Professor at the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) since 2008, where she leads the Systems Neuroscience group; and co-directed by Arnau Manasanch Berengué, Data Analyst at IDIBAPS and Biomedical Engineer. The tutor of this project is Agustín Gutiérrez Gálvez, PhD in Computer Engineering and Associate Professor at the Department of Electronics of Universitat de Barcelona.

The project starts with a planning period, in which the objectives are detailed and the chronogram is elaborated. Then, some literature related to the topic of study will be reviewed, in a bibliographic research stage, as well as a market analysis will be performed. After that, a specialized slow oscillations analysis (Slowpy) pipeline developed by Mr. Manasanch will be used for the central study of the project. After analyzing the mice data, some results will be discussed, and a conclusion

will be stated. The progression of this project will be followed up by several periodic meetings with the directors of the study.

1.4. Limitations, scope and span

The scope of this project is the analysis of slow wave local field potential (LFP) signals recorded from a population of mice with different ages. From this analysis, some highlighted metrics will be chosen and discussed. Moreover, some metrics will be added to the Slowpy pipeline in order to achieve a wider glance of the effect that aging may have on brain slow oscillations.

The span of the project is to contribute to cortical neuroscience. This contribution will be performed in coordination with the Systems neuroscience lab at IDIBAPS, the main biomedical research institute of Hospital Clínic de Barcelona, a leader hospital in Catalonia and Europe.

Some of the limitations of this project are related to the temporal duration of the project itself, as it is limited. Furthermore, the dataset used in this study only records the cortical activity of mice with two electrodes, in contrast of other datasets with more electrodes per subject.

2. Background

Over the last two decades, the study of brain oscillations has emerged as a relevant topic in neuroscience, critical for neural communication, cognitive functions, altered in various neurological and psychiatric disorders and thus, a target for therapeutic interventions. In particular, brain slow oscillations have been a subject of study in the field of slow wave sleep, although they are also associated to states of anesthesia and even of brain lesions. Slow waves play a crucial role in various brain functions related to sleep, including processes like replay and memory consolidation. In this section, the state of the science for slow waves will be reviewed, and the state-of-the-art analysis pipeline used in this project will be described.

2.1. State of the science

Before introducing the theory of slow oscillations, it may be interesting to review the different stages sleep is divided in.

2.1.1. Sleep stages

While sleeping, the brain, far from remaining inactive, goes under several characteristic stages along the sleeping process. In short, there are two types of sleep: non-rapid eye movement (NREM) and rapid eye movement (REM), which alternate cyclically (Colten et al., 2006). In physiological conditions, sleep episodes begin with four distinct stages of NREM, and 80 to 100 minutes later they start a REM period. From this point, NREM and REM sleep will follow successively in cycles of approximately 90 minutes throughout the night (Carskadon & Dement, 2011), with NREM taking usually 75% to 80% of sleep time.

REM sleep, which is defined by EEG activation, muscle atonia and episodic bursts of rapid eye movements (Carskadon & Dement, 2011), usually gets more prominent in the last third of the sleep time.

In contrast, in NREM sleep various brain functions are regulated. It is divided in four stages –1 to 4–, having the lowest arousal threshold at stage 1 and the highest one at 4. Indeed, sleep at stage 1 is superficial and susceptible to easy awakenings, while in stage 2, the EEG is characterized by sleep spindles and K-complex waves. In stages 3 and 4, high-voltage low-frequency oscillations (>75 μV, ≈1 Hz) dominate the EEG recordings. These stages are known also as deep sleep or slow-wave sleep (SWS) (Carskadon & Dement, 2011).

2.1.2. Slow waves

In 3 and 4 NREM sleep stages, slow waves oscillate at approximately 1 Hz in the cerebral cortex, a complex and multi-connected network that encompasses billions of neurons which form multiple circuits of precise connectivity (Pfeffer et al., 2013). These waves dominate the cortex, even in periods of functional or anatomical disconnection, to the point that they have been described as the default mode of the system, in both *in vitro* and *in vivo* experiments (Sanchez-Vives & Mattia, 2014).

Slow waves oscillate between periods of activity and silence, known as Up and Down states. In the Down phase, virtually all cortical neurons remain deeply hyperpolarized and stay silent for a few hundred milliseconds. On the other hand, in the Up state, there is a generalized depolarization for several hundred milliseconds in which the membrane potential reaches the firing threshold, the thalamocortical system is seized by intense synaptic activity, and neurons fire at rates even higher than in quiet wakefulness (Massimini et al., 2004).

This oscillatory state is not trivial, and it has several physiological implications. First, down states are useful for recalibration of the signal and cellular maintenance, while the neuronal activity of the up states is necessary for the preservation of the cortex properties and its synaptic connectivity (Sanchez-Vives & Mattia, 2014). Taking into account that the cortical network is recurrently connected (Lorente de Nó, 1934; Singer, 2021), it could function isolated provided that there is some intrinsic excitability. Despite that, recurrency has the risk of generating activity outbursts, i.e. epilepsy; therefore, the potential consequences of re-entering excitation are regulated by inhibitory neurons and hyperpolarizing currents. As a result, excitation and inhibition are maintained and balanced during cortical function (Sanchez-Vives & Mattia, 2014).

Statistically, a bistability model can be applied to this oscillation, regarding features such as the membrane potential, which is followed by a bistable firing rate, and the relative regularity of Up-Down cycle duration. This specific bimodal distribution is explained by three different factors (Sanchez-Vives & Mattia, 2014):

1. The first property is the synaptic reverberation in neuronal networks in combination with nonlinear amplification of pre-synaptic input by neurons, which can explain the firing rate bistability. In particular, this feature can be characterized through a mean-field approximation by the dynamics of the instantaneous firing rate $v(t)$ of the network, i.e. the MUA signal, usually taken from the power of the frequencies between 200 and 1500 Hz, in 5 ms windows (Dasilva et al., 2021). From this signal, the energy function $E(v)$ can be

extracted, showing two minima at high and low *v*, that is, two stable Up and Down states, respectively.

2. The second key model feature is the intrinsic fluctuations of *v*(t) that happen in this mean-field dynamics model with two attractor states. This randomness, which is a Poissonian stochastic process proportional to the amount of neurons, makes the system capable to overcome barriers and to switch between states by chance.
3. However, this synergy between non-linear dynamics and endogenous noise does not completely describe the experimental results, as Up and Down states alternate rather regularly. This point is supported by the evidence of relatively low coefficient of variations of their durations, in contrast with the exponentially-distributed permanence times in the stable states of the model. Therefore, we have to add an activity-dependent mechanism of self-inhibition based on the relationship between cell fatigue and firing rate. After the increase of network activity, cell fatigue also rises until a maximum is reached and the Up state ends. As the activity decreases, so does the cell fatigue, and the cycle starts again.

Taking into account these properties, a model based in a relaxation oscillator has been proven to fit the experimental oscillations (Sanchez-Vives & Mattia, 2014).

Regarding the biological function of slow oscillations, they act as a dynamical regime of the cortical tissue that follow a cycle for a relatively short time, leaving room to restorative functions that remove the neurotoxic waste products accumulated during wakefulness. Moreover, up states activate different functional networks depending on the sensory response of the cortex (Kenet et al., 2003), by exploring multiple network states of the cortex, and thus, being the basis for memory consolidation (Peyrache et al., 2009) and retention, through long-term plasticity (Chauvette et al., 2012).

