POLITECNICO
MILANO 1863

SCUOLA DI INGEGNERIA INDUSTRIALE
E DELL'INFORMAZIONE

HiPSC analysis with Multi-Electrode Array technology: setup and methods for a case-control study

Tesi di Laurea Magistrale in
Biomedical Engineering - Ingegneria Biomedica

Kevin Sangalli, 944941

Advisor:
Prof. Alessandra Pedrocchi

Co-advisors:
Andrea Menegon, PhD
Benedetta Gambosi, Eng.

Academic year:
2022-2023

Abstract:
Electrophysiological studies are the base to acquire information about the brain and neuron activity. Different techniques can give information about the anatomical structure and physiology of the central nervous system (CNS). Neuronal cultures can be studied using a multi-electrode system (MEA). This system records the extracellular activity, through a matrix of electrodes, giving us information about the overall activity of the cell network. Human induced pluripotent stem cells (hiPSC) were employed to obtain hiPSC-derived neurons, that can be used to build human functional models. These cells bring the DNA of the person they are from, carrying an eventual disease. A pathology that can be studied with these technologies is the Prater-Willis syndrome (PWS), a genetic disorder that affects chromosome 15.
hiPSC-derived neurons coming from a subject affected by PWS and a control subject were used in this work. A longitudinal study and a comparison between the two groups have been done regarding basal activity. Cells were recorded every week for the duration of the study. Other experiments involve the use of electrical high-frequency stimulation (HFS) to verify if the network display sign of long term potentiation (LTP). A basal recording and a post stimulation recording were done. As last experiment, glutamate was added to the culture to see the response of the cell network, then the cells are dismissed. Features regarding spiking and burst activity were extracted from the recordings using a developed software written in Python. Given the reduced sample size due to difficulties and long time to obtain hiPSC-derived neurons the dataset used has a small size.
Results seem to show greater activity in both spikes and burst features in the pathological subject in the longitudinal study. No evident changes were seen between the pre and post HFS protocol. Although the results seem promising, they don't show a statistical significative difference. The glutamate response shows interesting results, a response was seen in both cases, increasing the activity in the control, as expected, but decreasing in the pathological, probably due to synaptic fatigue.

Key-words: multi-electrode array, MEA, induced pluripotent stem cells, iPSC, Prater-Willi syndrome, PWS

1. Introduction

1.1. Neurons and action potential

In recent years, there has been a growing interest in understanding the complexities of the brain, including its functioning and various pathologies. Cognitive disorders, genetic conditions, and neurodegenerative diseases have gained significant attention, especially considering the extended life expectancy in modern times. To investigate and develop functional models for these pathologies, researchers have encountered the challenge of obtaining human neuronal cells. Animal cells have been utilized for many years, but the resulting models were limited to the characteristics of that specific animal, necessitating further studies. The unavailability of human neuronal cells, both due to ethical concerns and biological implications, was a problem that needed a solution. To resolve the question, scientists developed a method to produce induced pluripotent stem cells (iPSCs) derived from human sources. iPSCs are generated from certain somatic cells and can be differentiated into various cell types, including neurons. hiPSC offers an important resource for generating human pathological neurons, presenting a powerful tool for studying diseases and establishing human functional models.
The central nervous system (CNS) primarily consists of two specialized cell types: neurons and glial cells. Neurons are responsible for receiving, processing, and transmitting information. They receive signals in the form of neurotransmitters from other neurons, process this information, and then transmit it to other neurons. The transmission of information within a neuron occurs through electrical impulses known as action potentials (APs). An action potential is a brief, rapid increase in the neuron's membrane potential. It propagates along the neuron's axon and triggers the release of neurotransmitters into the extracellular space. Other neurons receive these neurotransmitters, which can either excite or inhibit their activity. Neurons that produce excitatory neurotransmitters are called excitatory neurons, whereas those that produce inhibitory neurotransmitters are named inhibitory neurons. The resting potential of a neuron's membrane is approximately -70 mV. When the membrane potential becomes more positive and reaches abut -50 mV, an action potential is generated. This process is sustained by ionic currents that flow through specific ion channels in the neuron's membrane. The currents are primarily caused by the entering of sodium ions and the exiting of potassium ions. These ionic currents modify the extracellular environment, leading to changes in the voltage surrounding the neuron. By monitoring the extracellular potential and its variations resulting from these currents, information about the action potentials inside the neuron can be obtained[11]. In addition to neurons, glial cells play a vital role in supporting and protecting neurons. They do not carry information themselves but rather maintain the overall functioning of neurons, contribute to homeostasis, form myelin (which enhances the efficiency of neuronal signaling), and provide structural support. One type of glial cell is known as astrocytes.

1.2. Electrophysiology

Electrophysiology is a widely used technique for studying neurons and brain activity. It captures a broad range of phenomena, from the spiking activity of single neurons to network oscillations in neuronal populations. It allows the study of electrical activity at different scales.

Microscale At the microscale level, patch clamp is the gold standard method for measuring cell membrane voltage and currents, including the measurement of single ion currents in individual neurons. This technique typically involves using sharp microelectrodes or patch-clamp techniques for intracellular measurements. However, it should be noted that the procedure itself can damage the neuron's membrane, leading to cell death and limiting the recording time. Additionally, the patch clamp technique has a practical limitation in terms of the maximum number of neurons that can be studied, typically limited to a few units. This limitation arises from the bulky nature of the instrumentation required for performing these experiments [14].

Macroscale At a macroscale we have direct measure as magnetoencephalography (MEG) and electroencephalography (EEG), as indirect measurements there are functional magnetic resonance (fMRI) and positron emission tomography (PET). These methods are used to acquire the anatomy and the functional activity of brain regions. These techniques have generally different time and spatial resolutions depending on the specific type used. They are most used to inquire about wide regions and the overall activity the same.

Mesoscale On the mesoscale level, it is possible to study small populations of neurons or specific brain sections. One tool commonly used for this purpose is the multi-electrode array (MEA) system. The MEA system allows for the recording of extracellular potentials surrounding the cells, providing information about neuronal activity. Additional details about the MEA system will be discussed in subsection 2.1.

2. State of the art

2.1. Multi-Electrode Array

Multi-electrode array (MEA) systems consist of a matrix of small electrodes disposed in a specific pattern and are used to record the extracellular voltage surrounding cells. They find applications in both in-vivo studies as part of neural interface systems, aimed at recording central nervous system (CNS) activity and transmitting signals within the system [9], as well as in in-vitro investigations of cell cultures, including neurons and cardiomyocytes, as well as tissues such as brain sections or cardiac tissue. Essentially, any cell or tissue exhibiting electrical activity can be studied using MEA. This work will focus on in-vitro multi-electrode array (MEA) systems and neurons derived from human induced pluripotent stem cells (hiPSC), these will be covered in subsection 2.2.
MEA systems are utilized to record the extracellular field potential (EFP) [16], also known as the extracellular action potential (EAP) [14] or local field potential (LFP), although the last term is not entirely accurate. This signal is generated by the spiking activity of individual neurons or the superposition of rapid action potentials (APs). The electrical currents involved in the AP process induce slight fluctuations in the extracellular voltage, which are captured as the EFP. Previous studies, as [11], confirmed the relationship between AP to the EFP. As a result, from extracellular recordings we can obtain information about the intracellular process that is the action potential. About this aspect a consideration must be done, MEAs system cannot record an eventual sub-threshold phenomena, neurons that do not produce any AP aren't detectable by the system and their eventual activity is lost. [16]
Another aspect of a MEA system is that electrodes record electrical activity from all the surrounding volume, there is no selectivity of the neurons recorded, but they capture all the activity that reaches the electrode. In the case of an electrode capturing the activity of multiple neurons the discrimination of the spike belonging to each of them must be done via software in a successive analysis. A similar issue is present when the electrode of the MEA is used for the stimulation, this can't be well contained to specific regions due to the conductive property of the extracellular medium.
One advantage of MEA systems is that they perform a non-invasive extracellular recording, which does not damage the neuronal structure. As a result, on the MEA chip cell culture can be grown over long periods of time, enabling the possibility to study over multiple weeks the activity of the cells or tissue on the MEA chip. Proper environmental conditions for the cells' survival must be ensured. This system offers the possibility to provide insights into neurodevelopmental processes that function at longer timescales. Also, MEA can be used to perform functional phenotyping experiments for disease modelling studies using iPSC. [13]

The dimensions, distances, and number of electrodes in a MEA system can vary depending on the specific model and the type of electrodes used [14]. The area of an electrode typically ranges from 2.5 x 2.5 μm^2 to 30 x 30 μm^2, while the distance between electrodes falls within the range of 10 to 100 μm. With advancements in technology, the number of electrodes can exceed 10,000, utilizing approaches like CMOS technology. The overall area covered by the electrode matrix is typically a few mm^2. Regarding electrode manufacture, various materials can be used, including metals, titanium nitride (TiN) for example, and also non-metal materials [14]. The system can also stimulate the cells from specific electrodes or from the one used for the recording. In this case, the electrode needs to be able to mediate reaction at the electrode-electrolyte interface and permit the electron current passing in the electrode to be transformed into an electrolyte flow toward cells nearby [8]. The choice of electrode material is crucial to obtain biocompatibility, especially for prolonged contact with cells, so as not to cause toxicity or other adverse effects.

