UNIVERSITAT DE BARCELONA

Final Degree Project

Biomedical Engineernig Degree

“ Study of neural circuits using multielectrode arrays in movement disorders ”

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ABSTRACT

Neurodegenerative movement-related disorders are characterized by a progressive degeneration and loss of neurons, which lead to motor control impairment. Although the precise mechanisms underlying these conditions are still unknown, an increasing number of studies point towards the analysis of neural networks and functional connectivity to unravel novel insights. The main objective of this work is to understand cellular mechanisms related to dysregulated motor control symptoms in movement disorders, such as Chorea-Acanthocytosis (ChAc), by employing multielectrode arrays to analyze the electrical activity of neuronal networks in mouse models. We found no notable differences in cell viability between neurons with and without VPS13A knockdown, that is the only gene known to be implicated in the disease, suggesting that the absence of VPS13A in neurons may be partially compensated by other proteins. The MEA setup used to capture the electrical activity from neuron primary cultures is described in detail, pointing out its specific characteristics. At last, we present the alternative backup approach implemented to overcome the challenges faced during the research process and to explore the advanced algorithms for signal processing and analysis.

In this report, we present a thorough account of the conception and implementation of our research, outlining the multiple limitations that have been encountered all along the course of the project. We provide a detailed analysis on the project's economical and technical feasibility, as well as a comprehensive overview of the ethical and legal aspects considered during the execution.

Key words: *Chorea-Acanthocytosis; VPS13A; multielectrode arrays; signal processing; neural networks; movement disorders; electrophysiological recordings.*

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GLOSSARY OF ABBREVIATIONS

ChAc	Chorea-Acanthocytosis
DBS	Deep Brain Stimulation
GUI	Guider User Interface
GFAP	Glial Fibrillary Acidic Protein
ICC	Immunocytochemistry
ISI	Inter Spike Interval
KD	Knock-down
KO	Knock-out
MBR	Mean Burst Rate
MCS	Multichannel Systems
MEA	Multielectrode Array
MFR	Mean Firing Rate
MSN	Medium Spiny neuron
PERT	Program Evaluation and Review Technique
SNR	Signal-to-Noise ratio
SWOT	Strengths Weaknesses Opportunities Threats
TiN	Titanium nitride
VPS13A	Vacuolar Protein Sorting 13 Homolog A
WBS	Work Breakdown Structure

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1. INTRODUCTION

1.1. Motivation

Movement-related disorders are chronic neurological conditions that hamper the control and coordination of voluntary motions, resulting in substantial limitations and a reduced quality of life for people who suffer from them [1]. Because these conditions pose a significant challenge to patients, families, and healthcare systems, there is an acute need for deeper developments that combine multiple areas of investigation, including genetics, cellular and molecular biology of neurodegeneration, and bioinformatics. Movement disorders may have a genetic foundation, since several mutations have been identified as the cause of these specific medical states, such as Parkinson's or Huntington's disease, by directly disrupting various brain systems [2]. As a consequence, genetic mutations can lead to alterations in cellular metabolism and neural networks mechanisms, which could be responsible for cell damage and the subsequent deterioration of brain functioning. For example, loss-of-function mutations in the VPS13A gene cause Chorea-Acanthocytosis (ChAc), a poorly understood and complex neurodegenerative disease with a plethora of adult-onset progressive symptoms that include motor disturbances [3]. Yet, the brain processes behind movement diseases remain unclear and effective therapies are frequently limited.

In this regard, analysis employing multielectrode arrays (MEAs) can assist to solve some of the outstanding concerns related to neural network electrophysiology by allowing simultaneous recordings in cultured cells or even brain slices [4]. Increased recordings of multiple neurons' activity simultaneously might lead to the identification of dynamic and particular patterns of action associated with movement disorders, promoting novel therapeutic strategies to improve patients' lives.

In light of movement-related diseases, electrophysiology and electrical activity of neurons using MEA technologies is a very interesting and promising area of study for multidisciplinary teams, which contributes to the development of innovative approaches and the implementation of scientific advances into valuable clinical practice [5]. Hence, bringing multiple disciplines' expertise together may enhance research in neuroscience given the advances on new and higher-resolution technologies. Also, the development of better software algorithms for analyzing and classifying data, may supply investigators with new tools for studying the neural mechanisms underlying movement disorders and, in turn, collaborate with healthcare professionals to possibly uncover new and effective treatments.

1.2. Objectives

The major focus of this project is to understand the cellular mechanisms associated with unregulated motor control, and to examine how neural networks and connectivity may be affected by such pathological conditions using MEAs to analyze the electrical activity of neurons.

However, we specified minor objectives across the multiple phases of the project's lifecycle, as it is an efficient strategy to guarantee that the project continues on track to achieve the broader research goal. Specific objectives to be accomplished in this work are described below.

- To define a standard procedure for neuron culture on MEAs to enhance cell growth and survival, including essential parameters such as seeding density.
- To create cell lines and primary cultures of mouse brain cells and perform immunochemical techniques for particular biomolecules recognition and cell culture characterization.
- To investigate the electrophysiological differences between control and knock-down (KD) cultures, as well as the influence of VPS13A deficiency on neuronal activity and connectivity in vitro.
- To understand the operating mechanisms of MEA electronics in order to achieve optimal results and remove substantial noise boundaries.
- To use signal processing software to extract interesting features of the recorded data: spike rates, burst analysis, ISI computation, network functional connectivity, among other statistical parameters.
- Discuss and interpret the results to uncover patterns or physiological alterations in brain activity that are unique to movement disorders.

1.3. Structure

This technical report covers every aspect of the project and provides a full description of the process's follow-up. Starting with the introduction, we identify the motivations and goals for the work, as well as the limiting variables that might restrict the project's scope. The report continues with a background frame that outlines the research problem with reference to the existing literature, including a general overview of movement disorders, especially ChAc, and MEA technology. This section also highlights the research's current state of the art, with an emphasis on the most recent and significant results in the field. The project's initial presentation concludes with a market analysis that presents a contextualized perspective on the sector and reveals the potential market for technology supply companies, mentioning current trend advancements in the industry.

The engineering design process is described in the next section of the report. Conception engineering provides multiple strategies for brain activity measuring techniques, current MEA manufacturers, and software for posterior analysis of data, taking into account the benefits and drawbacks of their application. Furthermore, the overall configuration of the chosen approach is described, commonly known as the materials and methods, with a full explanation of the project's techniques and processes. In the detailed engineering section, the approved conceptual design is then executed, and the findings obtained in cell cultures and MEA recordings are analyzed and discussed thereafter.

Project management in terms of execution, as well as technical and economic feasibility, have been undertaken in order to determine project timeframes, quality, and cost. We offer the execution timetable,

the SWOT analysis, and the estimated budget for the overall project execution. Finally, Section 9 covers the project's legal and regulatory environment.

Please note that the structure of this document has been slightly modified to resemble a scientific paper, while still adhering to the conventional format of engineering design reports. Also, it is worth mentioning that although the procedure has been thoroughly explained as methodology in Conception Engineering section, the strict protocols to be followed during the laboratory practices can be found in Appendices.

1.4. Limitations

There may be limitations owing to constraints on research design, methodology, and other external variables that can have an influence on the study's final outcomes and, as a result, they must be explicitly acknowledged throughout the project duration.

Generally, the main issues are time and budget. The project is limited to 5 months of work, which might have a considerable effect on the scope and conclusions of the study. Data collection and analysis are time-consuming activities, and it may be difficult to get sufficient trustworthy data from MEAs to properly analyze it.

Furthermore, because the integration of MEA equipment into the lab is still relatively new and unfamiliar, there is no standardized protocol for cell cultures that identifies the optimal variables (e.g. cell densities, culture media, etc.) for each type of cell. Poor experience and limited resources are also a relevant handicap in this scenario since technical challenges, such as the existence of noisy signals, might disturb the results and lead to inaccurate conclusions. Noise may have originated from a variety of sources, including subway stations and movement artifacts, as well as electrical noise from both the equipment and the surroundings, which make registration and analysis of the resultant signals problematic. Hence, when employing MEAs to examine brain activity and network connectivity, processing huge quantities of data with significant noise is an attainable restriction that should be addressed.

Given the nature of this project, it is critical to highlight the ethical concerns associated with the use of animals in research in terms of their welfare and treatment, together with the importance of designing experiments that minimize pain for animals while maximizing scientific findings [6].

2. BACKGROUND

2.1. General knowledge

2.1.1. Principles of multielectrode array devices

Neural recordings and stimulation with high spatiotemporal resolution are potential data sources in the study of several neurological diseases and research [7]. Hence, a MEA is a device that enables electrically active cells, such as neurons or cardiac myocytes, to be recorded and stimulated [8]. It has a tiny grid of closely spaced electrodes on the culture surface, where cells are plated and grown. When a neuron fires an action potential, an electrical signal is generated, and the extracellular voltage fluctuation is monitored on a microsecond timeframe by one of the electrodes. Furthermore, neural cells are cultured and attached to the array forming cohesive networks; MEAs can detect the individual action potential and its propagation throughout the cell network with spatial and temporal precision, resulting in the analysis of brain activity synchronization. As the network matures, neurons may exhibit bursts, defined as the dynamic state of activity in which cells repeatedly fire groups of spikes. Therefore, MEAs offer extracellular recordings of action potentials while remaining noninvasive to cells [8].