Research both in humans and animals shows that the normal aging process may have a particular role in slow waves, as their density, power and amplitude decrease substantially with age (Carrier et al., 2011; Latreille et al., 2019), whereas there is also a decrease in cortical excitability and a significant thinning of the cortex, both related to cortical maturation and cognition changes (Gaggioni et al., 2019; Natu et al., 2019; Ong et al., 2022). Last but not least, aging relates to the cortex by damping its structural and functional connectivity across different cortical regions (Dubè et al., 2015).

With all this literature background, it is remarkable that aging may play a crucial role in slow oscillations. Therefore, the aim of this project, as stated in Sections 1.1. and 1.2., is to study how

age affects the properties of the cortical slow oscillation in 3, 7, 15 and 20 months of age sedated mice.

2.2. State of the art: Slowpy pipeline

For the analysis of the slow oscillations, there are several toolboxes and packages on Internet, such as Spike2 (Cambridge Electronic Design Limited, 2019), NeuroExplorer (Nex Technologies, 2021) and FieldTrip (Oostenveld et al., 2011). In this project, we will use Slowpy, a pipeline developed at the Cortical Networks Lab in IDIBAPS - Institut d'Investigacions Biomèdiques August Pi i Sunyer (Manasanch, 2020).

The Slowpy pipeline consists of two self-explanatory Python notebooks (one for the detection of slow oscillations and another one for the analysis of them), along with four original Python scripts, a metadata Excel file, a .txt file with the module requirements and a guidelines document (Figure 1). From the brain signal to be processed, which must reflect a clear slow oscillation, obtained from sedated or asleep subjects, Slowpy extracts several metrics across different conditions (e.g. different levels of anesthesia, wild-type vs. knockout subjects, aging conditions, etc.).

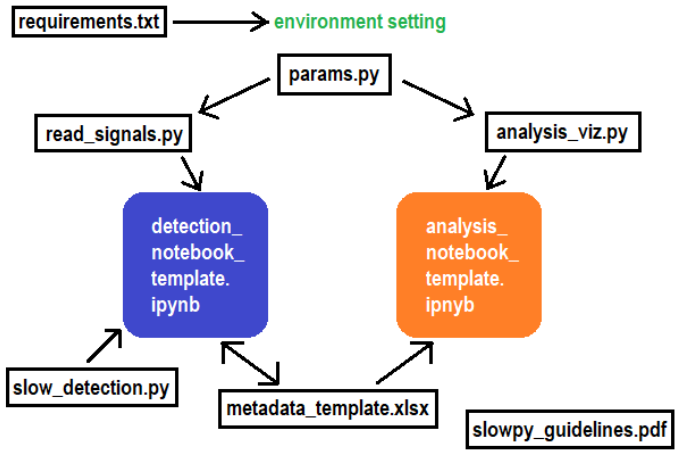

Figure 1. Schematic diagram of the files of the default pipeline, and their interactions. Arrow heads indicate data or parameter dependency.

As depicted in Figure 2, some previous steps must be performed before any analysis, which are:

- Setting up of the files and directories, as well as installing the required working environment.
- Downloading and inspecting the data to analyze to choose the best time periods.
- Setting up the data folder.
- Setting up the metadata.
- Defining the array configuration.

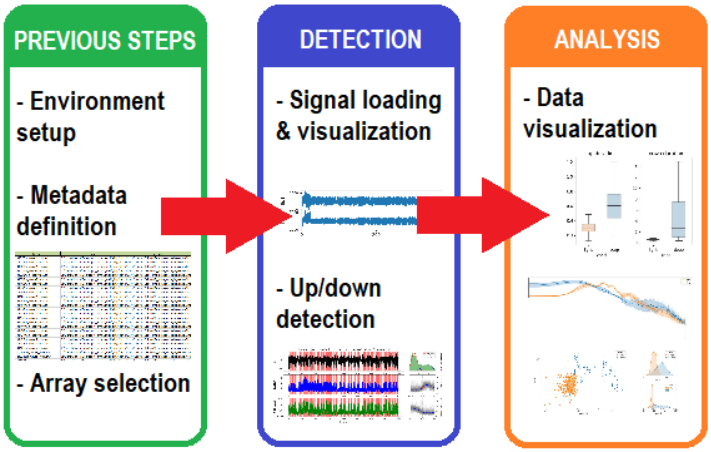

Figure 2. Flowchart of the process followed by the pipeline.

Once these steps are completed, we continue the pipeline workflow (Figure 2), now opening the detection notebook in order to separate the up from the down states. After importing the required libraries, and defining the metadata path, we can visualize in a Graphical User Interface (GUI) the signals of one of the subjects, check their quality and load a specific time period from one or more channels of the subject (Figure 3).

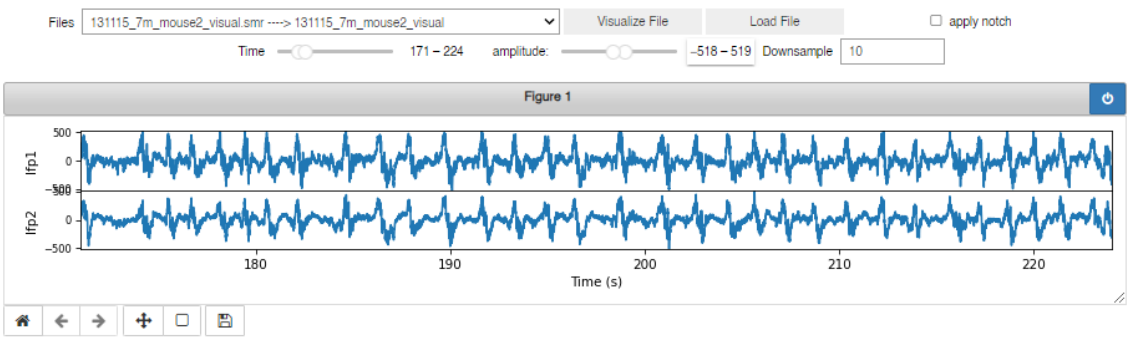

Figure 3. Visualization of two LFP traces taken from a specific subject.

Then, we can perform the detection of the Up and Down states in the next embedded GUI. This detection step has to be done semiautomatically for each recording, and validated through visual inspection (Figure 4). For this detection, we must choose one or more channels to detect and later analyze, and set the following hyperparameters and options:

- The duration of Up and Down states.
- The threshold that discriminates between states. There is also the option of choosing the integrated automatic threshold.
- The method of detection of the peaks (Section 4.2.).

Once the options are set, six plots are displayed (Figure 4), in order to visually validate the quality of the detection. On the left, we have three signals with the up states marked in red. The top-left plot depicts the raw signal of the selected channel in the desired time period, and the other two are filtered signals (see Section 4.2.). On the right part, there are three more plots. The one on the top shows a bimodal distribution of the filtered signal, the adjustment of the model and the previously set threshold. Below this, there are two plots that display the averaged down-to-up and up-to-down transitions of the wave, as well as the slope of the transition at its center.