Depending on cell's morphology around the electrode, the electrical signal measured by each electrode is composed of different signal sources. The signal recorded by the electrodes depends on some factors, the distance from the source, the area in contact with a neuron, the extracellular medium in which cells are and the same electrode properties. When a neuron is in contact with the electrode there is still a small gap between them that is filled with medium. An electrical equivalent of the coupling electrode-neuron is presented in Figure 1. In the figure can be seen different elements, the cellular membrane of the neuron in contact with the medium (in red) and its electrical equivalent composed by the resistance R_nj and the capacity C_nj. The cellular membrane of the neuron in contact with the electrode (in blue) and its electrical equivalent composed by the resistance R_j and the capacity C_j. The electrode (in orange) impedance is composed of a resistance,R_e and a capacity C_e. The extracellular medium (pink) is simply modelled with a resistance R_seal. [16]
Two major issues are presented in the MEA system that affect negatively the measurement. The first is that the extracellular solution is highly conductive, its resistance is small but present. When an AP creates a difference in the extracellular potential, the resistance of the medium (R_seal) cause the formation of a current between

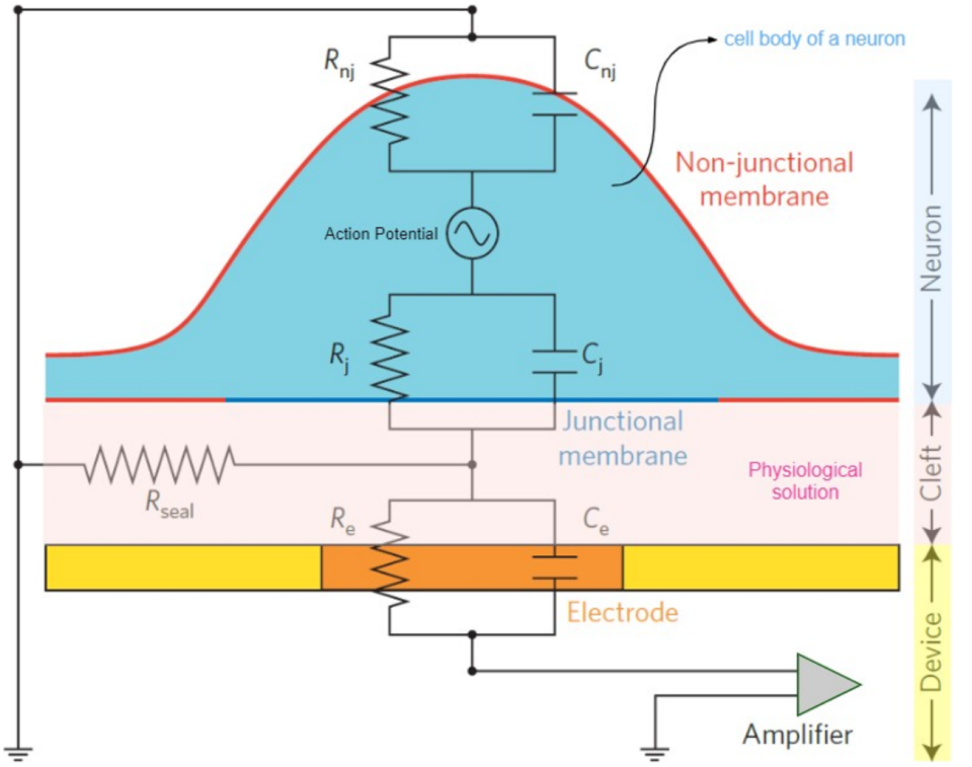


Figure 1: The body of a neuron (light blue) leans on a sensing electrode (orange) integrated into the culture substrate (yellow). The electrode is coupled to an amplifier (gray). A gap filled by the cell medium (it is an ionic solution) lies between the cell membrane and the electrode-substrate (pink). The neuron's plasma membrane is subdivided into two regions: the part that faces the electrode (blue) is defined as the junctional membrane and is represented by the junctional membrane resistance (R_j) and the junctional membrane conductance (C_j). The rest of the membrane, defined as the non-junctional membrane (red), faces the bathing solution and the culture substrate. This part of the membrane is represented by the non-junctional resistance (R_nj) and the non-junctional capacitance (C_nj). The electrode (orange) impedance is represented by the electrode resistance and capacitance R_e and C_e, respectively). The electrode can be a passive element or a transistor. Under physiological conditions, current is generated by transient changes in membrane conductances. Figure adapted by [16]

this potential and the ground. This current produces a small difference in voltages where it flow that can be recorded by the electrodes, introducing noise in the measurement.
The other aspect that affects the measure is the electrical coupling between the electrode and the neuron. This coupling is affected by the area of contact, the shape of the electrode, the property of the cell membrane. The impedance of this junction depends on R_j, C_j and if the value of this resistance is high and the value of the capacity is low it means that only a portion the current generated across the neuron's membrane flows through the membrane in contact with the electrodes. To reduce this impedance different solutions can be used, from reducing the distance between the electrode and the neuron by applying a negative pressure on the cell with openings in the substrate, others suggest mushroom-shaped electrons or other changes in the geometry of the electrode. Also, the impedance of the electrode can be taken into consideration, this depend on the material composing the electrode. Electrodes must have a low impedance and between them this value must be uniform. Reaching a low impedance results in a better signal-to-noise ratio, improving the quality of the recording.[16]

2.2. Induced pluripotent stem cells

Certain somatic cells, such as skin fibroblasts and peripheral blood cells, can undergo reprogramming using specific factors to transform into induced pluripotent stem cells (iPSC). These iPSC have the ability to differentiate into various cell types, including those found in the central nervous system, such as neurons, as well as cardiomyocytes and more. One significant advantage of utilizing iPSC is that they have the exact DNA of the individual from whom they are derived. In the case of individuals with pathological conditions, iPSC brings

the genetic information associated with the specific pathology, allowing for the creation and study of precise disease models.

iPSC-derived neurons Due to biological and ethical reason, it is impossible to obtain human neurons, so the researcher try to remedy this fact by using human iPSC (hiPSC) and reprogramming them into neurons. Another advantage of using hiPSC is that all the research and eventual therapy or treatment are done directly onto human cells, not animal cells, so the problem of an eventual species gap is eliminated.
iPSC-derived neurons present an electrical activity that can be studied similarly to neuronal networks, from the study of single neuron to the study of a culture of cells, to study the connectivity between them and eventually some network analysis. In general, the features linked to single spike activity of iPSC-derived neurons give an idea of the overall activity. Burst activity can be studied, a burst is defined by a minimum number of spikes and the maximum inter spike interval (ISI) between spikes in it.
There is a plethora of features that can be extracted from an analysis of these cells, regarding the analysis of signals coming from a MEA system Gramowski identified 30 different parameters that can be used. [10] There are parameters for the spike activity, the burst activity and for the network activity. From all of them six were taken into consideration for the analysis, see (subsubsection 3.4.2. Parameters about the spiking activity give general information about the activity of the culture. Burst features are linked to the functional and structural maturation of the cell network. [17] Also from these features, information about the balance between the excitatory and inhibitory system can be derived.
The phenomenon of long term potentiation (LTP) is a process involving persistent strengthening of synapses that leads to a long-lasting increase in signal transmission between neurons. It is at the base of synaptic plasticity and it is correlated with memory and learning. It is present in cortical neuron culture and studies induced it in iPSC-derived neurons. If present is interpreted as a sign of functional circuit formation between cells [3].

2.3. Prater-Willis syndrome

Each cell in the human body contains 23 pairs of chromosomes; one half is inherited from the mother and the other half from the father. Some portions of the DNA present genomic imprinting, this is present particularly on chromosomes 15 and 11. Genomic imprinting is a process where only one copy of a gene (either from their mother or their father) is expressed, while the other copy is silenced. Genomic imprinting does not affect the DNA sequence itself, the gene expression is silenced by the epigenetic addition of chemical tags to the DNA during egg or sperm formation. Epigenetic tags on imprinted genes usually stay in place throughout an individual's lifetime.

Prader-Willi syndrome (PWS) is a multisystemic complex genetic disorder caused by a lack of expression of genes on the paternally inherited chromosome 15q11.2-q13 region. There are three main genetic subtypes in PWS: paternal 15q11-q13 deletion (65–75 % of cases); maternal uniparental disomy 15 (20–30 % of cases), where the two chromosomes come both from the mother and imprinting defect (1–3 %)[2].
It has an incidence of about one in 10,000 and one in 30,000 with an equal incidence between males and females [5]. The syndrome affects various aspects such as appetite, growth, hormonal system, metabolism, cognitive functions, and behaviour. Individuals with PWS generally experience some level of cognitive impairment, delayed motor milestones, and later language development. Infants often have weak muscle tone (hypotonia) and feeding difficulties, while young children exhibit excessive appetite and gradually develop obesity. The mortality rate is around 3% per year, due to mostly secondary effects of the pathology linked to diabetes and cardiovascular problems. [2]

Within the region affected by the PWS there are the genes encoding the α_5, β_3 and γ_3 subunits of the gammaaminobutyric acid type-A ($GABA_A$) receptor. [12] The principal neurotransmitter that react to this receptor is the gamma-aminobutyric acid (GABA); this substances is produced by inhibitory neurons, and its effect is to reduce neuronal excitability. These receptors are expressed also by the maternal genes, so the subjects affected by the PWS have half the genes to encode them. Altered ($GABA_A$) receptor composition or number in some brain regions, such as the frontal and temporal neocortices, may be the cause for the neurobehavioral abnormalities introduced before in PWS subjects[12].

2.4. Aim of the work

In the present thesis, we present the setup and analysis protocol for a comparative study of human iPSC from one control and one pathological subject affected by PWS. In particular, the work will focus on assessing

differences between the control and pathological subject in terms of

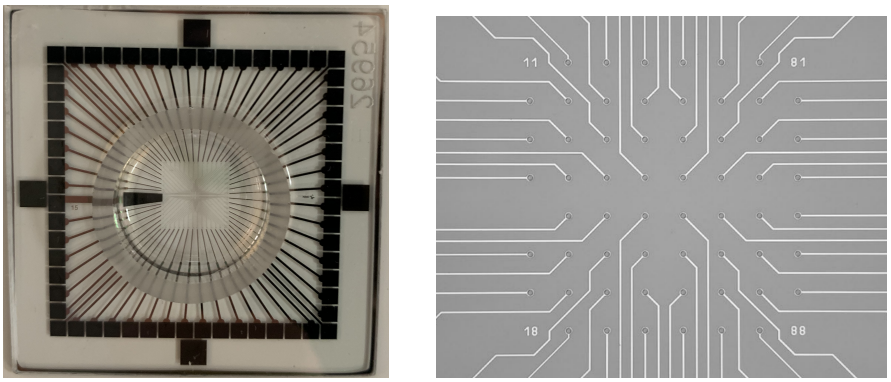
1. maturation of the culture, analysed through the basal activity of the samples;
2. response to high-frequency stimulation;
3. response to glutamate stimulation.