In terms of biosensor design and manufacturing, typical MEAs are made of conductive materials that are compatible with microfabrication processes. [9] Among these are silicon-based MEAs, which allow for precise control over size and shape while also being a good conductive and biocompatible material; glass-based devices, useful for imaging applications due to their transparency; metals, such as gold, platinum, and titanium owing to their excellent conductivity; polymers, since they are flexible and can be easily molded into various shapes and sizes; and recently, nanomaterials or nanocomposites with unique electrical properties. The material used in a MEA, together with the geometrical and technical design, is determined by the specific application, as well as the desired features of the biosensor, such as sensitivity and biocompatibility [10].

MEAs use electromagnetic principles to collect data on the electrophysiological activity of excitable cells [11]. When a neuron fires an action potential, the ions moving across the membrane generate an electric field, which, in turn, induces a current that can be measured as voltage (V). Therefore, it enables the monitoring of extracellular voltage or current generated by a cell during a spike. MEAs accurately reconstruct equivalent circuits of the neuro-electronic junction [12], which consists of neurons directly coupled to microtransducers embedded in an insulator surface, in order to prevent interference. Microtransducers, such as microelectrodes, serve as the fundamental sensing or stimulating elements within an MEA. They are composed of tiny electrodes connected to a differential amplifier that converts the electrical signal generated by a nearby cell into quantifiable voltage or current signals with respect to a common reference [12].

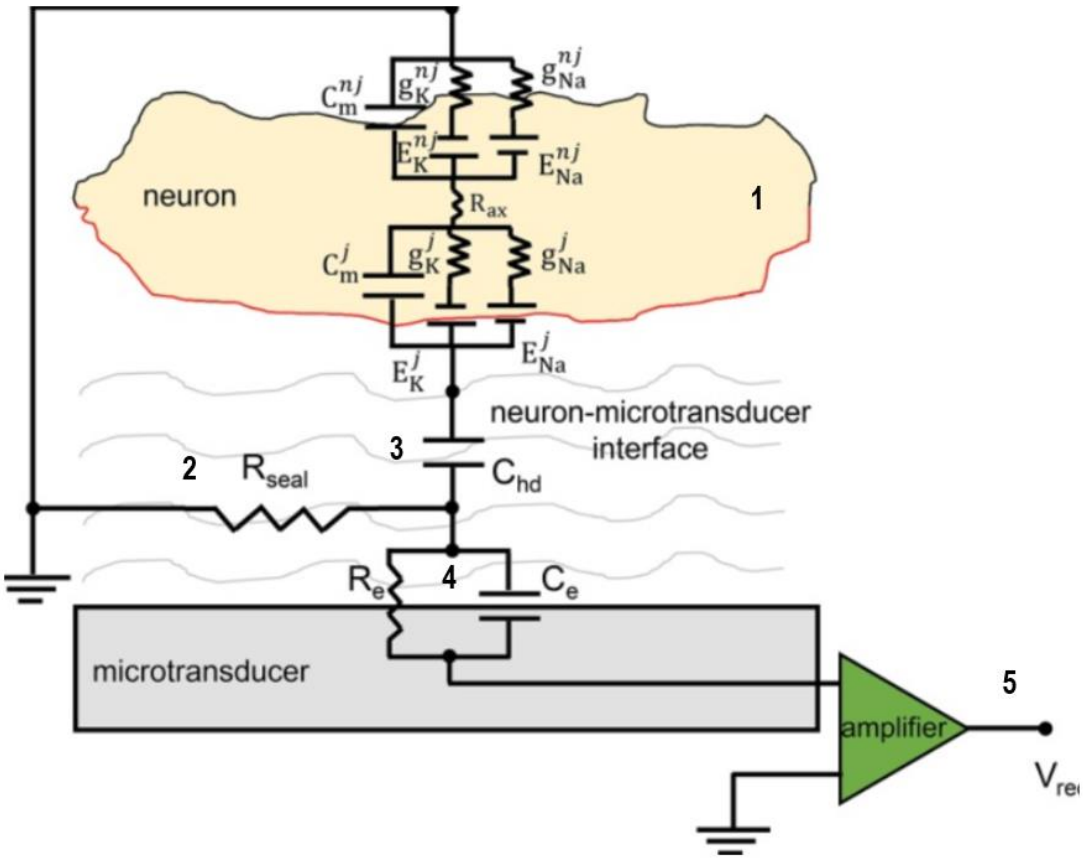

Figure 1. Equivalent circuit of the neuro-electronic junction. It is described by the sealing resistance (R_{seal}), neuron membrane-to-electrolyte capacitance (C_{hd}), the capacitor (C_e), leakage resistor (R_e), the Nernst potential for the two ion channels ($E_{Na/K}$), the conductances for those channels ($g_{Na/K}$) and the capacitive behavior or the cell membrane (c_m). The circuit is divided in (1) the electrophysiology of the neuron, (2) and (3) concerning the neuron-electrode interface, (4) electrolyte to microtransducer interface and (5) the output voltage. [Massobrio, P.et al. Interfacing Cultured Neurons to Microtransducers Arrays: A Review of the Neuro-Electronic Junction Models, 2016]

The schematic on Figure 1 displays the equivalent circuit of the neuro-electronic junction, which refers to the coupling between a living neural tissue and a generic microtransducer. According to Figure 1, the different elements of the electric circuit are described in Table 1 [12].

Table 1. Description of the electrical components in the neuro-electronic junction according to the specified numbers in Figure 1.

1	Electrical phenomena within the cell membrane are based on unequal ion distributions between the intracellular and extracellular spaces, as well as ion transport via gradient concentrations. The circuit works by identifying the electrical activity of the neuron in response to changes in the membrane potential utilizing the conductance (g) and capacitance (c), which are the ability of ion (Na, K) flow and the ability of the membrane to store energy, respectively [12]. Therefore, when the membrane potential changes, the conductance associated with each ion channel also change, causing ion transport across the cell membrane and further altering the membrane potential [13]. The Nernst potentials represent the resting membrane potential and are used to calculate the direction and magnitude of this flow of ions [12].

2 R_{seal} is placed in parallel between the cell membrane and the microtransducer [14]. It models the electrical resistance between the cell membrane and the microtransducer surface, describing the separation between them. This parameter has a major role during signal recording with MEAs because it affects the strength of the signal that is transmitted between the neuron and the electrode.

3 C_{hd} stands for the neuron membrane-to-electrolyte capacitance. This parameter models the capacitive part associated with the portion of the membrane that interacts with the electrode. This slightly contributes to the ability of the cell membrane and its ability to store electrical charges across its surface, separated by the insulating layer of the extracellular solution. Additionally, it is used to simulate the polarization effect of the electrolyte solution that surrounds both the neuron and the electrode. When an electric potential difference is applied across the membrane and the electrolyte, the charged particles in the solution split into layers, influencing the electrical characteristics of the system [12].

4 The RC parallel circuit models the interface between the electrolyte and the microtransducer. On the one hand, the resistive part of the circuit (R_e) is derived from the electrolytic resistance and its ability to conduct electrical current. On the other hand, C_e describes the electrical capacitance of the double layer between the electrolyte solution and the microtransducer's surface. The interaction between a solid surface and an electrolyte induces the formation of an electrical double layer, that is the charged surface and the ions in the solution [12].

5 A differential amplifier is connected to the microtransducer, which measures the difference between two electrical signals. In this case, it records the voltage (V_{rec}) or current generated by the microtransducer with regard to a common reference point [12].

Multichannel data gathering from neural networks is accomplished through the use of sophisticated electronic components that amplify and filter the electrical signals collected from the electrodes and are either incorporated into the MEA or externally linked to a PC [15]. Nonetheless, to analyze the results software solutions for huge data management and signal processing must be implemented.

2.1.2. Overview on movement disorders: Chorea-Acanthocytosis

MEA data analysis in a disease modeling context is a powerful tool for characterizing neural networks with high spatiotemporal resolution. Thus, monitoring the electrical activity of neurons in the brain regions associated with motor control is critical to study the neurological pathology of interest; in our case, Chorea-Acanthocytosis. This is an exceedingly uncommon hereditary disease with an estimated global incidence of 500 to 1.000 individuals worldwide [16]. Because of its rarity, limited literature has been reported and further research is necessary to fully understand the pathophysiology of the disorder and, consequently, optimize diagnosis and treatment.