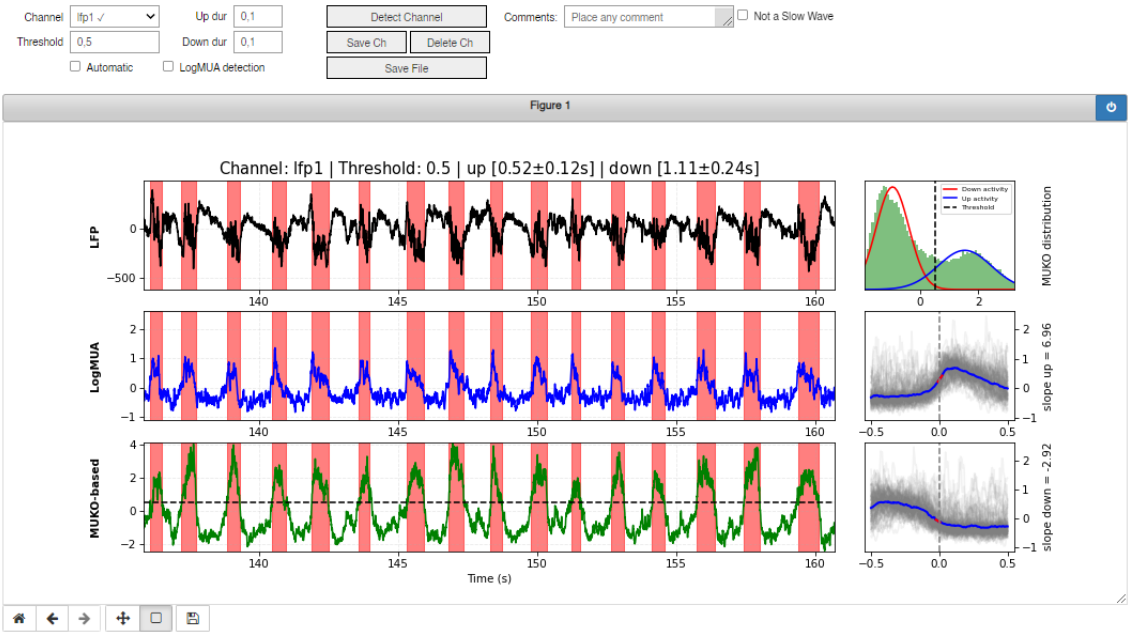

Figure 4. Detection GUI of a single channel.

Once the detection is done for all the desired subjects and channels, we can start analyzing the data with the analysis notebook. For this notebook, the detection performed previously is extracted and used to compute different metrics of the up and down states, either by subject or in group. In this case, we also need to import the libraries, set the metadata path and load the analysis GUI (Figure 5). Now, we can visualize several statistical analyses of the data, separated by condition and even channels, if desired. The available metrics are described in Table 1 (Section 4.3.).

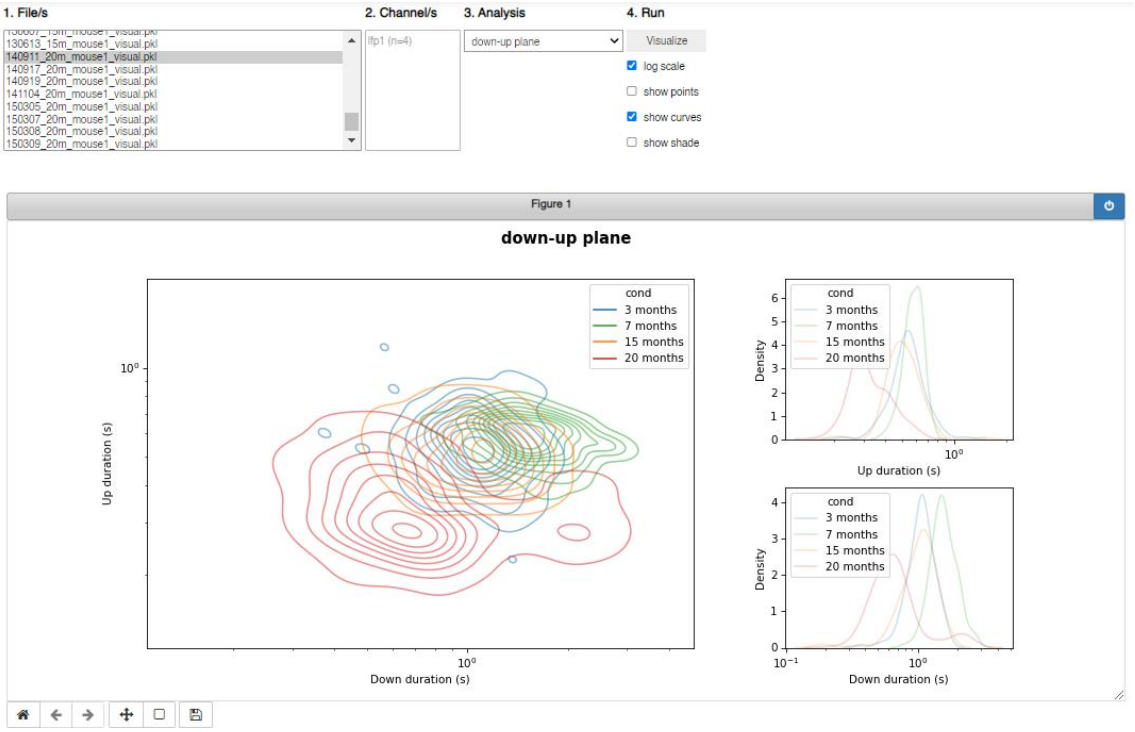

Figure 5. Example of a plot obtained from the analysis GUI.

In this pipeline, the boxplots are statistically evaluated by the Mann-Whitney U test, which tests for differences between two groups on a single, ordinal variable with no specific distribution (Mann & Whitney, 1947). It is usually used for determining whether two sampled groups are from a single population, without using parameters (McKnight & Najab, 2010).

As it can be observed, this pipeline allows us to perform an end-to-end data analysis and visualization of slow oscillations, which is of great interest for the project. Furthermore, it offers a vast range of metrics that can be insightful for extracting results.

3. Market analysis

This section is focused in the market perspectives of this project, and the context of it in a global and interconnected society.

3.1. Addressed sector

As this project aims to contribute to research done in cortical neuroscience, the primary sector this project addresses is the research in this field. Nonetheless, as it is a growing discipline, this study could ultimately help in, for instance a new treatment for a neurological disease, having a more direct impact in general society.

3.2. Historical evolution of research

Cortical neuroscience is a growing field of study nowadays. By a basic search in PubMed, it can be observed that article publications related to this topic have been multiplied by 6 in the last twenty years.

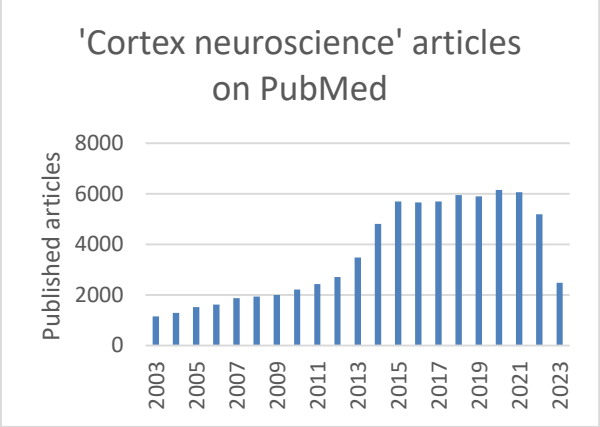

Figure 6. Historical evolution of Cortex neuroscience articles on PubMed.

This fact shows that there is room to actively contribute to this discipline in the future, as nowadays hundreds of laboratories around the world work towards finding out how this region of our brain works.