Additionally, this study aims to develop and test an apposite software for the analysis of the recorded data.

3. Materials and methods

3.1. Instrumentation setup

Standard MEA configuration The MEA system is composed by a pre-amplifier MEA1060-Inv-BC (Multichannel Systems GmbH, MCS GmbH, Reutlingen, Germany) with gain of 55 and bandwidth of 0.02Hz-8.5kHz, an amplifier (FS64S, MCS GmbH) with gain of 20 and bandwidth of 10Hz-3kHz and a data acquisition device (USB-ME64 MCS GmbH). An external power generator (PSW40), is used to supply additional power and to stabilize the voltages of the pre-amplifier and the amplifier. This system is also equipped with an external unit to control the temperature through a temperature-controlled plate (TCO2 by MCS GmbH) below the MEA1060.
For all the experiments 200/30iR-Ti-gr MEA (MCS GmbH) chip are used (Figure 2) There are 59 electrodes placed as a matrix, they are distant 200 μm and have a diameter of 30 μm (as shown in Figure 2b). There is an additional electrode that is used as reference: its purpose is to keep a stable and well-known potential (in this case to the ground voltage of the MEA1060-inv-BC). This electrode is physically wired to the electrical ground with an apposite cable.



(a) Photo of a MEA chip.

(b) Zoom of the 59 recording electrodes of the MEA chip.

Figure 2: MEA chip 200/30iR-Ti-gr.

Additionally, the setup is equipped with a a stimulus generator, the STG2004 (MCS GmbH), for the electrical stimulation.

MEA software control MC Rack (MCS GmbH) was used to do all the recordings during the experiment, it was used to see all the electrode channels to perform a visual inspection if the electronics worked fine and all the signals were acquired correctly. It was used to set the recording time length and the sampling frequency. Through the software MC-Stimulus II (MCS GmbH) a stimulus in current or voltage can be programmed with different waveforms and amplitudes. With the MEA-select (MCS GmbH) program it is possible to select the electrodes for the stimulation.

3.2. Biological preparation

3.2.1 hiPSC culture

The biological material used in the experiments was provided by the Neurogenesis and Stem Cell Unit Laboratory of San Raffaele Hospital, and the procedure to obtain the hiPSC used in this work was developed there. The protocol to obtain hiPSC was slight a modification of the protocol used by Banfi [4], instead of human skin

fibroblasts were used human peripheral blood mononuclear cells (PBMCs) and 250,000 iPSC-derived neurons were seeded onto a coated MEA.

Peripheral blood cells are reprogrammed into iPSC using apposite reprogramming factor introduced in the cells by the Sendai Virus using an apposite kit (CytoTune®-iPS Reprogramming Kit, Invitrogen), and then the virus is removed. From iPSC, with a specific procedure, neuronal progenitor cells (NPC) are derived, these cells are precursors of neurons, astrocytes and other cells of the nervous system. These NPC are differentiated in neurons and seeded on MEA with astrocytes coming from mouse, neurons are 250,000 and the astrocytes are 50,000 cells. The addition of astrocytes is done to help the maturation of neurons and prevent their detachment from the MEA chip.
To preserve the sterility of the cell culture, the culture cells in the chip are covered with a gas selective permeable membrane (ALA MEA-MEM, MCS GmbH). The membrane also is used to reduce the evaporation and the changes in pH of the medium; it is kept during all the lifespan of the cells.

3.2.2 hiPSC dataset

hiPCS were derived from two subjects: a pathological, affected by the PWS (subsection 2.3), and a control one. This specific pathological subject has a big deletion of about 4-5 megabase pair on the paternal region of chromosome 15, typical of the PWS. This deletion covers also the segment in the 15q12 region, which involves the deletion of the sequence that encodes for the GABRB3 gene. This gene is responsible for the encoding of a subunit of the GABA receptor as told before.

Throughout the study, experiments were performed on three sets of samples, derived from those two subjects. So, each set contains MEA chips, with hiPSC-derived neurons, from both the pathological subject, and the control one. In Table 1, further details about the sets are summarized: the number of chips belonging to the control and pathological group are presented, as well as interval in which recordings were performed (in terms of the maturation weeks when the recording have started and ended). Weeks are counted starting from the day cells were seeded on the MEA chip. Set 1 was the first set recorded, for the following two we decided to start recording from earlier weeks. Lastly, the week in which recordings are stopped corresponds to the chemical experiment with the glutamate, after that the sterility of the culture is compromised and the cells cannot be used anymore.

	Pathological (PWS)	Control	Starting week	Stop week
Set 1	3	2	4	6
Set 2	2	2	2	5
Set 3	3	3	1	5

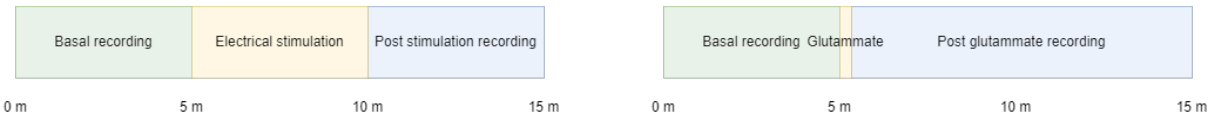
Table 1: Dataset characteristics: three sets obtained from one pathological subject and one control. The "Control" and "Pathological" columns represent the number of MEA chips used for each set. The "Starting Week" and "Ending Week" columns indicate the beginning and end weeks during which the experiments were conducted.Weeks are counted starting from the seeding of cells on the chip.

3.3. Data acquisition

The recordings were made using MC rack (MCS GmbH), beside the recording of the signal, the software allows the user to see in real time what is being acquired by all the electrodes through a video where the spatial geometry is kept in the representation. In this way the user is able to check the proper functioning before starting the recording.
During the recordings, the chips were kept at a temperature of 37°C[15] and acquired at 20kHz. The standard protocol for data acquisition is to place the MEA chip on the MEA1060, read the acquired signal and wait until the noise level is stable on all the electrodes before starting the recording. In the case that some electrodes are too noisy the chip is extracted and the pins and contact are cleaned with alcohol. Recordings were performed only if there was at most only two electrodes that remained noisy after the cleaning procedure.

High-frequency protocol Before any recording, the MEA chip with hiPSC is kept in an incubator with oxygen (O2) around 20% and carbon dioxide (CO2) at 5%. The day before the experiment half of the culture medium, corresponding to 500 μL, is changed.
For each chip a recording of the baseline of 5 minutes is done. The first basal record of the day will also be used for the longitudinal study. Experiments with a high-frequency stimulation (HFS) were done to try to induce long potentiation (LTP), see subsection 2.2. The stimulation pattern used was biphasic, each phase lasts 300 μs, negative phase before the positive one. This stimulation unit was repeated four times every 9400 μs, so 100Hz, this group of four units (Figure 4a) was repeated ten times in total every 200 ms(Figure 4b). This block was repeated ten times every 30 seconds. This pattern was inspired by some previous works[1, 3, 6]. In total this HFS protocol lasted for 5 minutes. The stimulation was supplied through all electrodes as in [3]. Different voltages were tested, ranging from 100 mV to 1 V, the most used protocol was the one that used 500 mV, as utilized in [3] and suggested by MCS support. Its results will be presented later in subsection 4.2. Others stimulation values were used mostly for testing. After the stimulation a post stimulus recording of 5 minutes was made. A summary of the whole protocol is shown in Figure 3a.

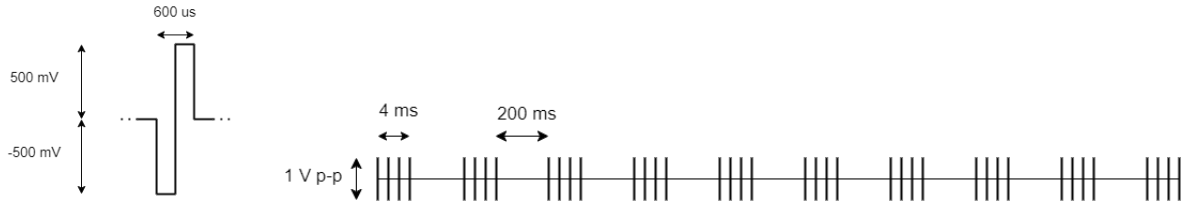
Glutamate As already introduced, in the final week of recording, a chemical experiment was carried out. For this case, we used compound, e.i. glutamate, to perform chemical stimulation. For these experiments, a unique recording was performed consisting of 5 minutes of basal plus 10 minute of post glutamate injection recording, as seen in Figure 3b. Glutamate was included in the solution with the following procedure: a small amount (50μL) of medium was taken from the culture to which 1μL of 0,1M of glutamate was added and finally this solution was injected to the culture on the MEA chip.



(a) HFS protocol, 5 minutes of basal recording, 5 of electrical stimulation and 5 of post stimulation recording. These are three different recordings.

(b) Glutamate protocol, 5 minutes of basal recording, then the glutamate is added and other 10 minutes are recorded; all in a single continuous recording.

Figure 3: Summary of the two protocols used, HFS on the left and glutamate on the right.



(a) Zoom on the voltage pulse used for the stimulation.

(b) Stimulus block composed of ten repetition of 4 pulses every 200ms. All this is repeated every 30 seconds for 10 times.

Figure 4: HFS protocol for stimulation, on the left a zoom of the pulse and on the right the stimulation protocol that is repeated every 30 seconds for 10 times.