Particularly, ChAc is an autosomal recessive condition defined by the absence or significantly reduced expression of the human Vacuolar protein sorting 13A (VPS13A), the only gene known to be relevant for the disorder [17]. This gene encodes for the chorein protein, which has been identified at the cellular level in the soma and neurites in mouse models, co-localizing the endoplasmic reticulum and mitochondria contact sites and allowing intracellular transport of lipids [18]. Under this context, the prior manifestation of onset symptoms in ChAc human patients occurs throughout adulthood, around the age of 35, with a slow progress over time and inevitably leading to premature death. Although ChAc may first present with neuropsychiatric abnormalities, such as obsessive-compulsive disorder (OCD), depression, irritability or hyperactivity, other clinical symptomatology associated with the disorder include involuntary and hyperkinetic movements, cognitive impairment, and seizures [19].

As with other neuropathologies, the emergence of symptoms frequently drives the use of neuroimaging diagnostic tools that allow characterization and diagnosis. In particular, computed tomography (CT) and Magnetic Resonance Imaging (MRI) scans show that the major neuropathological hallmark in ChAc patients is selective striatal atrophy [20], with a large reduction in volume of the caudate nucleus and putamen when compared to control populations, therefore alluding to a severe cell death of medium spiny neurons (MSNs). Thus, these striatal abnormalities, notably in the putamen and caudate nucleus, are responsible for the disruption of motor function, along with the characteristic behavioral and cognitive impairment seen in the illness. However, it is interesting to mention that mouse models of ChAc have been developed and have shown minor expression of VPS13A in the striatum, while being abundantly found in the pons, cerebellum, and hippocampus. This suggests that the condition is directly tied to particular striatal activities and MSN cell processes disrupted by the VPS13A mutation, rather than the amount of cellular protein [18].

Additionally, ChAc may be also distinguished by the presence of misshapen erythrocytes in peripheral blood, known as acanthocytes. Therefore, morphological, and mechanical anomalies in red blood cells may disrupt blood circulation across microvessels and capillaries, resulting in a lack of oxygen supply to certain brain areas, until the tissue is totally altered [21]. For this reason, laboratory tests and image segmentation algorithms can be useful in assessing confirmatory data for the disease. Unfortunately, there is no clear diagnostic criteria or genetic testing to identify ChAc cases at this time, indicating that further research is needed [20].

2.2. State of the Art

In recent years, the study of neuronal activity and, as a consequence, the insights into movement disorders have witnessed remarkable progress thanks to the advancement in technological resources. In the field of neuroscience, a fundamental objective is to unravel and identify the intricate relationships between functional connectivity maps of neural circuits and their physiological or pathological functions to further comprehend the cellular mechanisms driving movement disorders. As previously pinpointed, the prime methodology to conduct electrophysiological measurements is to use MEAs, which enable the

assessment of neuronal activity under in vitro conditions, providing deeper understanding of the complexity concealed in the nervous system [22].

Here, the current toolsets in research for uncovering functional activity and connectivity within neural networks are discussed. Additionally, we provide a brief overview of the latest findings concerning the application of MEA technologies in investigating movement disorders, such as Parkinson disease (PD), Huntington's disease (HD) and ChAc.

2.2.1. Current technology

Current MEA recording devices are a useful tool for in vitro research of network synaptic activity and plasticity. It is worth mentioning that numerous types of MEAs are available for a variety of applications involving neuronal cultures and brain slices, each with its own set of benefits. Planar MEAs and high density MEAs, for example, integrate a certain number of electrodes in a flat substrate and are frequently employed to record multisite activity from neurons simultaneously due to their ease of usage, providing high resolution outcomes. However, advancements in microfabrication techniques have resulted in the development of 3D MEAs, which are composed of tip-protruding microelectrodes that enable the capture of electrical signals from multiple depths within a three-dimensional neural network [4] [23]. Figure 2 presents the graphical conception of current technologies, that is planar and 3D MEAs, retrieved from Ming-Gang Liu et al. research.

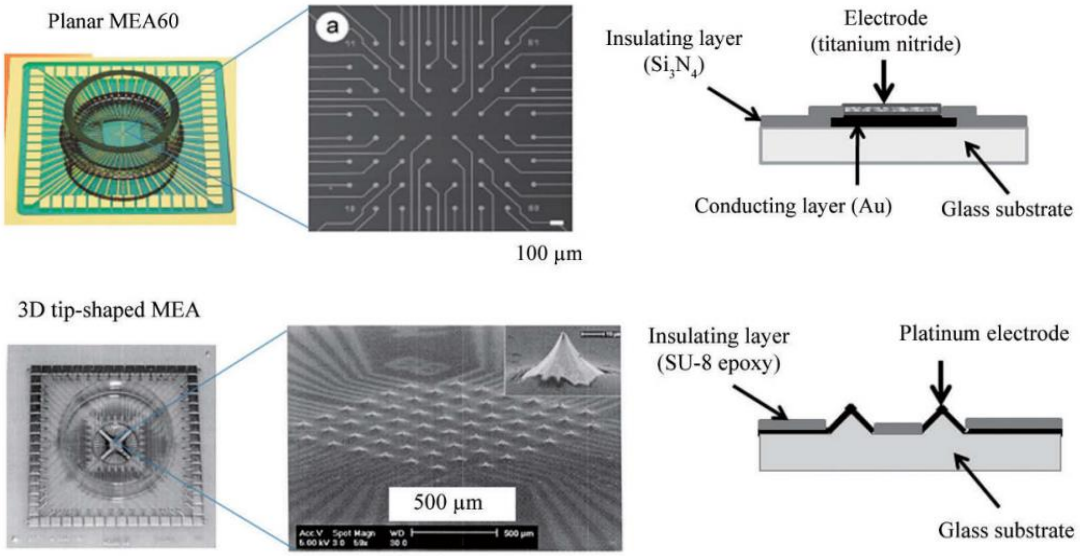

Figure 2. Planar 60-MEA (top) and 3D tip-shaped MEA (bottom) with the respective designs. [Liu, M., et al. Use of multi-electrode array recordings in studies of network synaptic plasticity in both time and space. Neuroscience Bulletin, 2012]

Alternatively, calcium imaging is another widely used technique in nowadays-neuroscience that allows for the assessment of neuronal activity by indirectly monitoring dynamic changes in intracellular calcium levels. Particularly, it uses fluorescent activity sensors to measure the activity of multiple neurons simultaneously, in such a way that changes in fluorescence reveal fluctuations in intracellular Ca^{2+}, which

is in turn an indicator for neural activity. Hence, Ca^{2+} imaging has gradually become a potential strategy in the context of the brain functioning, since it enables the recording of activity patterns in local circuits, facilitating the analysis of network dynamics [24]. Thus, by identifying correlated Ca^{2+} signals, researchers can assess functional connections and gain insights into the underlying circuitry. Moreover, calcium imaging in freely-behaving animals during electrical stimulation of deep brain structures allows for the characterization of cellular responses to specific stimuli or behavioral tasks. [25]

Additionally, calcium imaging combined with other promising techniques, such as optogenetic manipulations, provide a more complete background of cellular activity. According to Jennings J. H. et al. (2014), the combination of color-shifted indicators, optogenetics, and functional in vivo calcium imaging enables researchers to manipulate specific neurons while simultaneously monitoring the calcium activity of the neurocircuitry, providing valuable insights into functional brain dynamics [26].

2.2.2. Current research

Recent advances in neuroscience research have shed light on the pathogenesis of movement disorders such as PD, HD, and ChAc. These research rely on deciphering the complex brain circuitry and molecular processes at work in these conditions, revealing light on the underlying pathological mechanisms.

One area of research for PD is the glucose-sensing properties of dopaminergic neurons in the substantia nigra, which may be assessed through their electrical activity [27]. Based on physiological glucose levels, scientists designed MEA methods for long-term recordings, allowing them to discriminate between dopamine-inhibited and excited neurons. Although their significance and selective damage in PD remain unknown, this research gives unique insights into the disease and the role of glucose in modifying brain function, based on the activity patterns and regulatory mechanisms of dopaminergic neurons.

During the presymptomatic stage of HD, striatal connections are damaged. However, HD affects additional brain areas, and the synaptic activity of these extrastriatal zones, which is linked to cognitive failure, is unknown. MEA-based research on the excitatory synapse structure and function in HD mice revealed synaptic abnormalities not only in the striatum, but also in other areas such as the hippocampus [28], which is key for understanding the complexity of the brain in early HD samples for potential therapeutic strategies that take a brain-wide approach rather than focusing simply on the striatum.

In the context of ChAc, there is limited knowledge about the disease and the expression of VPS13A in the brain. However, the current state of the situation for ChAc sheds light on the molecular mechanisms underlying the pathology. Recent investigations of García-García E. et al. (2021) elucidate the spatiotemporal distribution of this protein in mouse brain, offering unique insights into the pathogenesis of ChAc [18]. VPS13A was found with distinct expression patterns in different brain regions, exhibiting remarkable stability in neuron cells, located in the soma and neurites, while co-localizing with the endoplasmic reticulum and mitochondria.

3. MARKET ANALYSIS

With the growing percentage of patients suffering from neurodegenerative illnesses involving motor control, the worldwide movement disorders market has experienced significant growth, and is expected to continue developing in the coming years. In this context, continuous research and the emergence of new techniques work in favor of market expansion, being a potential industry for technology suppliers.