4. Conception engineering

In this section, several topics related to the conception of the project are discussed, as well as different executive choices are compared. First, there is a description of the dataset that has been used for this project. After that, two filters for the raw signal are compared. Finally, the set of features that will be evaluated are explained in detail.

4.1. Dataset

The dataset used in this project consists of a set of Local Field Potential (LFP) extracellular recordings from a population of (n=45) 3, 7, 15 and 20 months old wild-type mice. These cortical recordings were performed between April 3, 2012 and March 9, 2015, following a set of regulations detailed in (Castano-Prat et al., 2019).

For their acquisition, a continuous infusion of ketamine was delivered subcutaneously to each animal to maintain a constant level of anesthesia. LFP recordings from the visual cortex were obtained by means of two tungsten microelectrodes per subject. The signals were amplified with a multichannel system, and acquired with Spike2 software (Cambridge Electronic Design Limited, 2019).

Once the data files are loaded to the pipeline, only one single channel per subject will be used for detection and analysis. Multi-channel analysis is dismissed for this dataset, as there are only two measures per mouse. Moreover, only a time period of about 200 seconds will be used, because enough up-and-down samples will be extracted.

The condition of each mouse and the desired period of measure, as well as the data and result filenames, have to be previously detailed in the metadata excel file.

4.2. Peak detection algorithms

The Slowpy pipeline, utilized for the detection and analysis of Up and Down episodes of the recorded slow waves, uses two distinct filters to pre-process the signal.

On the one hand, the firing of the local network, related to the multiunit activity (MUA), is estimated by band-pass filtering the LFP signal between 200 and 1500 Hz in 5 ms windows (Ruiz-Mejias et al., 2011; Sanchez-Vives et al., 2010; Torao-Angosto et al., 2021). This filter, which uses the logarithm of the MUA (logMUA), achieves an effective separation between Up and Down states. This discrimination can be validated with the bimodal distribution of its histogram.

On the other hand, the second filter considered is the one proposed by (Mukovski et al., 2007). This filter, also known as MUKO (Torao-Angosto et al., 2021), is based on the differential spectral composition of the LFP in the beta/gamma frequency band (20–100 Hz). Like the previous one, it also separates active from silent states in a bimodal distribution.

The Slowpy pipeline offers both logMUA and MUKO filterings for the detection of peaks (i.e., separating between Up and Down states), while it only uses logMUA filter for the analysis step as part of some of the metrics (see Section 4.3.), as it provides a quantitative measure of the total amount of firing of the neurons.

By inspecting how the filters perform for some of the recordings, at a given threshold and up and down durations (see Section 5.2.), it can be observed that, although both methods effectively discriminate between states, MUKO filter has a better outcome as noise in the Down state is less amplified (Figure 7). Therefore, less noisy Down periods are misclassified.

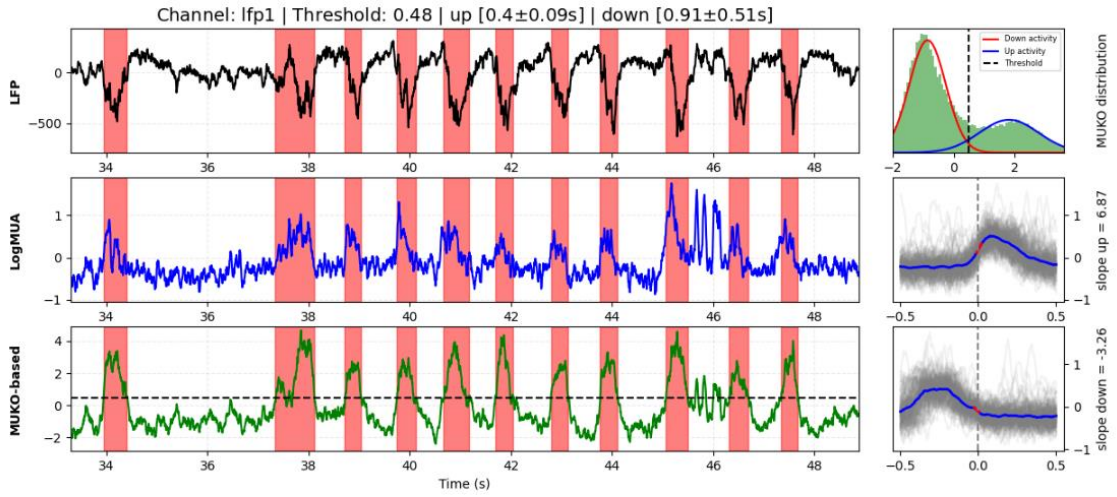

Figure 7. Example of a peak detection performed by the MUKO filter (bottom-right plot). Around t=46, LogMUA filtering performs poorly, as it amplifies a noisy Down state. If LogMUA detection was used, however, it would not be a bad classification in general, as these kind of noisy episodes are relatively infrequent.

4.3. Metrics

The metrics that will be the first to be analyzed are the ones already integrated in the pipeline, described in Table 1:

Metric type	Metric name	Description	Selectable options
Signal traces	LFP	Display of the original LFP signal of a single subject.	☑ show detection
	Log MUA	Display of the signal after a logMUA filter.	
Down-up plane		Scatter distribution plot of the up vs. the down durations of one or more subjects and channels. Each color belongs to a condition. Complementary histograms of up and down durations by separate, equivalently.	☑ log scale ☑ show points ☑ show curves ☑ show shade
Durations	Up duration	Boxplot of the up-state durations. One or more subjects and channels. Split by condition.	☑ split channel analysis ☑ fliers and significance ☑ show statistics
	Down duration	See "Up duration".	
	Slow frequency	Boxplot of the wave frequencies: $\frac{1}{dur_{up} + dur_{down}}$	
	Up CV	Boxplot of the coefficient of variation of the Up state: $\sigma_{up} / \overline{dur_{up}}$	
	Down CV	See "Up CV".	
	Cycle CV	See "Up CV". $\sigma_{up+down} / \overline{dur_{up+down}}$	
Firing rate	Average FR up	Boxplot of the mean, for each channel, of the logMUA during an up. One or more subjects and channels. Split by condition.	☑ split channel analysis ☑ fliers and significance
	Average FR down	See "Average FR up".	

	Std. FR up	See "Average FR up".	☑ add logMUA reference ☑ show statistics
	Std. FR down	See "Average FR up".	
	Max peak up	See "Average FR up". Max value, for each channel, of the logMUA during an up.	
	Cycle FR	See "Average FR up". Mean, for each channel, of the logMUA during an up and the consecutive down.	
	Relative FR	See "Average FR up". Mean, for each channel, of the logMUA during an up vs. the mean for the consecutive down.	
	FR per second	See "Average FR up". $\frac{\sum_t logMUA}{t}$	
PSD bands	Full trace	Boxplot, for one or more frequency bands, of the power spectral density of one or more subjects and channels. Split by condition.	☑ log scale ☑ z-score
	Full up	See "Full trace". PSD of the Up episodes.	
	Full down	See "Full trace". PSD of the Down episodes.	
PSD traces	Same as "PSD bands"	Like PSD bands, but in a continuous trace, split by condition.	☑ log y-scale ☑ log x-scale ☑ z-score
Spectrogram		Representation of PSD for separate channels through several time windows.	☑ add traces ☑ log y-scale ☑ log spectrogram

Table 1. Description of the metrics originally included in the analysis module of the Slowpy pipeline.