3.4. Data analysis protocol

The software (SW) used for data analysis is a custom one developed in Python to be open source and more oriented to the analysis needed. All the parameters used in the code can be changed for a custom setting, giving all the users the possibility to adapt the analysis to the specific situation. The SW can be updated easily, with new algorithm implementation and further customization.
All the signal analysis was performed offline. The general flowchart of all the processes for the data analysis is reported in Figure 5. Firstly, the files were converted from the mcd format, the output format of MC Rack, to an HDF5 file using the apposite program (MC DataManager) from MCS. This output file can be read and data are extracted by the SW.

3.4.1 Filtering

The signals from each channel are initially filtered using a second-order Butterworth filter, retaining frequencies within the band of 100Hz-3kHz, in a manner consistent with the work of Obien [14].
The software evaluates outliers in the recorded channels in two ways based on two thresholds. The signal is divided into windows of 500 ms and the standard deviation (STD) of each of them is calculated. If the STD of a window is lower than 0.15 μV the channel is discarded, this first filtering step discards channels that aren't active. This condition can happen if the contacts are dirty, ruined or broken. The other filtering procedure exploited a second threshold (set to 6 μV) which is compared to the STD of the entire signal, if this value is greater than the threshold the channel is discarded because considered too noisy.

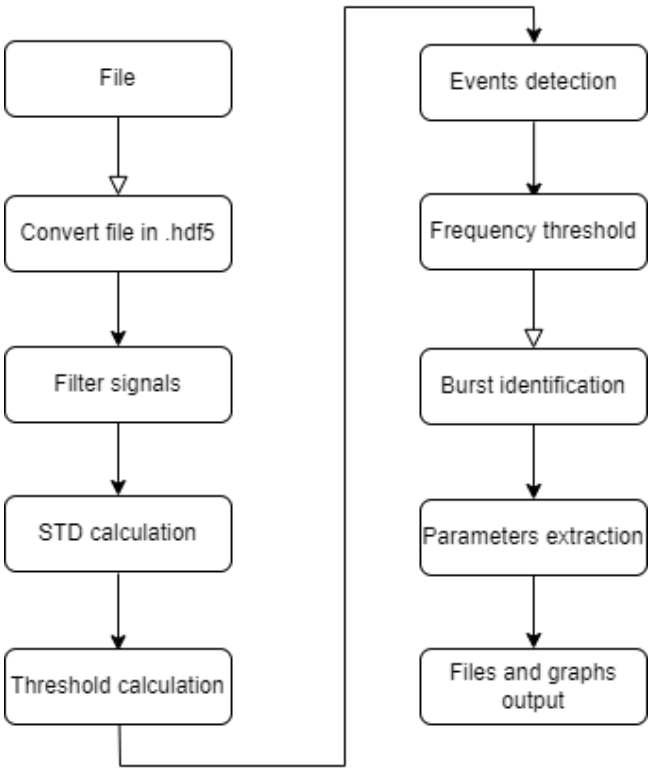


Figure 5: Analysis flowchart, the output file from MC Rack is converted to a HDF5 format and then the software performs different steps for the analysis, including filtering out noisy channels, performing event detection and feature extraction.

3.4.2 Event detection

On the channels that pass the previous checks, event detection is performed. This is achieved via a thresholding method: an additional threshold is calculated as the mean of the five smaller STD calculated on the 500ms windows multiplied by 5. The main assumption is that when the signal crosses the threshold, it is not a result of noisy oscillation, but an event occurred. In this work was used a value of 5, but values range usually from 5 up to 7. The threshold obtained is used both for a positive threshold and for a negative one. If the signal crosses the positive or negative threshold the SW searches for the maximum or the minimum in a short time range, that is 0.8 ms, and that peak is considered an event. If two events occur in a too short time window, of 1.8 ms, the latest event is discarded. Thresholding methods are commonly used in literature, although other methodologies exist. [14]

The identified events are stored in a file and used for further processing. They are the starting point for the calculation of all the features used for the comparison between the control and pathological signals. Particularly, the selected features, among the plethora used in the literature [10, 13] and presented in subsection 2.2, are:

- number of active channels
- total events per minute
- mean amplitude of events
- number of channels with bursts
- bursts per minute

- mean length of bursts

Number of active channels For each channel, the mean frequency of the spikes is calculated as total event number divided by the duration in seconds of the recording. Channels with a mean event frequency lower than 0.1 Hz are discarded [3] as if there is a mean event frequency greater than 300 Hz, this upper threshold is set because cells cannot fire that fast.

Total events per minute As the sum of all the events per minute of all active channels in the chip.

Mean amplitude of events Calculated as the mean of the absolute value of all events from all active channels.

number of channels with bursts The number of channels with burst, defined as the number of channels that shows a burst activity; Bursts are identified using criteria from Chiappalone[7]: all spikes or events within a burst must exhibit a minimum inter-spike interval of 100 ms. Furthermore, to satisfy the burst composition requirement, a minimum of 10 spikes must be present within a burst.

Burst per minute The total burst per minute is the sum of all the bursts per minute of all active channels.

Mean length of bursts The average above all active channels of the mean length of all the bursts in a channel.

3.4.3 Basal activity in weeks

The longitudinal study on the basal activity has been done for the purpose of evaluating the degree of activity of the hiPSC, the objective is to find the week that presents the maximum activity of the cells and when the activity tend to decrease. The idea is that in that week will be performed the chemical stimulation. Also some information about difference in maturation time between the two groups can be derived by this analysis. The presented features (see Event detection above) were extracted from the first basal recording of the the day for each chip and used to do two analysis: one is the longitudinal study of the activity within the same group and the other is a comparison between the two groups in the different weeks of the study. All data from the different sets are pulled together maintaining the separation between control and pathological and the specific week of recording. The numerosity of the two group can be found in Table 2.

	1 week	2 weeks	2 weeks	4 weeks	5 weeks	6 weeks
Control	3	5	5	7	7	2
Pathological	3	5	5	8	8	3

Table 2: Numerosity of the groups used for the time course analysis for the control and the pathological subjects.

3.4.4 High-frequency stimulation (HFS) protocol

The specific HFS protocol is explained in subsection 3.3. The objective of stimulating with a high-frequency stimulation protocol is to verify if the cells can show the effects of long term potentiation (LTP). This phenomenon is at the base of memory and learning mechanisms, well established neural networks show it. If iPSC-derived neurons show LTP is proof that the cells are well differentiated in neurons and there is a well connected network. For the HFS protocol a comparison between features from the basal recording and the ones from the post stimulus recording was done. All the data about one specific protocol are grouped together if the acquisition corresponds to the same week for the different sets; control and pathological were kept separated. In Table 3 the dimension of the samples in the different weeks is shown for both the pathological and control subjects.

	1 week	2 weeks	2 weeks	4 weeks	5 weeks	6 weeks
Control	0	3	3	3	6	0
Pathological	0	5	5	3	8	3

Table 3: Numerosity of the groups used for the HFS protocol for the control and the pathological subjects.

3.4.5 Glutamate

Glutamate is an excitatory neurotransmitter, one of its effect is to make the cellular membrane more permeable to ions, making the generation of action potential possible and easier. For the glutamate out of the entire recording were compared the 5 minutes of the basal recording and the 3 minutes after the injection of glutamate right after the electrode signals recover from the noise caused by the injection. Expecting an increase of the activity in the period right after the injection of glutamate the analysis done before was considered not fully adequate to take this fact into consideration. The use of ISI histograms to capture and evidence well this transitory has been decided to be more useful. The ISI histograms utilized are composed by the ISI of all the active channels in the chip recording. As further support for this analysis will be shown plot of the count of spikes from 150 seconds of the basal recording and the same time for the post injection recording.

3.4.6 Software comparison

The developed SW was compared to MC Rack, a program made by MCS because there was the will to compare the developed software to one used in literature. MC rack can also do some spike and burst analysis, but it is a program owned by MCS and also its implementation can be different by the one implemented. Both programs were told to use 5 as the multiplier of the STD; depending on the channel MC rack used a positive or negative threshold decided by the user. MC Rack were used only to obtain the time stamps of the event detected. The developed SW did the same and also the next steps of the comparison. The features used for the comparison are the threshold calculated, the number of event detected, the mean inter spike interval and number of burst detected. Two channels were taken into consideration from the same recording and present two different condition, in case there is a channel that has visually distinguishable spikes, with little background noise. Case two presents a second channel that has significant background noise along with the firing activity. The STD of the entire signal was considered to somehow characterize numerically this difference. The STD is a measure of how far the signal fluctuates from the mean. The STD of the signal in case one is 3.06 μV and for case two is 3.94 μV.

3.4.7 Statistical analysis

Once the features have been extracted from all the recording, the comparison between the control and pathological subjects was performed. Toward this aim, a statistical analysis was carried out. Two different test were used depending on the specific case. The Mann–Whitney U test, a non parametric test given the low number of samples, was used to evaluate wheter the samples could be considered as drawn from the same distribution as null hypothesis or wheter this hypothesis should be rejected. This test is used when the two samples are independent. It was used for the comparison of the features extracted from the basal recording of the control and pathological subjects at different weeks. For the longitudinal analysis the Wilcoxon signed-rank test was used, it is a non parametric test to compare two related samples or two samples at different time points. The null hypothesis is that the median of the population of differences between the paired data is zero. This test was also used in the HFS protocol, to compare the pre and post stimulation features of the two groups.

4. Results

4.1. Basal activity in weeks

As previously introduced in subsubsection 3.4.3, a longitudinal analysis of the basal activity of the two groups was performed and a comparison between the pathological and control subject is done. The graphs relative to all the parameters extracted can be found in Figure 6, in light blue there are box plot of the control subject and in light red the box plot of the pathological one. In Figure 5a the data about the total spike activity can

be seen, emerge the fact that the pathological subject show an higher activity starting from the 3rd week but in particular in the 4th and 5th weeks. In Figure 5c are presented data about the number of channels that present spiking activity, the pattern resembles the previous one, the pathological subject shows more active channels on most of the weeks but in particular on the 3rd and 4th. Figure 5e shows the mean amplitude of spikes of all the chips, should be noted that the pathological subject shows a value generally higher than the control subject. Figure 5b regards the number of burst per minute, also here the subject affected by the PWS shows this feature with higher values. Figure 5d is a plot about the number of channels with burst, can be seen an increase in this parameter with time passing; an increase in burst activity can be associated to an increase of maturation of the network. Figure 5f shows information about the mean length of the burst, this parameter could be linked to the excitatory and inhibitory balance, long burst indicate a less present inhibitory activity.
Although some consideration could be made visually, a statistical study must be done. The two test cited in subsubsection 3.4.7 were used for this reason, in both cases the results doesn't confirm the deduction done by visual inspection of the plots. An important thing to be noted is the number of samples that composes the two groups as reported in Table 1, the pool size for each group does not reach the ten samples.