The market for movement illnesses is expected to be worth USD 12.800 million in 2021 [29]. Furthermore, the compound annual growth rate (CAGR) predicts that the market will grow by 9.80% between 2021 and 2030 [29], indicating the potential expansion for related medical device manufacturing, pharmaceutical companies, and research institutions to develop innovative solutions. Graphically, North America is projected to experience the fastest growth with 45% of the total contribution to the market [30], followed by Europe. This is driven by several factors, among them the high prevalence of disorders, current advancements in medical technology and research, as well as an increased awareness regarding the existing clinical conditions and their effect on patients' quality of life.

The worldwide market for movement disorders is characterized by fragmentation [30], implying that it is made up of a multitude of small to medium-sized companies developing distinct solutions that adapt to their respective desired outcomes. In terms of prevalence and market share, PD dominates the market, although other prominent diseases include HD, dystonia, chorea, and others. Similarly, the market is further segmented based on treatment types, that is drugs, non-invasive therapies and Deep Brain Stimulation (DBS), which has emerged as an effective modality in the market. Finally, the global market for movement disorders can be fragmented based on the key end users, including research entities (i.e. universities, academic laboratories, pharmaceutical companies), point-of-care centers (i.e. hospitals, clinics, and ambulatory surgical centers), as well as commercialization of medical devices within the field. Indeed, each end user category contributes to the overall market dynamics to address these neurological conditions.

Relative to ChAc, the potential market is relatively small compared to more common movement disorders due to its rarity and reduced prevalence, in such a way that research is limited. However, the discovery of genetic mutations, the investigation of biomarkers for diagnosis and treatment and collaboration between professionals from different disciplines (e.g. researchers, physicians, engineers, etc) have resulted in valuable knowledge of some underlying mechanisms of ChAc. Therefore, measuring the electrical activity of neuron populations using MEAs has the potential to yield significant insights and contribute to the market evolution.

3.1. Historical evolution

The development and further improvements of MEA designs has followed the historical evolution of the market for research in neuroscience, including neurodegenerative disorders such as ChAc, since MEAs have made major contributions to our understanding of brain activity and its relationship to these clinical

conditions. Therefore, in this section we include the temporal evolution of ChAc, together with a brief overview of MEAs initial designs.

On the one hand, various chorea-related movement disorders have been identified since the late 19th century. For instance, Jean-Martin Charcot characterized chorea but failed to distinguish it from HD. The discipline of neurology emerged as an independent specialty of medicine, paving the way for expert neuroscientists (i.e. as Osler, John Hughlings Jackson, and George Huntington, among others) to provide an understanding and definition of choreas as a group of movement disorders characterized by involuntary behaviors. Genetic research identified distinct mutations related with chorea across time, suggesting that the condition had several etiologies. [31]

On the other hand, the systematic development of MEAs was hampered by a lack of suitable microelectronic approaches and challenges in managing massive data streams until the latter half of 20th century. Significant progress has been made in recent years in making MEA strategies more accessible to the broad scientific community, leading to more widespread application in diverse areas of investigation.

The pioneering work by Thomas et al. (1972) successfully demonstrated the effectiveness of their early planar MEA design in recording the electrical activity from contracting cardiac myocytes in vitro. Their approach consisted of a 30-element MEA fabricated through photolithography processes, wherein thin gold/nickel film conductors were etched onto glass coverslips. [32, 33] Individual neural activity from mammalian central SN cultures was first reported using Pine's design (1980), containing two rows of 16 planar gold electrodes. Furthermore, in 1985 Gross et al. introduced a significant advancement in electrode materials for MEAs by employing transparent indium tin oxide (ITO) electrodes. ITO was used as a transparent conductive coating that allowed researchers to integrate electrophysiological recordings with optical imaging techniques, providing comprehensive insights of neural activity and the functional connectivity of networks. [33]

Aside from advancements made by commercial enterprises such as Multichannel Systems in Germany, Panasonic USA, and Ayanda in Switzerland, the ease of use and availability of photolithography techniques has enabled many researchers to fabricate MEAs with precise and customized electrode layouts tailored to their specific research needs. This is particularly valuable in brain slice recording, where electrode placement is adjusted for the individual experiment to target and record activity from specific brain areas of interest.

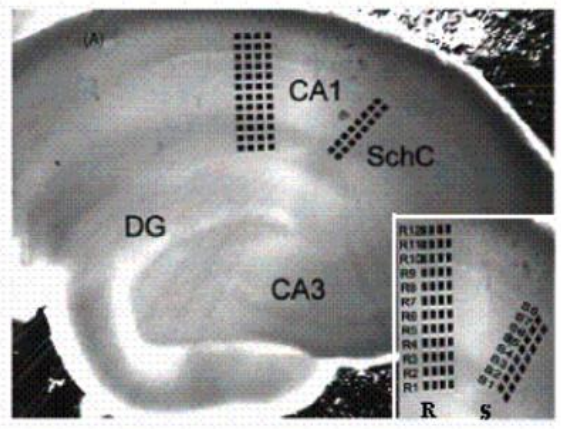

Figure 3. Conformal MEA for use with hippocampal slices

Figure 3 depicts one example of hippocampal MEA conformation (Gholmie et al., 2006) [34].

3.2. Future perspective

Continued research in this area holds promise for the development of targeted treatments and interventions to enhance the lives of individuals affected by neurodegenerative diseases, including chorea-related disorders. Additionally, new medical technologies are reshaping the industry, leading to further understandings and new therapies.

Future market prospects for movement disorders are influenced by technological innovations seeking to improve early diagnosis via advanced neuroimaging techniques and other devices, in such a way that new generations of these techniques are expected to increase sensitivity and specificity in detecting small structural and functional modifications associated with particular movement disorders.

The integration of Artificial Intelligence to this framework can contribute in a variety of ways. Machine learning and deep learning techniques can be used to create predictive models that assist in early detection and diagnosis by analyzing large datasets, including the neuroimaging scans to quantify structural and functional changes in the brain, resulting in more precise and accurate interpretation of the results. AI paves the way, as well, for predicting disease progression and treatment outcomes.

Researchers look forward to finding novel techniques for treating movement disorders by focusing on particular processes and pathways implicated in the condition. Some of these strategies include pharmacological approaches seeking to restore cellular activities and gene therapy to address genetic mutations.

Collaboration between technology developers will rise in the market for movement disorder research. Future MEAs might provide improved spatial resolution by increasing the number of electrode channels, allowing for more detailed recordings of neural activity. Likewise, as nanotechnology advances, compact and biocompatible MEAs might be produced and implanted in specific brain areas to permit long-term recordings and stimulations.

Overall, deeper understanding on the underlying mechanisms of movement disease and the incorporation of advanced technologies for early detection and optimized treatment will promote the role of personalized medicine as a potential future trend in the field, leading to improved patient outcomes and quality of life.

4. CONCEPT ENGINEERING

Over the life cycle of a project, a series of decisions between alternative strategies must be undertaken. In this section of the report, we define the direction and foundation for the procedure's design by comparing the potential of several implementable solutions depending on the limitations and the scope of the project. The concept engineering comprises an in-depth examination of electrophysiology techniques based on their advantages and disadvantages. Similarly, a review of MEA provider companies is presented, together with the analysis of software tools used for image processing.

Based on the research of alternative solutions compatible with our project, the chosen strategy is detailed as the description of materials and methods that are used in the project.

4.1. Study of the solutions

4.1.1. Electrophysiology techniques

The purpose behind electrophysiology is to explore the electrical activity of living neurons, tissue, or organs, and investigate the molecular and cellular processes that mechanisms their signaling. Hence, the techniques imply the detection of the electrical impulses utilized by neurons to communicate, allowing scientists to get a deeper understanding of intracellular and intercellular processes. Electrophysiology lab setup may be different depending on the requirements of the experiment, among other aspects.

This section of the report examines alternative solutions for detecting electrical activity in neuronal cultures in order to select the most suitable one for this specific project. Table 2 summarizes the main pros and cons of the different electrophysiological recordings used nowadays in neuroscience.

Table 2. Comparison table for the different electrophysiology techniques

Technique	Advantages	Disadvantages
Intracellular recording [35]	Exceptional stability is required for high-quality recording. Provides measurement of the electrical activity of a single neuron. High molecular resolution.	High professional skills. Invasive technique since it entails inserting a microelectrode directly into a neuron. Inability to study the activity of neuronal populations or networks.
Patch clamp [36]	Provides measurements of single-neuron electrical activity and individual ion channel activity	High professional skills. Inability to study the activity of neuronal populations or networks.