Once all the analysis is performed, only the metrics that have a minimal statistical significance (in Mann-Whitney U test, only metrics with p-values smaller than 0.05) will be taken into account.

The pipeline is designed in a modular way, so it allows the addition of new functionalities. One of the functionalities that has been developed in this project is the computation of the Functional Connectivity across different brain areas, as described in (Dasilva et al., 2021). However, as the mice of our initial dataset only have been recorded by two electrodes in the visual cortex, no significant data can be extracted from them. Thus, another dataset will have to be used for the study of this metric (see Section 5.4.).

5. Detail engineering

In this section, the implementation of the project is explained in detail, as an application of the different choices done in Section 4. First of all, the structure of the metadata file will be briefly explained, and then the chosen parameters for Up and Down states detection will be exposed. Finally, a selection of the more relevant results obtained will be presented.

5.1. Metadata definition

The first step of all, just after installing the needed libraries, is the setup of the metadata file, that will be called by both detection and analysis notebooks to interact with the downloaded, and then generated, data files.

For this pipeline, the metadata file is in excel format as it is intended to be used by researchers that are not necessarily familiarized with programming. This file consists of two sheets, one for loading the raw data to the detection notebook and saving the signal after detection in a separate directory (if preferred), called 'load'; and another one to be read by the analysis notebook and retrieve the pre-processed file, called 'analysis'.

For the sake of completeness of the explanation, the raw data files of this project are in .smr format, although the pipeline can also take as input other file formats such as .mat or .hdf. The filenames follow the structure *DATE_COND*_mouse*X_AREA*.smr, where *DATE* is the date of recording in YYMMDD format, *COND* is the abbreviated aging condition of the subject (that is: 3m, 7m, 15m and 20m), *X* is the ID of the mouse (useful when two subjects with equal characteristics are recorded the same day), and *AREA* is the cortex region where the electrodes were placed, in this case, visual.

In this file, each row corresponds to a mouse, while each column is a different feature, path or parameter. Column names of *load* and *analysis* sheets, and their descriptions, can be found in Table 2 and Table 3, respectively.

Column name	Description
filename	Name of the file to be detected.
path	Full directory path where the filename is located.
save_dir	Full directory path where to store the output of the detection. It can be the same one where data is located or a new one.
channels	Channels to detect, separated by commas or *all* if all channels are needed to be detected.

time_start	Starting point of the time period to analyze, in seconds. It must be an integer, and smaller than time_end.
time_end	Ending point of the time period to analyze, in seconds. It must be an integer, and larger than time_start.
array_config	Identifier of the array configuration. This identifier will be added to array_configs dictionary in params.py file.
output_name	Name that will be used to save the detection file and then fetched to perform the analysis. It is saved in pickle format (.pkl).

Table 2. Short description of the columns in the *load* sheet of the metadata file.

Column name	**Description**
filename	Name of the file to be analyzed. It must be equal to output_name.
path	Full directory path where the filename is located.
condition	Condition used for that specific file.
channels	Channels to analyze. Same rules as in the load sheet.

Table 3. Short description of the columns in the analysis sheet of the metadata file.

Consequently, after setting the metadata up, the params.py file has been modified, adding this array configuration to the array configurations dictionary:

```
array_configs['electrode_aging'] = {
'labels': ['lfp1','lfp2','Mi 1','Mi 2','HR'],
'coords': [[0,0], [0,1], [0,0], [0,1], [0,0]]}
```

5.2. Peak detection

For the detection stage, some hyperparameters need to be set to achieve a satisfactory output. As it can be observed in the left part of Figure 8, there are a few parameters and checkboxes to be set. First of all, a channel must be chosen. If no artifacts are found in that channel (lfp1), we proceed with the detection of it. Otherwise, we use the lfp2 channel. After detecting all subjects, lfp2 channel has only been used three times.

Regarding Up and Down duration parameters, they have been left at their default values (0.1, similar to (Torao-Angosto et al., 2021)), and the parameter evaluated by visual inspection of the outcome has been the threshold, starting at 0.5, its default value, and switching to the automatically

detected one if there is some defect. As stated in Section 4.2., LogMUA detection has not been used in this project.

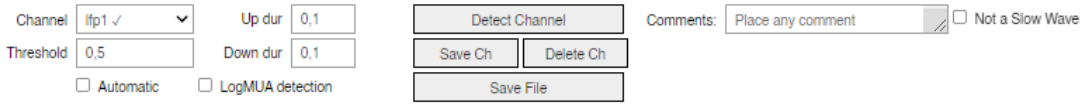

Figure 8. Options menu of the detection GUI.

5.3. Results and discussion

After analyzing the 45 subjects in function of their age, some insights on the influence of age to the slow oscillation properties can be extracted from it. Here are described the most relevant metrics found in the analysis stage.

5.3.1. Up and down durations

One of the measurements of interest in this project is the duration of up and down states for each condition (age range). As can be seen in Figure 9 histograms and Figure 10 boxplots, the duration of Ups and Downs slightly decrease with age. This can be attributed to several underlying biological mechanisms, such as changes in neuronal excitability and network connectivity. The balance between excitation and inhibition of the cortical network may contribute to the shorter duration of up and down states.

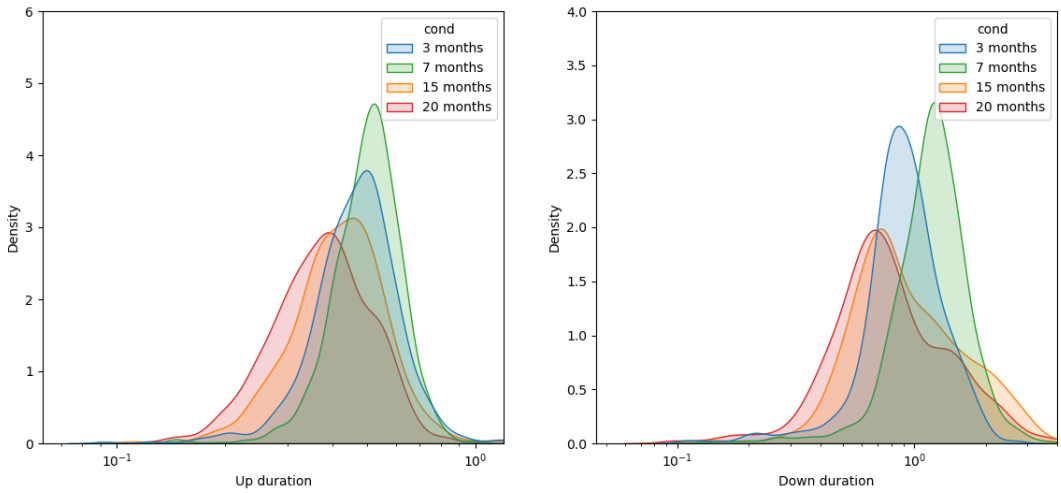

Figure 9. Histograms of the detected Up and Down state durations.