	Threshold value [μV]	Events detected	Mean ISI [s]	Burst detected
MC Rack	13.9	400	0.750	14
SW	12.53	418	0.718	11
% difference	-9.86 %	4.5 %	-4.26 %	-21.42 %

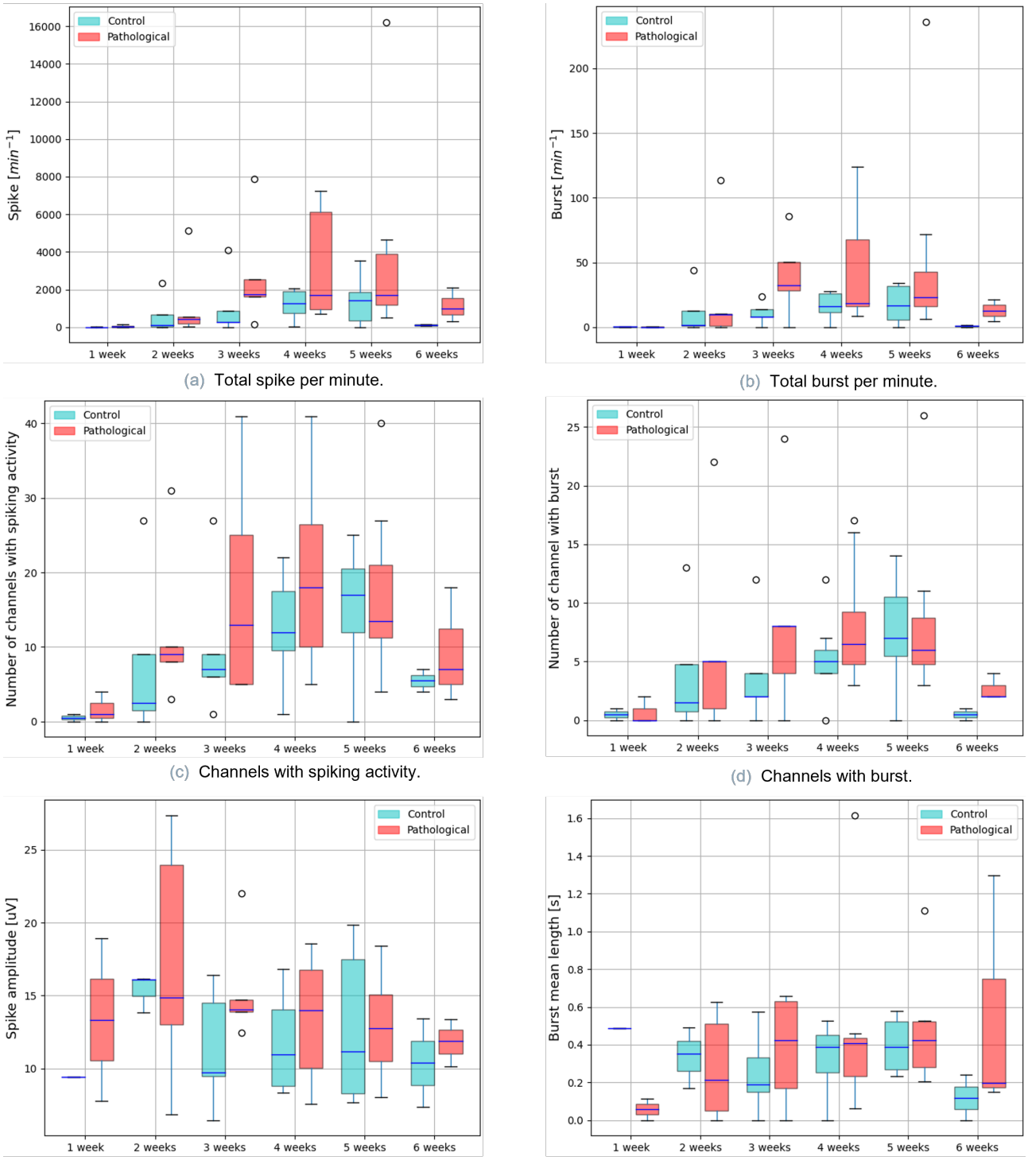
Table 4: In the first case, the signal is clean (the STD of the signal is 3.06 μV) and spikes are easily detectable by eye. Are reported the value of features extracted from MC Rack and the developed SW.

	Threshold value [μV]	Events detected	Mean ISI [s]	Burst detected
MC Rack	17.5	4143	0.072	20
SW	15.11	5982	0.050	24
% difference	-13.66 %	+44.3 %	-30.7 %	+20 %

Table 5: In the second case, the channel is noisier, in particular, while there is a spiking activity the noise slightly increases (STD is 3.94 μV). Are reported the value of features extracted from MC Rack and the developed SW.

4.2. High-frequency protocol

Here we present the result of the previously introduced analysis (subsubsection 3.4.4), in terms of change in the selected features between the basal and the post stimulation recording in the two groups. As told, the HFS protocol used for the stimulation protocol had a voltage of 500 mV(1V peak-to-peak(p-p). In Figure 7 can be seen the three features extracted for the control and pathological group regarding the spiking activity and in Figure 8 regarding the burst activity. From the plots doesn't emerge an evident pattern in the different features, but there are some cases in which the stimulation had an effect. In some particular cases, such as in Figure 8e in all weeks, Figure 7d and 7f at three weeks, and Figure 8f at three weeks there are some differences between the pre and post stimulus. Even with these few cases of dissimilarity, to be precise and rigorous, a statistical analysis has been done. For every set of pre and post stimulus recordings among all features and weeks, The Mann–Whitney U test has reported that the null hypothesis cannot be rejected, so the features before and after the stimulation aren't statistically different, as expected by seeing the graphs. An additional comment regards stimulation protocols that used 1 V (2V p-p) as voltage value in the HFS protocol, there was a drastic decrease in the total activity of the network, both for spike and burst parameters.



(a) Total spike per minute.

(b) Total burst per minute.

(c) Channels with spiking activity.

(d) Channels with burst.

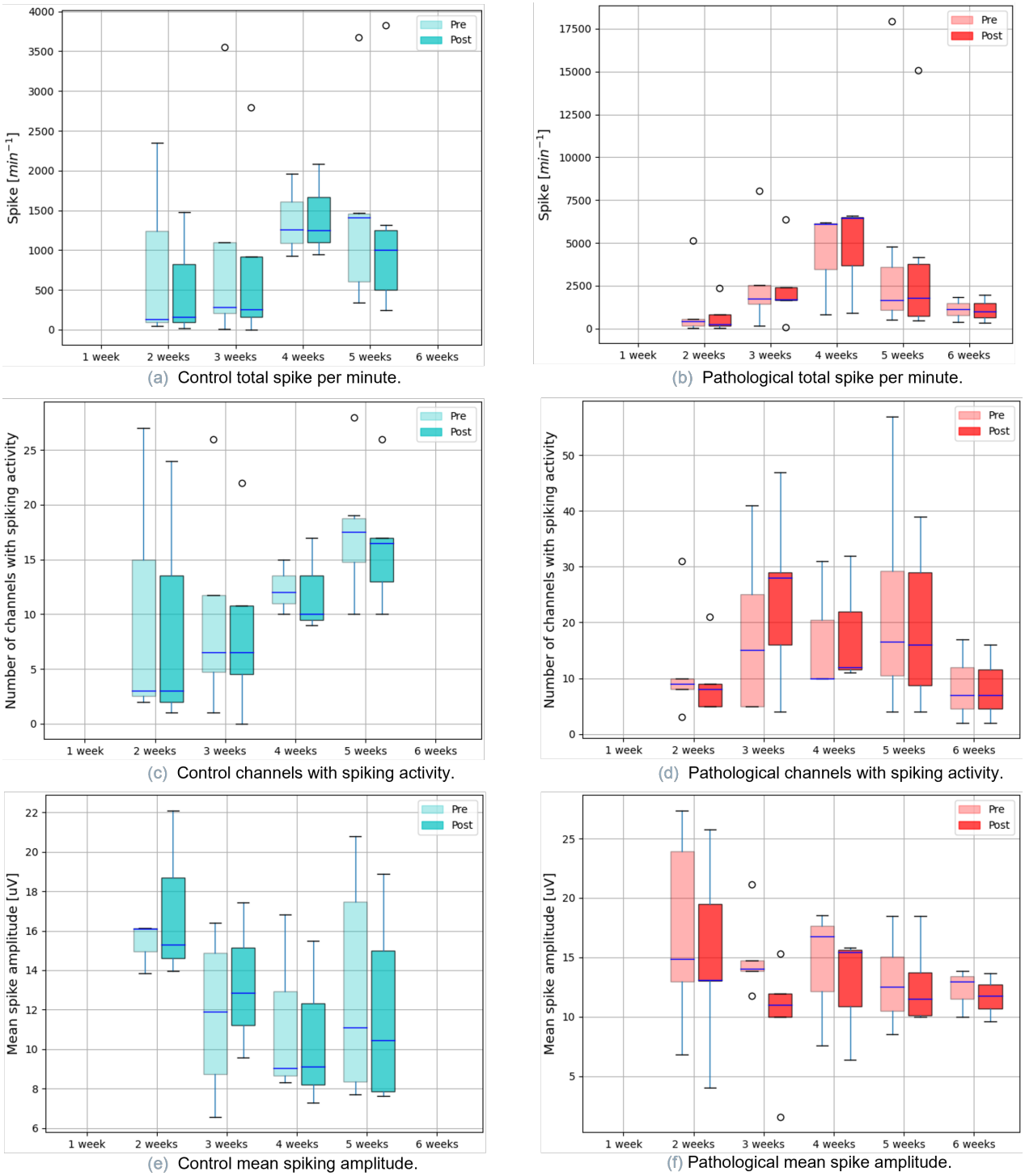
(e) Mean spike amplitude.