	Temporal resolution Electrical activity measurements under multiple conditions, such as drug testing. High molecular resolution.	Time-consuming, due to the rate of diffusion through the tip.
Extracellular recording [37]	The electrical activity of a single neuron or many neurons might be measured at once. Recordings can be conducted in vivo or in vitro.	Less details on signal processing inside a single cell. Its performance is limited by the interfering noise.
Multielectrode arrays [8]	Capability of gathering field potentials from a neuronal population rather than a single neuron. Non-invasive technique. Possibility to study the effect of drugs. Cultures and recordings may be sustained for an extended period of time, providing good temporal resolution.	High sensibility to noise and motion. Poor cellular resolution.

4.1.2. Multielectrode Array Suppliers

The study of MEA companies focuses on evaluating Multichannel Systems (MCS) and 3Brain. We compared various aspects (detailed in Table 3), including pricing, technical support for data collection, and design to make an informed decision regarding the most compatible MEA set up for our project.

Table 3. Comparison table for the different MEA suppliers

Model	Advantages	Disadvantages
MultiChannel System [38]	User-friendly software for collecting the data. Wide diversity of designs and setups. Affordable	No cellular resolution. Not so innovative or cutting-edge.
3Brain [39]	Easy software for collecting the data. High-resolution MEAs. Newest generation of MEAs and Innovative BioCAM platform, providing imaging and electrophysiology techniques. Customization options.	Expensive Some of their products require specialized expertise to use effectively.

4.1.3. Programming language

Ultimately, MATLAB and Python programming languages were evaluated. Although they offer extensive tools for image processing and analysis, MATLAB's user-friendly interface (SPYCODE, Bologna et al. 2010, Italy [40]) arises as a key and distinguishable advantage, enhancing accessibility for researchers with diverse programming backgrounds.

Table 4. Comparison table for the different programming languages

Model	Advantages	Disadvantages
MATLAB	SPYCODE platform for data analysis and visualization of MEAs. Multiple toolboxes for signal processing. Online resources and support.	Expensive license.

Python	Free programming language. More personal experience Online resources and support.	Non-existence of a GUI to analyze the data of the MEA recordings.

4.2. Materials and Methods

At this point, the materials and methods section is described based on the final choice, that is recording electrical activity of neurons seeded in MEAs from MCS and performing a subsequent analysis with SPYCODE interface.

Primary cell cultures

Cortical primary mouse cultures were conducted on E18.5 brain embryos in Neurobasal media (Fisher Scientific, USA) supplemented with Glutamax and B27 (Fisher Scientific, USA), allowing neurons to grow and maintain in serum-free conditions. The procedure requires expertise in tissue culture techniques. To promote cell survival, it must be performed rapidly and in the laminar flow hood under sterile conditions; ideally the process should not take more than three hours after tissue dissection. Cells were plated at a density of 60.000 cells/ml onto 12 mm glass coverslips that had been pre-coated with 0.1 mg/mL poly-D-lysine (Sigma-Aldrich, USA) one day prior under sterile conditions, as well as onto the sterilized MEAs platforms (MultiChannel Systems, Germany) at a density of 1500 cells per square millimeter and pre-coated with 1 mg/ml Poly-L-Lysine (Sigma-Aldrich, USA). Primary culture cells can be seeded directly onto the pre-coated MEA's glass substrate following the appropriate in vitro procedures, in such a way that the medium stays within the central cell culture chamber. Cultures were kept in an incubator with a relative humidity environment of 37ºC and 5% CO2 for 15 days in vitro (DiV) in both cases. MEAs medium was changed every 3 days to ensure optimal cell growth and they were incubated with tiny amounts of sterile milliQ water to avoid evaporation [18]. The specific protocol for cell culture procedures is described in Appendix I.

After 2 DiV, cells were infected utilizing VPS13A shRNA encapsulated into viral particles (Vector Builder, USA) to knock down VPS13A expression. The most effective MOI for this scenario was set at 1:20.000. Thus, the final medium, which is composed of Neurobasal, the shRNA virus, and reduced amounts of Glutamax and B27, is suitable for viral infection and has been identified after several experiments at different concentrations in Neurochemistry Lab, seeking to support virus entrance and reproduction while maintaining cell viability.

Characterization

Cortical primary cultures were fixed at DiV 15 with 4% paraformaldehyde (Thermo Fisher Scientific, UK) and stored at 4ºC in 0.02% sodium-azide (Sigma-Aldrich, Spain) PBS until further usage (detailed in

Appendix II). The samples underwent immunolabeling to visualize specific molecules within cells. Coverslips were permeabilized with PBS containing 1% BSA (Sigma-Aldrich, USA) and 0.1% saponin (Thermo Fisher Scientific, USA) for 10 minutes at RT, and incubated with a blocking solution of PBS and 15% NGS (Vector Laboratories Ltd., UK) for 30 min. At this point, samples were incubated ON at 4ºC with primary antibodies from Table 5, to recognize and bind to specific target molecules of interest. Then, the samples were washed with PBS, and incubated with secondary antibodies conjugated with fluorescent molecules for 60 minutes. After washing coverslips with PBS, samples were counterstained with DAPI to enable cell nuclei visualization (Thermo Fisher Scientific, USA) and after 15 minutes, they were mounted using Fluoromount-G media (Southern Biotech, USA) and stored at -20ºC until visualization on the inverted fluorescence microscope (Zeiss, Germany). For further detail, consult ICC protocol in Appendix III.

Table 5. List of antibodies used for culture characterization.

Antibody	Target population	Company	Dilution
Primary antibody			
Rabbit Anti Iba1	Microglial protein Iba	Wako Pure Chemical Corporation, USA	1:500
Mouse Anti-NeuN	Neuronal protein NeuN	Sigma-Aldrich, USA	1:100
Mouse monoclonal Anti-Glial Fibrillary Acidic Protein (GFAP)	Astrocytic protein GFAP	Sigma-Aldrich, USA	1:1000
Secondary antibody			
AF555 goat anti-mouse IgG	Mouse IgG	Thermo Fisher Scientific, USA	1:100
AF647 goat anti-rabbit IgG	Rabbit IgG	Thermo Fisher Scientific, USA	1:100

Quantification

For NeuN quantification, three to five images of each coverslip were acquired using the inverted fluorescence microscope (Zeiss, Germany) and quantified with a specific ImageJ macro designed to automate the process of counting NeuN positive nuclei in the immunolabeled samples. The spatial scale of the images was calibrated prior to processing to guarantee accurate measurements. The customized macro relies on image thresholding algorithms to distinguish the immunolabeled nuclei from the background and posterior particle detection and analysis was computed. These automated techniques contributed to efficient quantification of neuronal density based on the number of detected NeuN-positive nuclei. For further detailed information about the specific steps of the ImageJ macro, please refer to Appendix IV. Statistical analyses were then performed with the GraphPad Prism 9.5.1 (733).

Alternatively, microglia and astrocytes were qualitatively analyzed based on the visual inspection and distribution of Iba1 and GFAP specific staining, respectively.

Multielectrode arrays setup

MCS constitutes a German company that specializes in the development of scientific data acquisition interfaces for electrophysiology research, offering various systems for extracellular recordings using MEAs and electrical stimulation.

The biosensors used in this project are glass MEAs 8X8 200/30, that is 60 titanium nitride (TiN) microelectrodes placed in an 8x8 grid with a particular geometry of 200 μm of interelectrode spacing and 30 μm electrode diameter. The TiN electrodes present a long lifetime due to the biomaterial's stability and compatibility to high temperatures, in such a way that they can be autoclaved and reused for several times. The material provides excellent electrical properties for application in electrophysiological recordings, such as lowering impedance and noise levels for accurate findings. For instance, low impedance electrodes allow for more current flow and higher signal-to-noise ratio (SNR) because the amplitude of the recorded spikes is increased, making them more discernible from background noise [41] [42]. The impedance of MCS 30 μm TiN electrodes is generally 100 $k\Omega$. Thus, the smaller the electrode, the higher the impedance and, in turn, thinner electrodes and shorter interelectrode distances result in higher spatial resolution. Despite the trade-off between resolution and signal quality, MCS devices perform properly under specific experimental needs, presenting an average noise level of around 10 μV peak to peak for 30 μm TiN electrodes [43]. Additionally, the 200/30 version is available with an internal reference electrode (IR) that has to be connected to the amplifier's ground. It behaves as the fixed reference point and enables the measurement of the electrical potential at any specific location.

Raw data was amplified using a 60-channel (MEA 1060-BC-PA) amplifier with a typical pass band of 300 to 3000 Hz for the recording of neural spikes, in such a way that small electrical signals are amplified while undesired noise and interferences are filtered out. MCS amplifiers incorporate a heating software to regulate the temperature of MEAs without requiring extensive user intervention. The TC02-Control can supply the desired temperature conditions into the cultured chamber with high accuracy (0.1ºC) by adjusting all the settings online [44].

Lastly, the amplifier's output signal is digitized using the USB data acquisition component (USB-MEA-System), which connects to a computer through standard shielded cables for subsequent data processing. MCS offers software tools for conveniently graphing and interpreting recorded data: MC_Rack optimizes recording setups and provides real-time feedback, spike detection analysis, and electrical stimulation control, among other features [45].