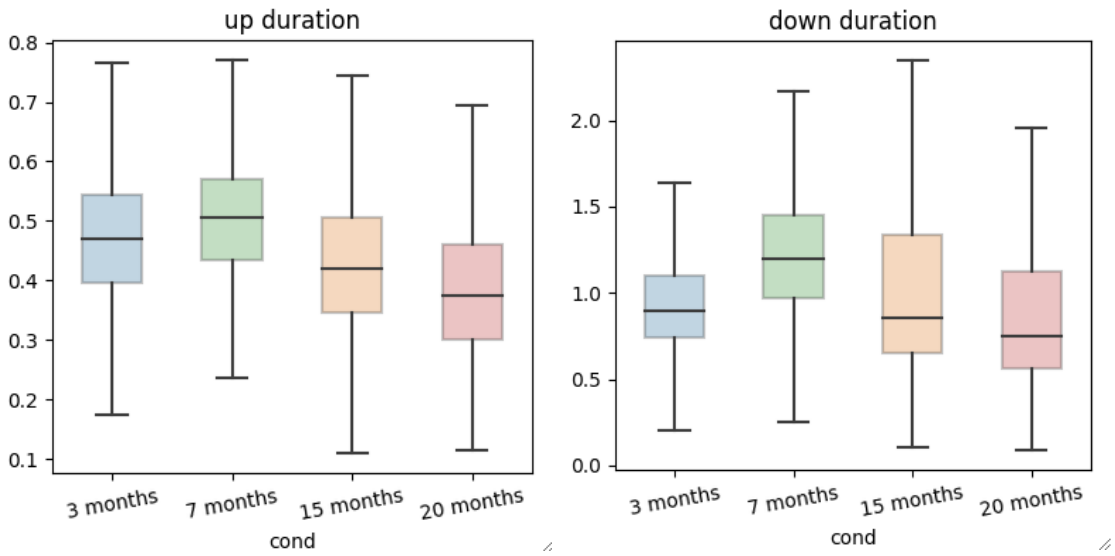

Figure 10. Boxplots of the detected Up and Down state durations.

5.3.2. Wave frequency

In this case, frequency slightly increases and has a larger variability with age (Figure 11). These features can be attributed to the same biological factors as described in the duration of Up and Down states, as smaller durations imply larger frequencies.

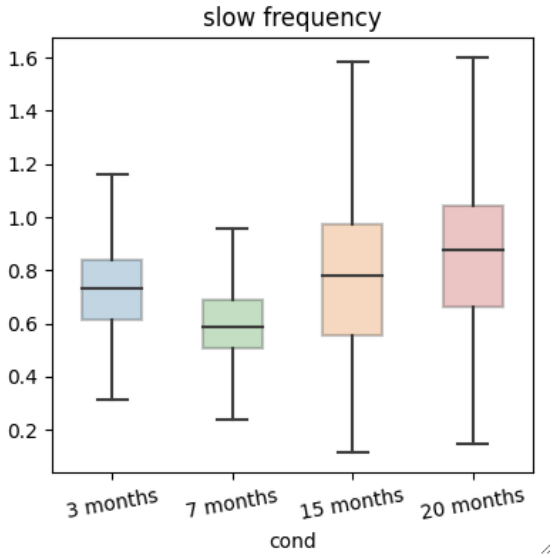

Figure 11. Boxplot of the frequency of waves.

5.3.3. Firing rate of the Up states

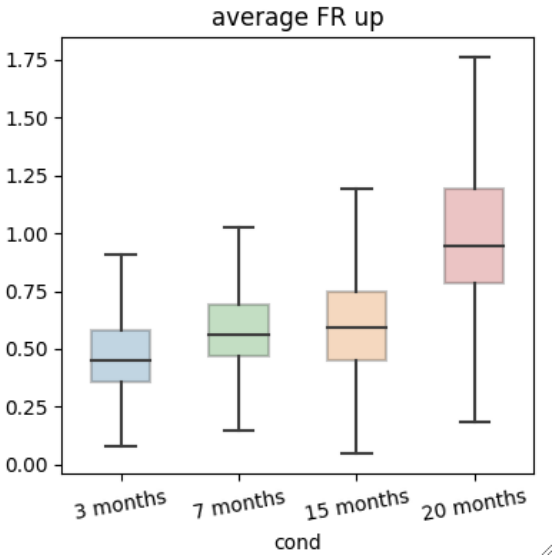

Figure 12. Boxplot of the average firing rates for the Up states.

The observed increase in firing rate of the Up states with age in Figure 12 can be attributed to the same biological factors as described in the duration of Up and Down states. Alterations in neuronal excitability, including changes in ion channel expression and membrane properties, can contribute to an increased firing rate during up states. A tightly related parameter to the firing rate is the maximum of the logMUA during up states. As can be seen in Figure 13, there is also an increase with age.

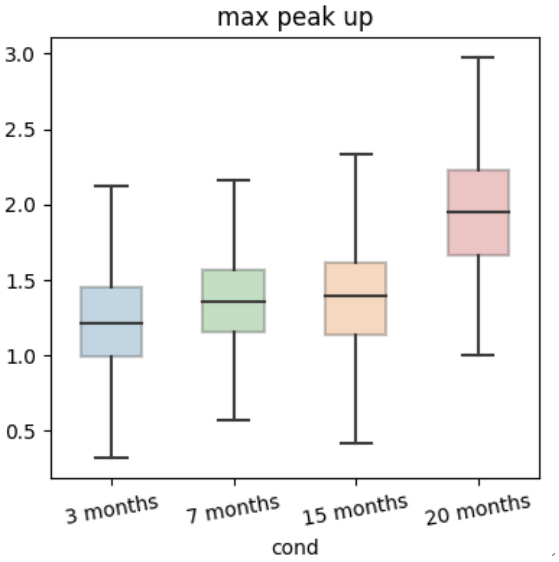

Figure 13. Boxplot of the maximum firing rates for the Up states.

Additionally, the increase in the firing rate of the Up states can also be observed in the PSD of the Up states at high frequencies (gamma band, 60-100 Hz) (Figure 14).

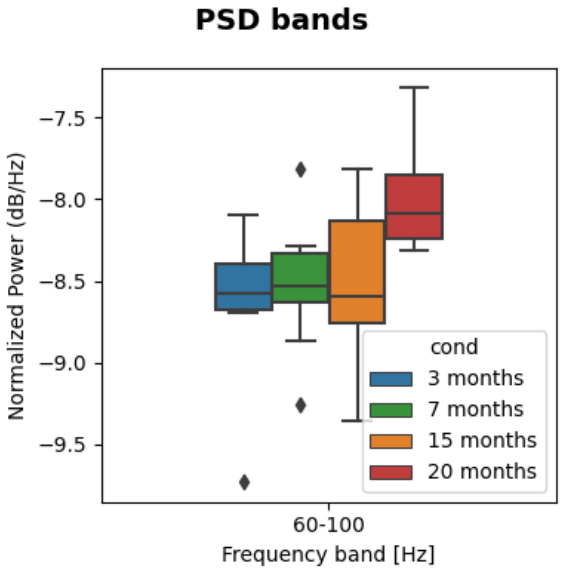

Figure 14. Boxplot of the PSD of Up states at frequencies 60-100 Hz.