(f) Burst mean length.

Figure 6: Plot of features extracted from the pathological and control group for the longitudinal study. On the left the one regarding the spiking activity and on the right the one regarding the burst. Box plots in light blu regard the control and in light red regard the pathological subject.

4.3. Glutamate

Glutamate is an excitatory neurotransmitter, it is added to record the response of the hiPSC-derived neurons to it. The expected response is an increase in the spiking activity. As we can see in Figure 9, the activity before and after the glutamate addition is quite different in the control subject. In the basal recording, there is a quite small activity, and the number of spikes is not high, after the glutamate we have an increment in the activity, as expected. The ISI histogram after the injection maintains a similar ISI distribution to the basal ISI histogram.



(a) Control total spike per minute.

(b) Pathological total spike per minute.

(c) Control channels with spiking activity.

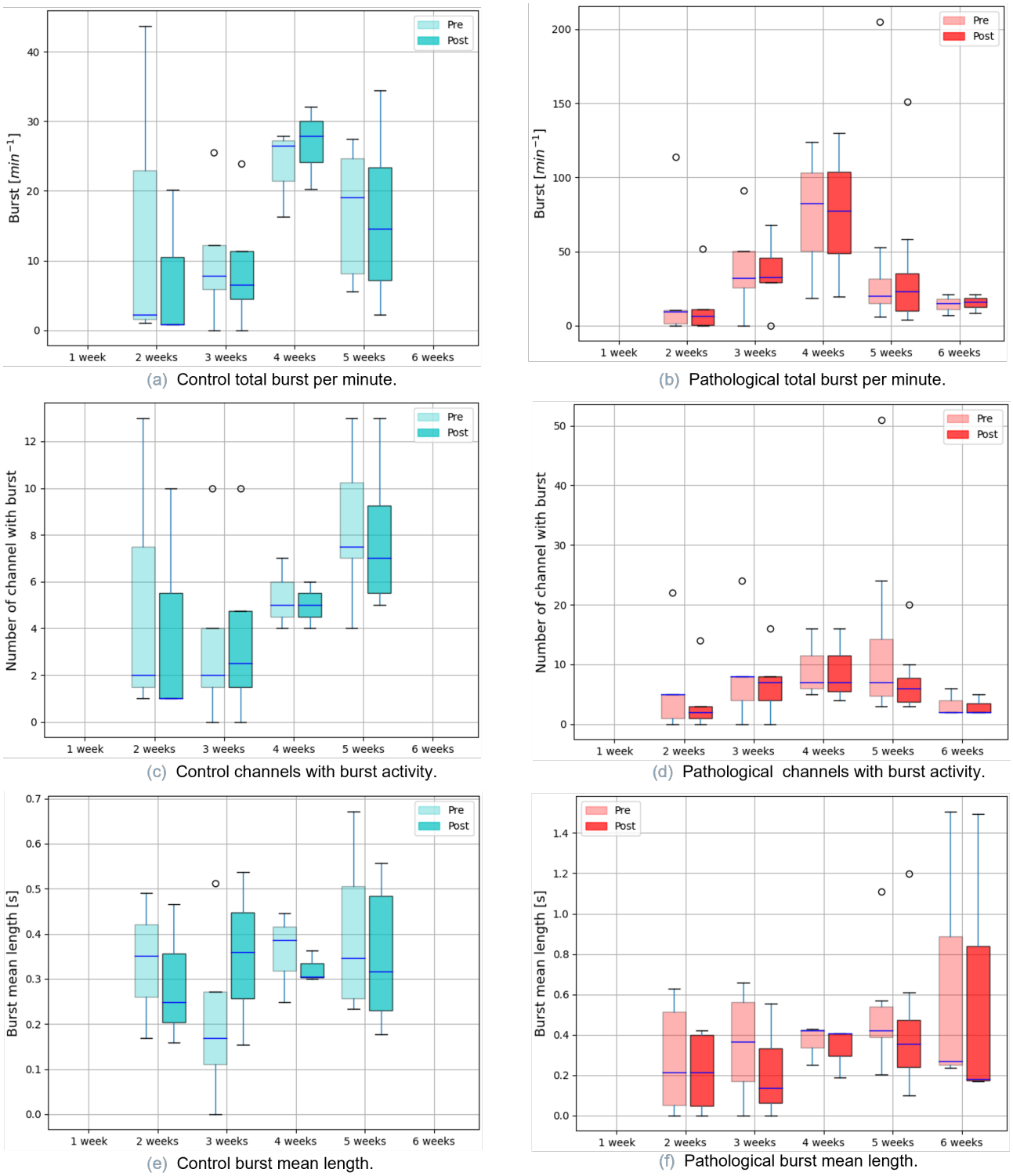
(d) Pathological channels with spiking activity.

(e) Control mean spiking amplitude.

(f) Pathological mean spike amplitude.

Figure 7: Graphs of the three variables extracted for the stimulation study, here are presented features regarding the spiking activity. Can be seen the control (left) and pathological (right) in comparison during the different weeks on the MEA chip.

The ISI interval seems to focus around 0.04 s from a baseline around 0.025 s. In Figure 10 can be seen the activity in the two recordings as the event counted in time. Focusing on the post glutamate, high activity is present right after the injection and gradually diminishes over time, reaching a low level after approximately 40 seconds (Figure 10b). The peak level after was greater than the peak of the pre recording (Figure 10a)
A quite different situation is shown in Figure 11, the activity is very high before the injection of the chemical substance. After the addition of glutamate, the overall activity decreases drastically, to be noticed the difference in the scale on the count axis. There is an evident chchangen the ISI distribution, the peak is shifted from around zero to around 0.05 seconds.



(a) Control total burst per minute.

(b) Pathological total burst per minute.

(c) Control channels with burst activity.

(d) Pathological channels with burst activity.

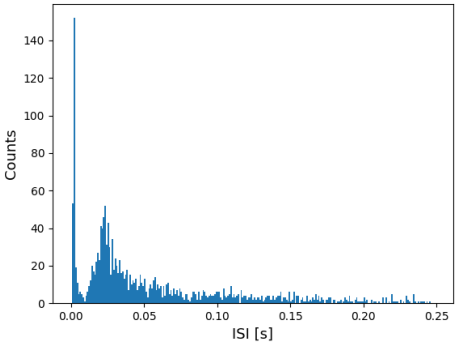
(e) Control burst mean length.

(f) Pathological burst mean length.

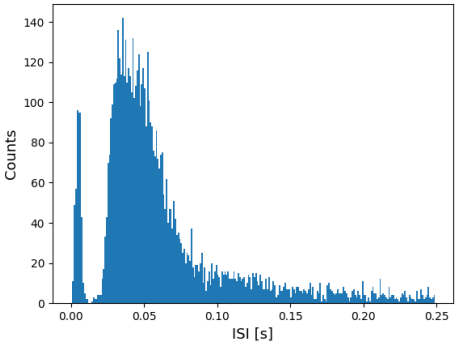
Figure 8: Graphs of the three variables extracted for the stimulation study, here are presented features regarding the burst activity. Can be seen the control (left) and pathological (right) in comparison during the different weeks on the MEA chip.

4.4. Software comparison

In Table 4 can be seen the numerical values of the first case and in Table 5 the second. The percentage difference is calculated as $(SW - MCRack)/MCRack * 100$. The results in the two cases are quite different, starting from the threshold value used to detect the events. The one calculated from MC Rack is higher than the one obtained from the custom SW. In the first case, the SW threshold is lesser than 9.86 % and in the second 13.66 %. This reflects directly on event detection, the developed SW detect a greater number of events in both of the

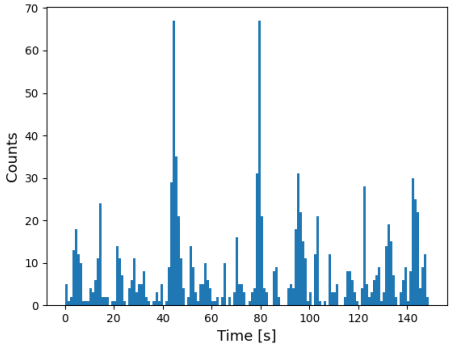


(a) ISI histogram before adding glutamate of the control subject.

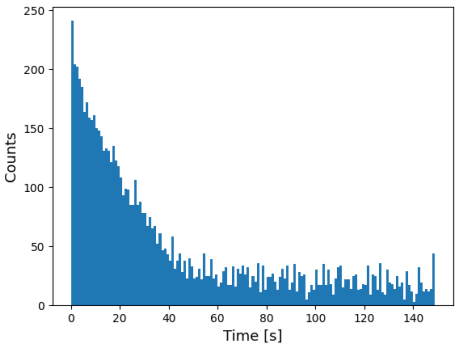


(b) ISI histogram after adding glutamate of the control subject.

Figure 9: Control subject ISI histogram, on the left before the addition of glutamate, on the right after.

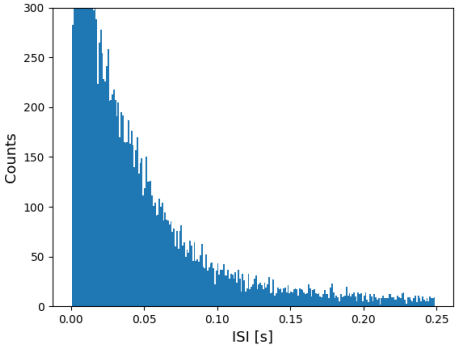


(a) Count of spikes of the control subject before adding glutamate.