Stimulation system

Voltage-controlled stimuli were generated using one channel from the STG 4004 (Multichannel Systems, Germany). Stimulus generators are software driven through MC_Stimulus II, which provides advanced features that enable precise and flexible control over the pulse generation. Thus, by selectively stimulating a specific electrode, one is able to investigate how the neural culture responds and exhibits coordinated activity. The combination of STG 4004 and MC_Stimulus II is suitable and adaptable for various applications, including neural networks and brain slices [46].

We tested rectangular voltage pulses of alternated positive and negative polarities, with a peak amplitude of 500 µV, every 30 seconds. Such configuration is displayed in Figure 4.

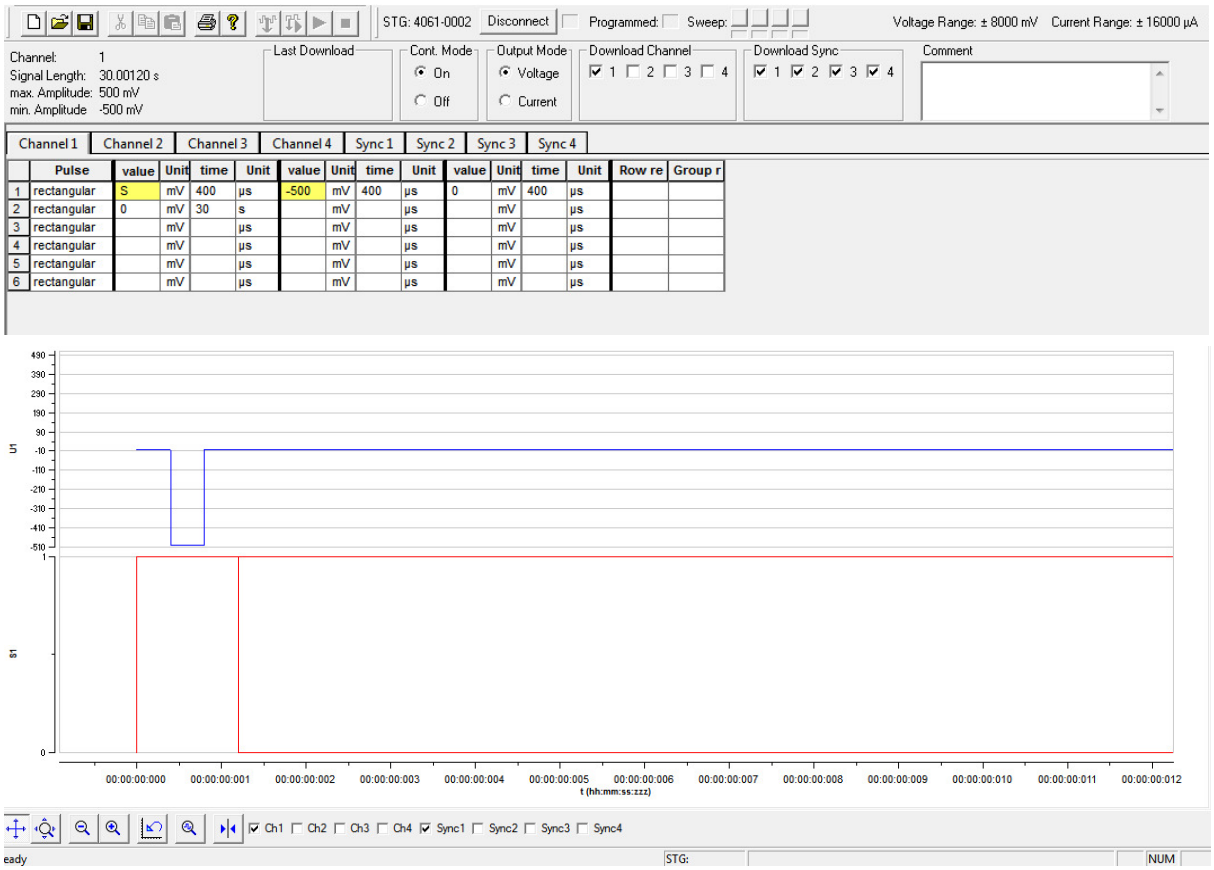

Figure 4. Main menu file with the stimulus protocol used (blue) and stimulus protocol display (red). [Guerras, Àngels. 2023]

Overall, the measurement of electrical activity from neural networks in MEAs involves a well-defined workflow that integrates all the individual components previously stated. Similarly, we provide the visual configuration of the entire setup in Figure 5.

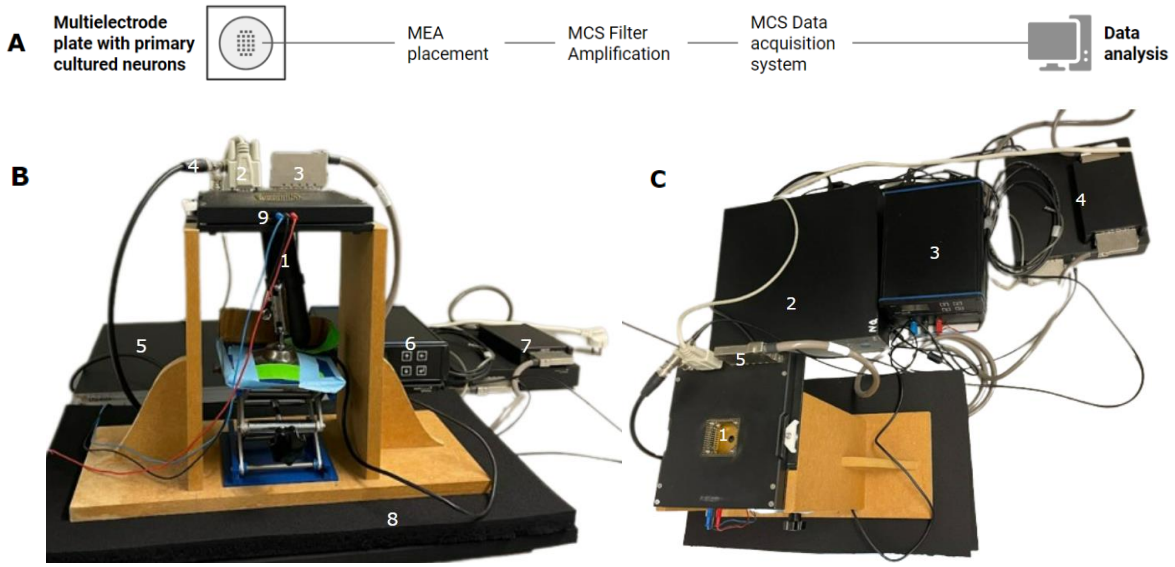

Figure 5. Setup configuration in the laboratory. (A) Workflow for data acquisition using MEAs with MCS components. (B) Front view of the setup where 1 is the optical camera (not used in this project), 2 is the connection to MEA_Select program, 3 is filter amplifier and data acquisition, 4 is Sync Out connection from STG 4004, 5 is the STG 4004 itself, 6 is T02 Controller, 7 is USB-MEA-Systems, 8: physical support with foam; 9: wire connection (blue = stimulus A, red = stimulus B, black = ground). (C) Plant view of the setup where 1 is the MEA placement, 2 corresponds to the STG 4004, 3 is T02 Controller, and 4 refers to USB-MEA-Systems. [Guerras, Àngels. 2023]

First, the ground is connected to the interface board. According to Figure 5, the neural culture is carefully placed on the headstage (1 from C), which allows for electrical activity detection. The recordings are amplified and digitized using the USB-MEA data acquisition systems for further processing. Note that the stimulation component, while not included in this workflow, can be integrated similarly.

Data processing software

SPYCODE is a MATLAB software [40] that provides a Graphical User Interface (GUI) for computational analysis of MEA recorded data. This platform was particularly developed for the analysis of neuronal activity from cultured cells, providing an operational environment capable of managing and processing big data, since it incorporates both conventional and sophisticated methods for signal analysis.