5.3.4. Slopes of upward and downward transitions

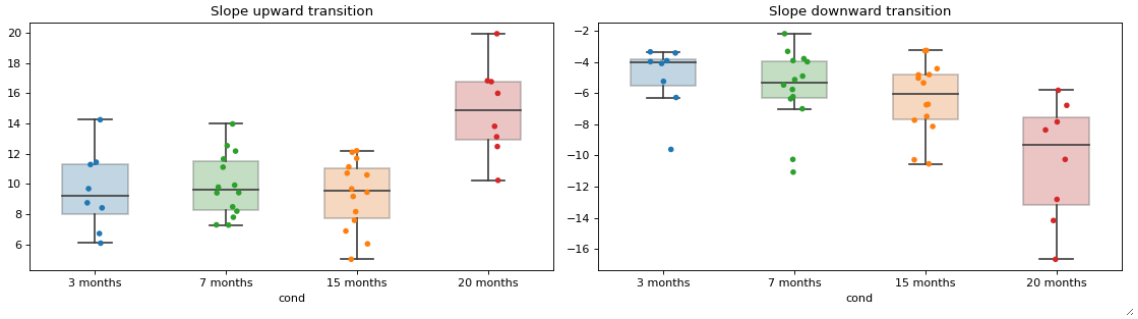

Figure 15. Boxplot of the slopes of the upward and downward transitions.

The slopes of the upward or downward transitions indicate how fast is the change from a down-to-up or up-to-down transition, respectively. What can be seen in Figure 15 is that both upward and downward slopes get higher (in absolute value), with age. This is again related to the higher firing rate occurring in older mice. As Ups and Downs are shorter, and the firing rate is larger, there is less time to achieve a higher firing pattern, thus making the network to rapidly switch from one state to another, when compared to younger mice.

5.4. Adding new functionalities

As stated in Section 4.3. Metrics, one of the functionalities that has been developed in this project is to allow the computation of the Functional Connectivity across different brain areas.

The example below shows how this has been implemented in the pipeline with another dataset that consists of recordings with 32 channels multi-electrode arrays in rats under different levels of anesthesia (light, mid and deep). As expected, and described in (Dasilva et al., 2021), areas get

more synchronized in deeper levels of anesthesia, thus resulting in an increase in the correlation between areas, as seen in the histograms below.

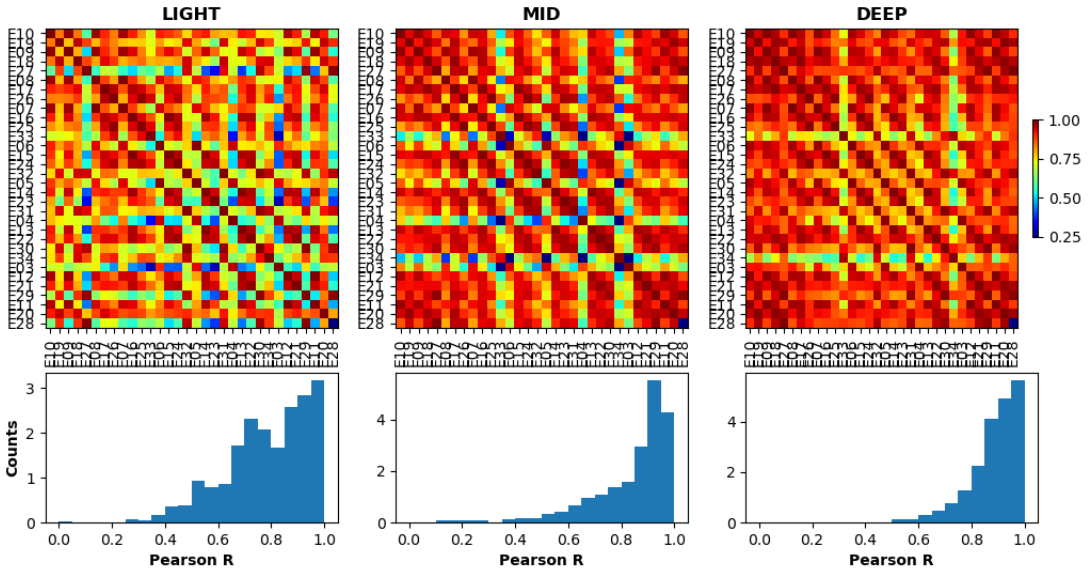

Figure 16. Graphical representation of functional connectivity in function of the level of anesthesia.

6. Execution chronogram

In this section, all the previous planning of the project is explained in detail. The project is divided in various sections that contain a few work packages related both to execution and managing.

6.1. Work breakdown structure (WBS)

This project is divided in 5 main parts (Figure 17):

- Documentation and managing, with the work packages: Planning, Project follow-up, Report writing and Technical study
- Bibliographic research, with the work packages: State of the science (Sleep stages, Slow waves), State of the art and Market analysis
- Previous steps, with the work packages: Previous setup and Metadata definition
- Detection, with the work packages: Signal visualization and Peak detection (Thresholding, Validation)
- Analysis, with the work packages: Plotting, Interpretation of results and Development of new functionalities

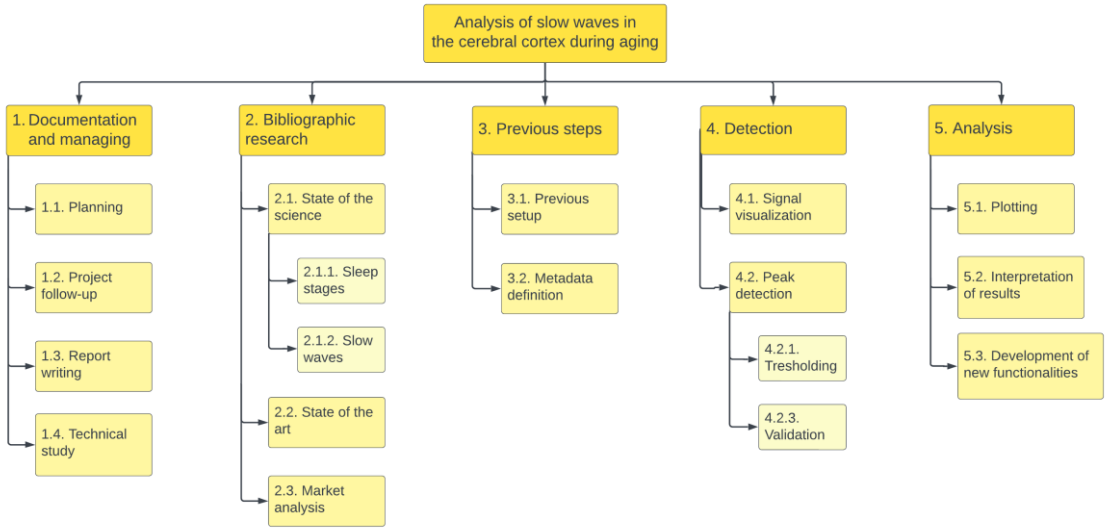

Figure 17. WBS of the project.

6.2. WBS dictionary

The work packages are described as it follows:

Task	Definition	Activities
1. Documentation and managing		
1.1. Planning	Planning of the project	- Study of the work packages - Elaboration of the chronogram
1.2. Project follow-up	Perpetual follow-up of the situation	- Ask directors for meetings - Ensure deadline compliance
1.3. Report writing	Writing of the report	- Compilation of documentation
1.4. Technical study	Study of the technical feasibility	- SWOT analysis
2. Bibliographic research		
2.1. State of the science		
2.1.1. Sleep stages	Bibliographic research on sleep stages	- Literature review
2.1.2. Slow waves	Bibliographic research on slow waves	- Literature review
2.2. State of the art	Understanding of the pipeline	- Read the pipeline documentation - Run the pipeline with test data
2.3. Market analysis	Research on market trends	- Know the addressed sector - Know its historical evolution - Search for future perspectives
3. Previous steps		
3.1. Previous setup	Setup of all the previous steps	- Environment creation - Download libraries and dataset - Create directories

3.2. Metadata definition	Setup of the metadata file	- Follow the documentation instructions on metadata
4. Detection		
4.1. Signal visualization	Visualize and load the data	- Check for abnormalities - Time period selection
4.2. Peak detection		
4.2.1. Thresholding	Tuning of the detection parameters	- Test different threshold values for each recording
4.2.2. Validation	Validate the parameter tuning	- Inspection of the detected peaks
5. Analysis		
5.1. Plotting	Visualization of the data	- Test all possible plots - Check if they deliver significant results
5.2. Interpretation of results	Discussion of the results	- Find a biological meaning - Discuss the results
5.3. Development of new functionalities	Include new metrics to the study	- Check state of the science - Implement new functionalities by modifying the source code

Table 4. WBS dictionary of the project.