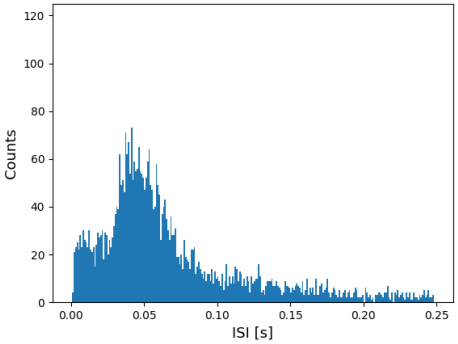


(b) Count of spikes of the control subject before adding glutamate.

Figure 10: Control subject count of events plots, on the left before the addition of glutamate, on the right after. To be noted the different time scales.



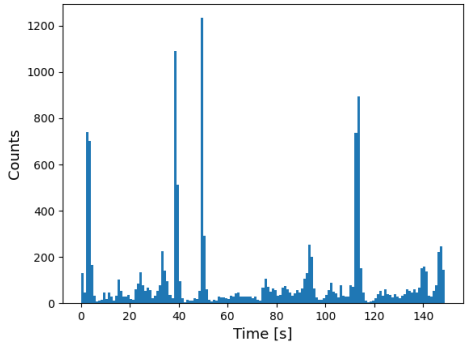
(a) ISI histogram before adding glutamate of the pathological subject.



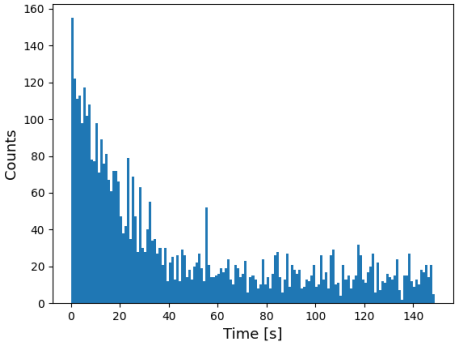
(b) ISI histogram after adding glutamate of the pathological subject.

Figure 11: Pathological subject ISI histogram, on the left before the addition of glutamate, on the right after. To be noticed the different scales of the count axis.

cases. In case one, the one with a clean signal, the amount of spike found differs by 4.5%. In the second case, the one with a noisy signal, this value is bigger, around 44 %. A similar pattern appears for the mean ISI but



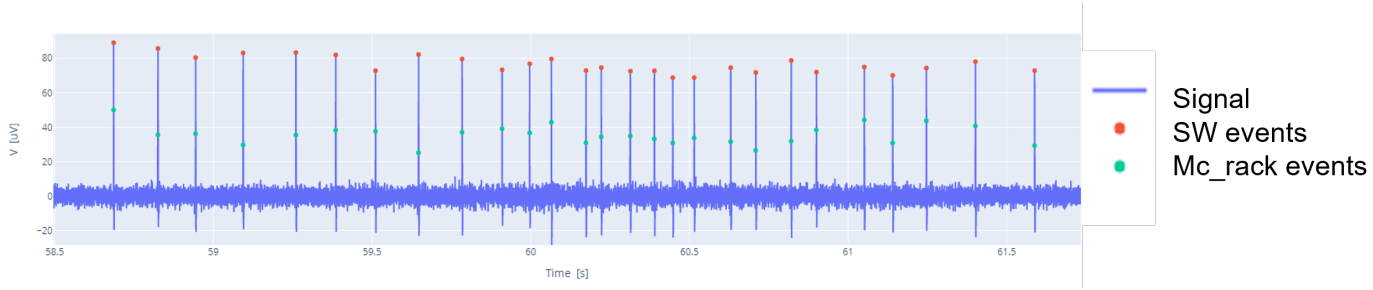
(a) Count of spikes of the pathological subject before adding glutamate.



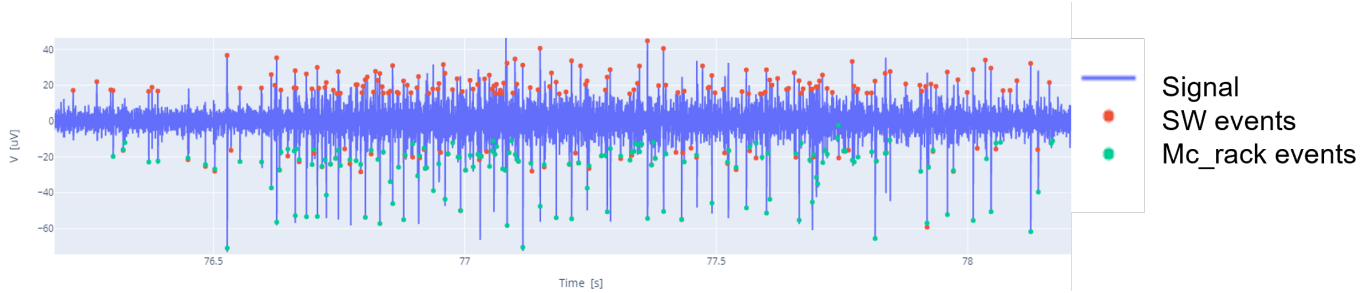
(b) Count of spikes of the pathological subject before adding glutamate.

Figure 12: Pathological subject count of events plots, on the left before the addition of glutamate, on the right after. To be noted the different time scales.

with a negative sign, the developed SW find a value lesser than the program of MCS by 4.26 and 30.7 %. For the number of burst detected results are quite the opposite, the difference is in the first case negative and in the second negative of about 20%.



(a) Portion of signal of case one, events are distant and well recognizable by eyesight.



(b) Portion of signal of case two, the events are very close and not well recognizable.

Figure 13: Two cases in comparison, to be noticed the two temporal scales that are different. As in legend, in blue there is the signal, in red the point considered events by the developed SW and in green the spikes identified by MC Rack.

5. Discussion

5.1. Basal activity in weeks

As can be seen in subsection 4.1, seems that the pathological subject shows a general activity greater than the control one, in particular, it has higher spiking activity on the 3rd, 4th and 5th weeks, with a particular predominance in these last two. This is also reflected in features regarding the burst, specifically on the number of channels that present burst and the general burst activity in different channels. Both of these elements

suggest that the pathological subject has a greater activity than the control one and also a major grade of interconnection between hiPSC-derived neurons. This conclusion tends to align with the fact that the PWS comport a less expression of genes that encode for the ($GABA_A$) β_3 sub-unit. Due to this condition there could be a less impact of the inhibitory neurons on the network resulting in more activity. This observation is reinforced by the finding that the mean length of bursts in the pathological subjects is slightly greater.
Unfortunately, the deduction made so far are not well sustained by the data: statistically the results of the test used for comparison doesn't confirm us any difference. The first and important thing to notice is linked to the sample size, the dimension of the two group suggest that to reach a more reliable result more experiments must be done. Another point is that we are using a single control subject, adding also data from a new control could eventually cancel bias belonging to the specific person used.

5.2. high-frequency stimulation protocol

The aim of electrical stimulation with a HFS protocol is to induce some LTP effects in the iPSC-derived neurons network. As a recall (subsection 2.2), the fact that if LTP is present is interpreted as a sign of functional circuit formation between cells. As shown in subsection 4.2, from the different plots doesn't emerge some evident differences in the different features. As a confirmation of this fact, the statistical test confirms that we can't reject the null hypothesis and that the population aren't statistically different. There are a couple of hypotheses that can explain this situation: the first is that the voltage of the stimulation protocol used in not high enough to stimulate the cells, the second is that the stimulation protocol is not adequate, the third one is that the iPSC-derived neurons are not connected enough to show long term potentiation.

5.3. Glutamate

Given the results of the experiments (subsection 4.3), the iPSC-derived neurons respond well to glutamate. As expected, the introduction of the excitatory neurotransmitter leads to an increase in activity within the network. In the control case, there is a small activity in the recording pre-glutamate and higher post injection. This increase in activity is expected within this experiment and confirms the reaction of the cells to the glutamate. The ISI distribution does not change drastically the shape, but the peak value shifts toward a greater value. A peak of activity is shown right after the injection and a slow decrease after, which can be a sign of neurons starting to suffer from synaptic fatigue, a temporary decrease in the efficiency of the synaptic transmission. The neurons can't produce and uptake fast enough the neurotransmitter released in the extracellular medium, so the capability of synaptic transmission decreases, also the neurons can become less sensitive to a certain stimulus if it is too intense.
In the pathological case, the basal activity is high and the injection of the substance causes a decrease in activity, this may be also due to synaptic fatigue, as the network cannot withstand an activity so intense. Also, the distribution of ISI seems to change, in the basal recording they are decreasing from low ISI to higher ISI. In the post glutamate the distribution has a small peak around 0.05 seconds. The overall activity decreased, but the response to glutamate is very similar to the one of the control subject.
The possibility that too much glutamate was added must be taken into consideration if the increment in activity arises right after the injection and lasts around half a minute. As a protocol side note, the injection of glutamate should be done with a fresh medium, also less noise is introduced in the recording and the procedure is faster.

5.4. Software comparison

Remembering that there is no true ground truth about the spike identification, we still decided to compare the SW developed to MC Rack. In case one where the events are well visible, in the second case it is not, also a human operator can find difficulties on distinguish spikes from pure noise if the noise level is comparable to the spike amplitude. This can be seen in (Figure 13). About the numerical differences, the first thing to be noticed is the difference in the threshold obtained, MC rack tend to have a greater threshold while the SW developed a lesser one. As a matter of fact, a lesser threshold tends to include more events as can be seen in subsection 4.4. Also MC Rack uses a single threshold, positive or negative chosen by the user, the coded SW uses both a positive and a negative one. This difference is reflected in all of the other features considered, in the first case this value can have a small variation. Major changes are present in the features considered in the other case. As expected if in one case more events are detected there will be a lesser mean ISI than the other case, this is well visible in Table 4 and Table 5. The results about the burst detected can be explained in two way, in the first case the SW detect more spikes and these spikes extra can form longer and lesser burst. For the second case the increased amount of spike detected can justify the increase of burst number.
The possibility that using 5 as multiplier of the STD in the developed SW has lead to include events that can

be noise is possible, this fact could be resolved by increasing the value to 6 or 7, in that case the event detection is more rigid and tend to consider events that are more probable real spike. Also the implementation of other features for event detection, such as template matching, or introducing spike sorting techniques could help in discriminating better events caused by noise.