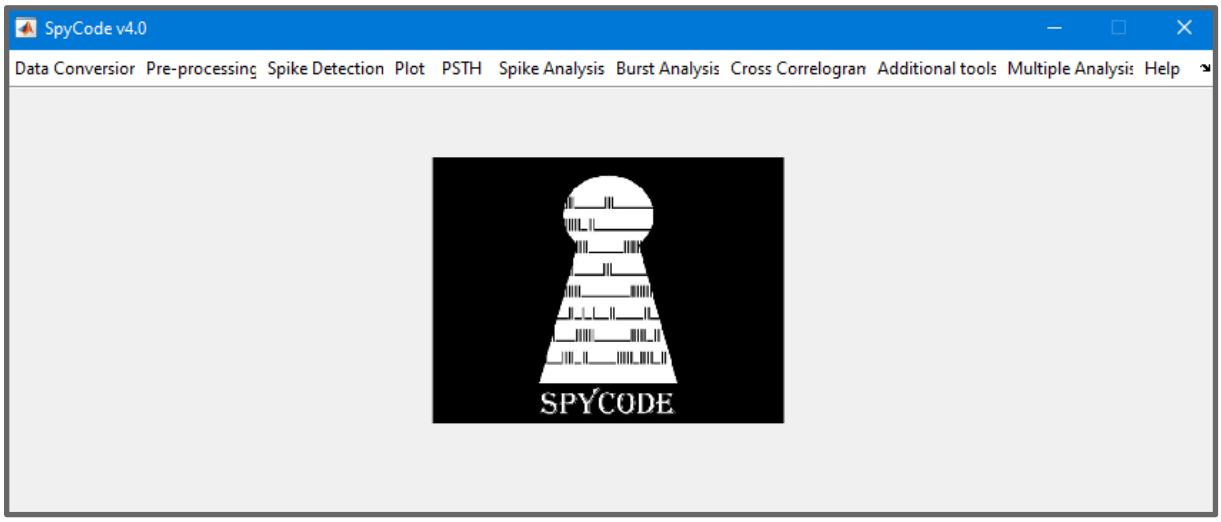

Figure 6. GUI of SPYCODE. [Guerras, Àngels. 2023]

The theoretical flow chart of its working system is schematized in Figure 7 and further explained based on Bologna et. al. specifications [40].

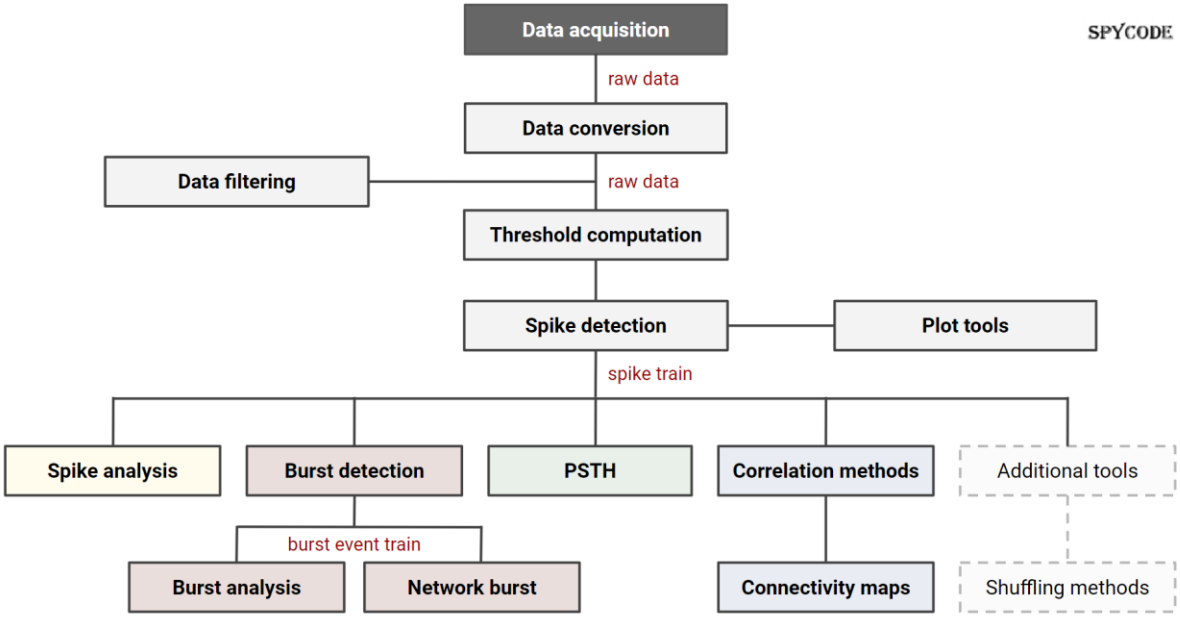

Figure 7. Schematic overview of SPYCODE's workflow and functional algorithmic blocks for data analysis [40]. Note that additional tools and shuffling methods have not been implemented in this particular study. [Guerras, Àngels. 2023]

1. Acquisition of raw MEA recordings

The raw recordings were collected in the widely used .mcd file format, which is primarily designed to store multi-channel data. This format makes the acquired data easily accessible for subsequent processing and analysis using specific software tools.

2. Preprocessing

2.1. Data conversion

Raw MEA data files must be converted from .mcd to MATLAB internal format (.mat). Single channels are kept in separate files, that is, with the current MEA setup in the laboratory, one MCD record yields 60 mat files corresponding to the 60 electrodes.

2.2. Data filtering

The existing SPYCODE versions (SPYCODE _v.3.8 & SPYCODE_v.4.0) provide an optional raw data filtering via bandwidth and sampling frequency adjustments, utilizing Butterworth low-pass (v.3.8), high-pass (v.3.8), and band-pass (v.4.0) filters. Particularly, we used the band-pass filter set at [300 3000] Hz, meaning that frequencies below 300 Hz or above 3000 Hz will be attenuated, while the sampling frequency is set at 25 kHz, that is the number of samples that were recorded per second. The specific filter was selected based on the objections from the MEA amplifier manual provided by MCS, stating that typical pass bands for action potential recordings are 300 to 3000 Hz. [47]

2.3. Threshold computation

The thresholding technique is based on applying a multiple of the standard deviation coefficient of the raw data signals, in order to differentiate neuronal activity from the baseline noise and increasing SNR. We applied a threshold of x7 SD.

3. Analysis

SPYCODE covers data analysis in three major steps: spike analysis, burst analysis and connectivity analysis.

3.1. Spike detection and analysis

An automated PTSD algorithm was used to detect spikes. It looks for a peak in the signal near the specified threshold within a certain time window, corresponding to the peak lifetime period. This option makes spike identification simpler since the algorithm looks for peaks inside the defined time range crossing the threshold. Peak lifetime period was set at 2 ms and any peak exceeding the threshold of 7x SD is considered a spike (1), otherwise no spike is found (0).

Next, SPYCODE offers additional tools to conduct an extensive analysis about cell activity, including computation of the Mean Firing Rate (MFR) per channel, and the Inter-Spike Interval (ISI). On the one hand, MFR gives important information regarding the degree of activity in neurons, which may be used to compare patterns across populations. It is defined as the number of spikes divided by the unit time (s). On the other hand, ISI refers to the time duration between consecutive spikes in a neuronal firing sequence generated by each electrode, revealing patterns for bursting behavior. Thus, we computed a logarithmic ISI histogram choosing 10 bins x decade on the x-axis.

3.2. Burst detection and analysis

Burst detection was performed using CH-v1 algorithm, which allows the detection of burst events in a spike train, that is the sequences of at least N consecutive intra burst spikes (N=5) that are spaced less than 200ms.

Various additional functions can be used to generate the Main Statistics Report of the analysis, providing quantitative information concerning burst activity and firing patterns. Among the usual statistics that may be evaluated are Mean Burst Rate (MBR), number of intra-burst spikes, burst duration in ms, and detection of random spikes.

At this point of the analysis, the Post-Stimulus Time Histogram (PSTH) functional block allows computing the response to the applied stimuli of one or more channels. PSTH represents the average behavior in response to a stimulation pattern while it shows the probability of firing as a function of time after the voltage pulse is onset.

3.3. Correlation methods and connectivity maps

The last block evaluated corresponds to the extraction of functional connectivity from a neural network using cross-correlation (CC) methods that consist of quantifying the similarity between each couple of neurons at a given time series. To differentiate between true and random (i.e. noise-induced) interactions, the strongest and statistically meaningful connections between each electrode pair must be identified via threshold-based algorithms. Bologna et al. (2010) took into account the K strongest links among the possible N connections; where K < N. Based on this approach, connectivity maps are created using the more selective threshold.

Through the GUI, one can adjust the necessary parameters and set the threshold for strong connections at 0.2 to obtain the connectivity map from the CC algorithm. The correlation was calculated from burst event trains.