6.3. Precedence analysis

The project lasts from February 20 to June 4, 2023. Thus, it lasts 15 weeks in total.

Task	**Precedence**	**Duration (weeks)**
1.1. Planning	-	2
1.2. Project follow-up	-	15
1.3. Report writing	1.1.	13
1.4. Technical study	1.1.	1
2.1.1. Sleep stages	1.1.	2
2.1.2. Slow waves	1.1.	2
2.2. State of the art	1.1.	3
2.3. Market analysis	1.1.	1

3.1. Previous setup	2.2.	1
3.2. Metadata definition	3.1.	1
4.1. Signal visualization	3.2.	1
4.2.1. Thresholding	4.1.	2
4.2.2. Validation	4.2.1.	1
5.1. Plotting	4.2.1., 4.2.2.	2
5.2. Interpretation of results	5.1.	2
5.3. Development of new functionalities	3.1.	4

Table 5. Precedence analysis of the project

6.4. GANTT chart

The GANTT chart of the project is the following, with the critical path highlighted in red.

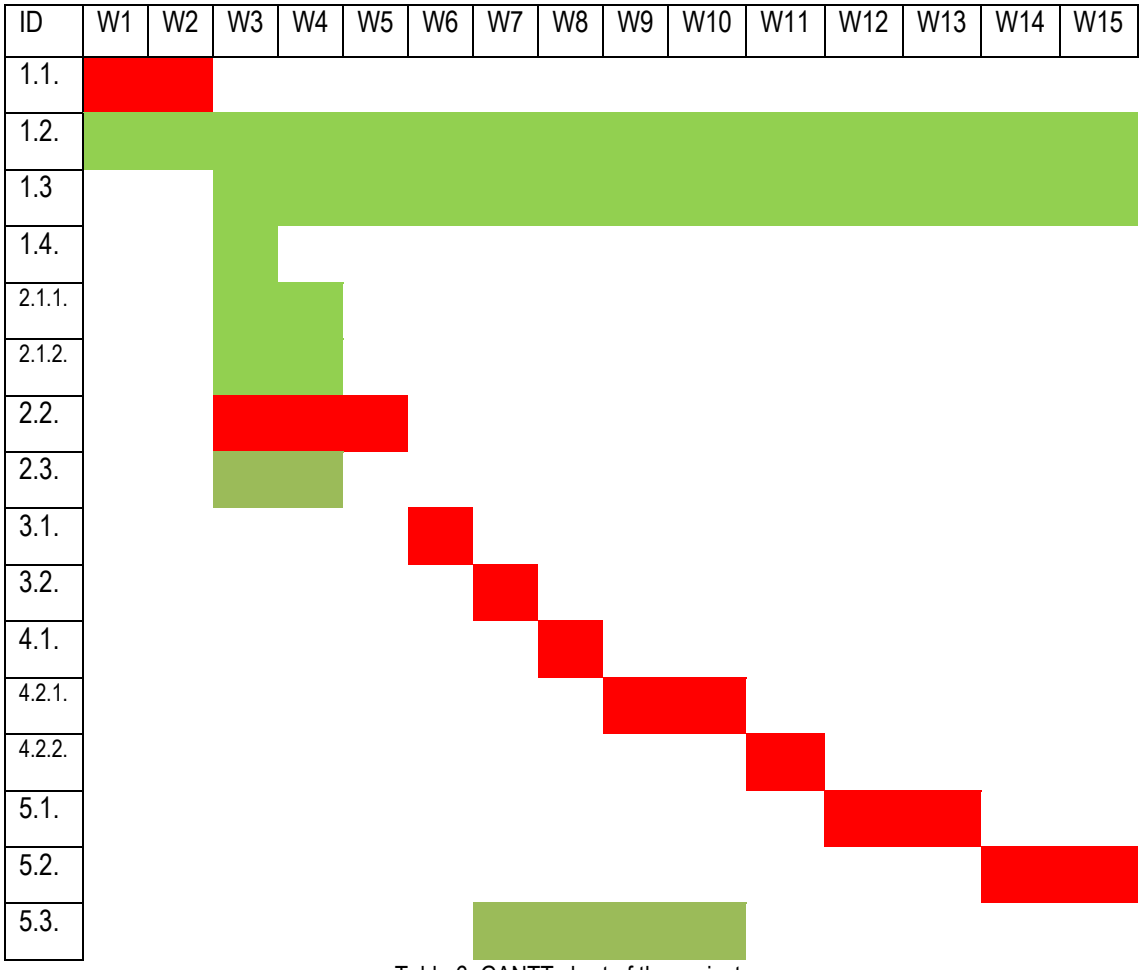

Table 6. GANTT chart of the project.

7. Technical feasibility

In this section, the technical feasibility of the project is evaluated and presented in a SWOT analysis, in order to study the strengths, weaknesses, opportunities and threats of the project.

7.1. SWOT analysis

S	INTERNAL **STRENGTHS**
1	Experience in data processing and Python before starting the project.
2	Good support by the Systems Neuroscience research group.
3	The pipeline has been previously tested and used for several journal articles.

W	INTERNAL **WEAKNESSES**
1	Limited time of execution.
2	This head of this project does not have exclusive dedication on it.
3	A cybernetic attack halted the normal functioning of the IDIBAPS facilities during several weeks.

O	EXTERNAL **OPPORTUNITIES**
1	Research in this field has been growing in the recent years.
2	There is enough literature that supports this research.
3	The understanding of aging mechanisms and their effects is still under investigation.

T	EXTERNAL **THREATS**
1	There is other and more generalist software that has more functionalities than the pipeline.
2	The processed dataset does not allow connectivity studies.
3	These kind of investigations need funding from governmental helps or private grants.

Table 7. SWOT of the project.

8. Conclusions and future work

Slow oscillations, which are the default mode of the cortex and the main oscillation in slow-wave sleep, may be affected by aging. In particular, significant effects have been noted in the duration of Ups and Downs, as they slightly decrease with age, possibly due to several underlying biological mechanisms, such as changes in neuronal excitability and network connectivity. Furthermore, the balance between excitation and inhibition of the cortical network may contribute to the shorter duration of up and down states.

Also, an increase in firing rate of the Up states with age has been observed. This may be explained by alterations in neuronal excitability, including changes in ion channel expression and membrane properties, which can contribute to an increased firing rate during up states. Other affected metrics are the slopes of the upward and downward transitions.

Some future work that could be performed could be using other brain areas (not only visual but also prefrontal, motor, sensory, auditory, etc.), comparing if changes in the connectivity across these different areas are also present and are related to aging, making the Slowpy pipeline available to the neuroscientific community and organically including other metrics, like functional connectivity, to the pipeline.

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