6. Conclusions

For this type of study the results seems promising, hiPSC-derived neurons present a good spontaneous activity for both spike and burst. Some differences in the pathological and the control subject can be seen, even though that the statistics test does not suggest that there is a difference between the two groups. The hypothesis that the PWS cells presents a higher activity for the moment remains only an assumption. To investigate further, more acquisition should be made for both the subjects, increasing the sample size should lead to a more robust and reliable statistical analysis. A crucial point also is that there is only one control subject, more should be used to have a more robust study of the pathology and cancel any eventual bias of the specific subject.

6.1. Future implementation

About the SW implementation some improvements can be made: spike sorting, maybe template matching method for event detection, and using an adaptive method to calculate the inter burst interval are the priority things to add to obtain more robust extracted features. Another point is to add more extracted parameters, first of all the inclusion of features that include the overall network activity and an evaluation of spike and burst synchrony or causality.

References

[1] Wickliffe C. Abraham and Ann Huggett. Induction and reversal of long-term potentiation by repeated high-frequency stimulation in rat hippocampal slices. *Hippocampus*, 7(2):137–145, 1997.

[2] M. A. Angulo, M. G. Butler, and M. E. Cataletto. Prader-Willi syndrome: A review of clinical, genetic, and endocrine findings. *Journal of Endocrinological Investigation*, 38(12):1249–1263, 2015.

[3] Kaveena Autar, Xiufang Guo, John W. Rumsey, Christopher J. Long, Nesar Akanda, Max Jackson, Narasimhan S. Narasimhan, Julbert Caneus, Dave Morgan, and James J. Hickman. A functional hiPSC-cortical neuron differentiation and maturation model and its application to neurological disorders. *Stem Cell Reports*, 17(1):96–109, 2022.

[4] Federica Banfi, Alicia Rubio, Mattia Zaghi, Luca Massimino, Giulia Fagnocchi, Edoardo Bellini, Mirko Luoni, Cinzia Cancellieri, Anna Bagliani, Chiara Di Resta, Camilla Maffezzini, Angelo Ianielli, Maurizio Ferrari, Rocco Piazza, Luca Mologni, Vania Broccoli, and Alessandro Sessa. SETBP1 accumulation induces P53 inhibition and genotoxic stress in neural progenitors underlying neurodegeneration in Schinzel-Giedion syndrome. *Nature Communications*, 12(1):1–21, 2021.

[5] Merlin G. Butler. Genomic imprinting disorders in humans: A mini-review. *Journal of Assisted Reproduction and Genetics*, 26(9-10):477–486, 2009.

[6] Julbert Caneus, Nesar Akanda, John W. Rumsey, Xiufang Guo, Max Jackson, Christopher J. Long, Frank Sommerhage, Sanya Georgieva, Nicholas M. Kanaan, David Morgan, and James J. Hickman. A human induced pluripotent stem cell-derived cortical neuron human-on-a chip system to study Aβ42 and tau-induced pathophysiological effects on long-term potentiation. *Alzheimer's and Dementia: Translational Research and Clinical Interventions*, 6(1):1–14, 2020.

[7] Michela Chiappalone, A. Novellino, I. Vajda, A. Vato, S. Martinoia, and J. van Pelt. Burst detection algorithms for the analysis of spatio-temporal patterns in cortical networks of neurons. *Neurocomputing*, 65-66(SPEC. ISS.):653–662, 2005.

[8] Stuart F. Cogan. Neural stimulation and recording electrodes. *Annual Review of Biomedical Engineering*, 10(1):275–309, 2008. PMID: 18429704.

[9] Morgan Ferguson, Dhavan Sharma, David Ross, and Feng Zhao. A Critical Review of Microelectrode Arrays and Strategies for Improving the Neural Interface. 8(19):1–28, 2020.

[10] Alexandra Gramowski, Konstantin Jügelt, Dieter G. Weiss, and Guenter W. Gross. Substance identification by quantitative characterization of oscillatory activity in murine spinal cord networks on microelectrode arrays. *European Journal of Neuroscience*, 19(10):2815–2825, 2004.

[11] Darrell A. Henze, Zsolt Borhegyi, Jozsef Csicsvari, Akira Mamiya, Kenneth D. Harris, and György Buzsáki. Intracellular features predicted by extracellular recordings in the hippocampus in vivo. *Journal of Neurophysiology*, 84(1):390–400, 2000.

[12] Giovanni Lucignani, Andrea Panzacchi, Laura Bosio, Rosa Maria Moresco, Laura Ravasi, Isabella Coppa, Giuseppe Chiumello, Kirk Frey, Robert Koeppe, and Ferruccio Fazio. GABAA receptor abnormalities in Prader-Willi syndrome assessed with positron emission tomography and [11C]flumazenil. *NeuroImage*, 22(1):22–28, 2004.

[13] Fraser P. McCready, Sara Gordillo-Sampedro, Kartik Pradeepan, Julio Martinez-Trujillo, and James Ellis. Multielectrode Arrays for Functional Phenotyping of Neurons from Induced Pluripotent Stem Cell Models of Neurodevelopmental Disorders. *Biology*, 11(2), 2022.

[14] Marie Engelene J. Obien, Kosmas Deligkaris, Torsten Bullmann, Douglas J. Bakkum, and Urs Frey. Revealing neuronal function through microelectrode array recordings. *Frontiers in Neuroscience*, 9(JAN):423, 2015.

[15] Alicia Rubio, Mirko Luoni, Serena G. Giannelli, Isabella Radice, Angelo Iannielli, Cinzia Cancellieri, Claudia Di Berardino, Giulia Regalia, Giovanna Lazzari, Andrea Menegon, Stefano Taverna, and Vania Broccoli. Rapid and efficient CRISPR/Cas9 gene inactivation in human neurons during human pluripotent stem cell differentiation and direct reprogramming. *Scientific Reports*, 6(June):1–16, 2016.

[16] Micha E. Spira and Aviad Hai. Multi-electrode array technologies for neuroscience and cardiology. *Nature Nanotechnology*, 8(2):83–94, 2013.

[17] Jyothsna Suresh, Mihailo Radojicic, Lorenzo L. Pesce, Anita Bhansali, Janice Wang, Andrew K. Tryba, Jeremy D. Marks, and Wim Van Drongelen. Network burst activity in hippocampal neuronal cultures: The role of synaptic and intrinsic currents. *Journal of Neurophysiology*, 115(6):3073–3089, 2016.

Abstract in lingua italiana

Gli studi di elettrofisiologici sono la base per acquisire informazioni sul cervello e sull'attività dei neuroni. Diverse tecniche forniscono informazioni sulla struttura anatomica e sulla fisiologia del sistema nervoso centrale (SNC). Le colture neuronali possono essere studiate utilizzando un sistema multi-elettrodo (MEA). Questo sistema registra l'attività extracellulare, attraverso una matrice di elettrodi, fornendo informazioni sull'attività complessiva della rete cellulare. Sono state utilizzate cellule staminali pluripotenti indotte umane (hiPSC) per ottenere neuroni derivati da hiPSC, utilizzabili per studiare modelli funzionali. Una patologia studiabile con queste tecnologie è la sindrome di Prater-Willis (PWS), un disturbo genetico che colpisce il cromosoma 15. In questo lavoro sono stati utilizzati neuroni derivati da hiPSC provenienti da un soggetto affetto da PWS e da un soggetto di controllo. È stato condotto uno studio longitudinale e un confronto tra i due gruppi per quanto riguarda l'attività basale. Altri esperimenti prevedono l'uso della stimolazione elettrica ad alta frequenza (HFS) per verificare se la rete mostra segni di potenziamento a lungo termine (LTP). Sono state effettuate una registrazione basale e una registrazione post-stimolazione. Come ultimo esperimento, è stato aggiunto glutammato alla coltura per vedere la risposta della rete cellulare. Le caratteristiche dell'attività di spike e burst sono state estratte dalle registrazioni utilizzando un software sviluppato in Python. Data la ridotta dimensione del campione, dovuta ai tempi lunghi per ottenere neuroni derivati da hiPSC, il set di dati utilizzato è di dimensioni ridotte. I risultati sembrano mostrare una maggiore attività sia in termini di eventi che di burst nel soggetto patologico nello studio longitudinale. Non sono stati osservati cambiamenti evidenti tra prima e dopi ul ptotocollo HFS. Sebbene i risultati sembrino promettenti, non mostrano una differenza statisticamente significativa. La risposta del glutammato mostra risultati interessanti: il'attività aumenta nel controllo, ma diminuisce nel soggetto patologico, probabilmente a causa dell'affaticamento sinaptico.

Parole chiave:sitema a multi elettrodi, MEA, cellule staminali pluripotenti indotte, iPSC, sindrome di Prater-willis, PWS

Acknowledgements

To begin with, I would like to thank my advisor, Prof. Alessandra Pedrocchi for the opportunity to work on this thesis and my co-advisor Andrea Menegon for the precious teachings about this interesting topic. Further, gratitude goes to the NEARLAB group, especially to Benedetta, who helped me a lot during this hard period. I have to thank also the people from San Raffaele Hospital, Camilla, Simone and especially Irina, for keeping me company, helping and teachings me about biology. I will thank my family, my father, my mother, and my siblings Danny and Nicole. They supported me in every moment of my academic career, giving precious advice, support and comprehension. I cannot but thank my friends, "I Pinguini", for the help, company, holidays, presence and encouragement when I needed it. I'd like to thank many people, everyone that studied with me during these years giving me the energy to continue even in hard times when I was not performing well during the exam period.