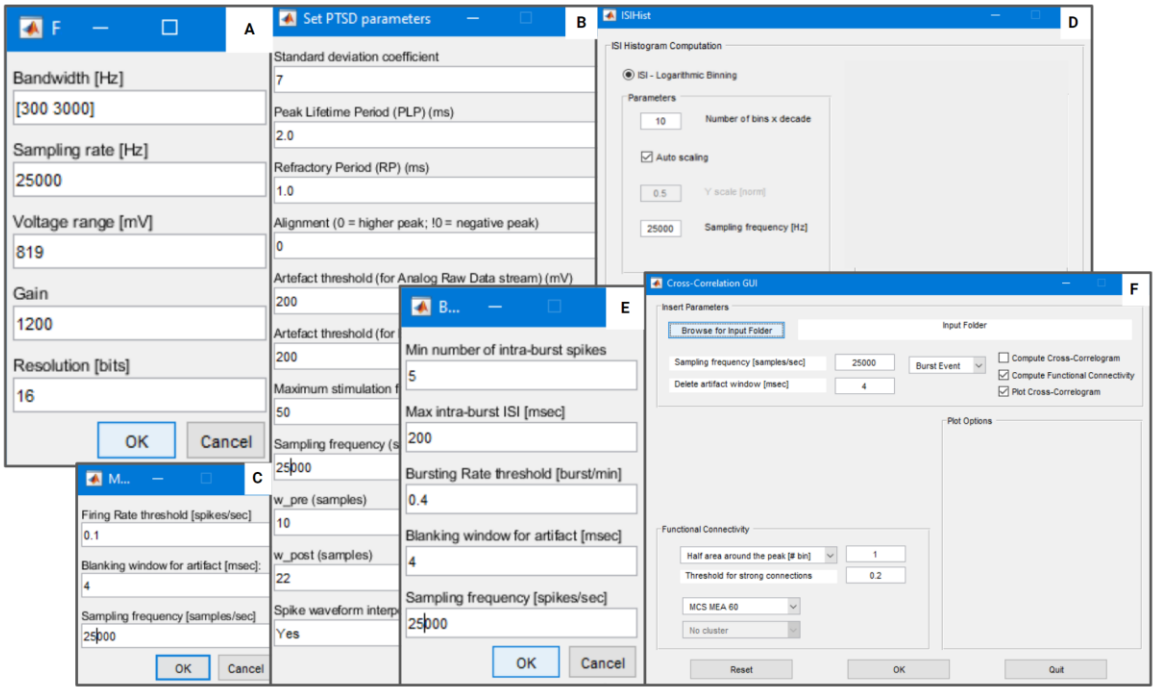

Figure 8. Multiple running GUI windows. (A) data filtering, (B) PTSD spike detection, (C) MFR computation, (D) Log ISI histogram, (E) Burst detection using CH-v1 algorithm, (F) cross-correlation GUI. [Guerras, Àngels. 2023]

Figure 8 displays the multiple GUIs showcased during the working course of the analysis, as well as the various variables chosen for our study. It must be pointed out that while some parameters were customized according to our experimental needs, as we have previously indicated (i.e. sampling frequency), most of the settings remained as default values because SPYCODE, being originally intended for the analysis of neuronal activity, already included optimal default parameters.

5. DETAIL ENGINEERING

Let's now dig into the results obtained from the implementation of the described techniques. This section provides a comprehensive analysis of the data, including the characterization of cell populations from neuronal primary cultures, the assessment of neuronal activity from the MEA recordings and any observed effects under investigation. It is worth noting that we faced several challenges throughout the experimental process, which will be addressed and detailed here. By understanding the encountered limitations, we can pave the way for improvements and future research on the study of ChAc.

Along the course of my internship, the availability of pregnant mice was limited, reducing the possibility to perform a number of cell cultures large enough to allow MEA analysis. Hence, a total of 5 neuronal primary cultures were successfully conducted. In order to provide a concise overview of the experimental results, we have summarized their respective progression during the different stages of viral infection, fixation, and recordings in Table 6.

Table 6. Summarized outcomes of the laboratory procedures.

ID	Date	Infection	Fixation	Recording	Comments
#1	21/02	X	X	X	All cells were dead by the day of infection.
#2	13/03	✓	✓	X	Cells were successfully infected with the virus on 15/03 and fixed after 15 DiV (28/03). However, cells cultured in MEAs turned out to be dead, that is no registration was conducted.
#3	20/03	✓	X	✓	Cultures were not fixed due to cellular death. Despite MEA recordings were performed 10 DiV to avoid death, no electrical activity was observed.
#4	27/03	✓	✓	✓	Cell fixation and MEA recordings took place after 15 DiV, that is 11/04. Only one electrode showed activity from a neuron.
#5	17/04	✓	✓	X	Cells were fixed on 02/05 for posterior usage in ICC. Cultures seeded on MEAs were dead when inspected under the microscope.

Characterization of cell cultures indicates no significant differences in cell viability between control and KD neurons.

To assess the cellular population of cortical primary cultures, immunocytochemistry (ICC) techniques were used in order to visualize specific cell types within the cultures based on their protein expression profiles. The results indicate that our cultures are majorly neuronal, with no detectable presence of astrocytes or microglia.

The ICC analysis revealed strong reactions throughout the cortical primary cultures when stained with the neuronal marker NeuN, demonstrating the presence of several mature neurons. The immunostaining of NeuN targets neuronal nuclei, but we unexpectedly observed the presence of cell bodies in our results, which can be attributed to ICC limiting factors and variations (e.g. antibody specificity, ICC protocol, etc.). Conversely, astrocyte and microglia markers, that is GFAP and Iba1 respectively, did not show any immunoreactivity within the cell cultures. These data point to the lack of astroglia and microglia cells, which support the evidence of the most predominantly neuronal nature of the cultures.

At this point, the effect of VPS13A KD on neuronal cells was evaluated by quantifying NeuN puncta with ImageJ macro, thereby measuring neuronal viability in terms of the number of living cells under the effects of genetic manipulation. The quantitative study was limited to neurons, while the absence of astrocytes and microglia was investigated qualitatively. Figure 9 displays the NeuN quantification process and gives a visual depiction of the results acquired, including the processed image and the counting analysis of stained nuclei.

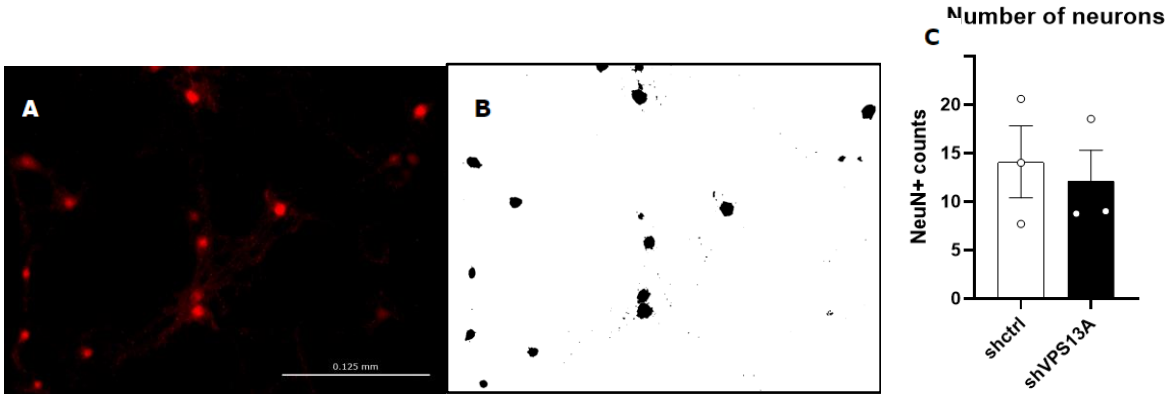

Figure 9. NeuN quantification. (A) NeuN staining in cortical primary neurons from mice. (B) Processed binarized and thresholded image using ImageJ software. NeuN positive puncta were quantified (C) in 10 images from control and 15 images from KD conditions from 3 different embryos as a function of the genotype group. All data is presented as the mean ± SEM and statistical analysis were performed with Student t-test. [Guerras, Àngels. 2023]

The statistical analysis (extended in Appendix V) demonstrated that the presence of virus does not cause considerable neuronal loss. The unpaired t-test resulted in p-value = 0.7, indicating that there was no significant difference in neuronal cell numbers detected between the control and KD groups. Nonetheless, it is worth noting that the study was conducted with a limited sample size of just n = 3 embryos, explaining the variability of the results. A statistical study of three animals can give adequate data to determine the absence of neuronal cell death. Increasing the n implies the sacrifice of more animals, which, in turn,

raises ethical concerns. With this sample size, researchers can reliably make conclusions about the lack of neuronal cell death by carefully planning the study and employing rigorous statistical methods.

Recordings with MEAs posed significant challenges to the experimental procedure.

As previously stated, MEAs were used to culture cortical primary neurons with the major objective of investigating the electrical activity of control and KD groups to unravel differences in their neural network and functional connectivity.

The recording methodology included multiple stages for capturing neural signals under different circumstances. A 10-minute period of spontaneous activity was undertaken to allow cellular adaptation to the new environment, with 5 minutes of no signal recording followed by 5 minutes of recording. During the following 10 minutes, cells were subject to stimulation recordings, and a voltage pulse was applied to a single electrode to examine the response of surrounding cells. At last, a 5-minute recording was conducted to look for any changes in neuronal activity caused by the stimulation procedure.

Despite all of our attempts, we experienced various difficulties throughout the experimental process, including massive cell death within MEA chambers and, as a result, the inability to recognize any electrical patterns during the recordings, as well as dealing with excessively noisy signals.

Particularly, we observed that neurons in the center of the MEA chamber, meaning in proximity to the electrode placement, experienced massive cell death; while neurons allocated in the periphery of the device seemed to remain viable and functional. In Figure 10, dead vs healthy neuronal cells can be visually inspected based on the distinctive characteristics of both conditions. Neurons that were identified as dead (A) look round and brighter, lacking the typical shape and complexity of their morphology. In contrast, healthy neuronal cells (B) appear elongated, with an extended cell body, and exhibited a distinct contrast when compared to the dead population.

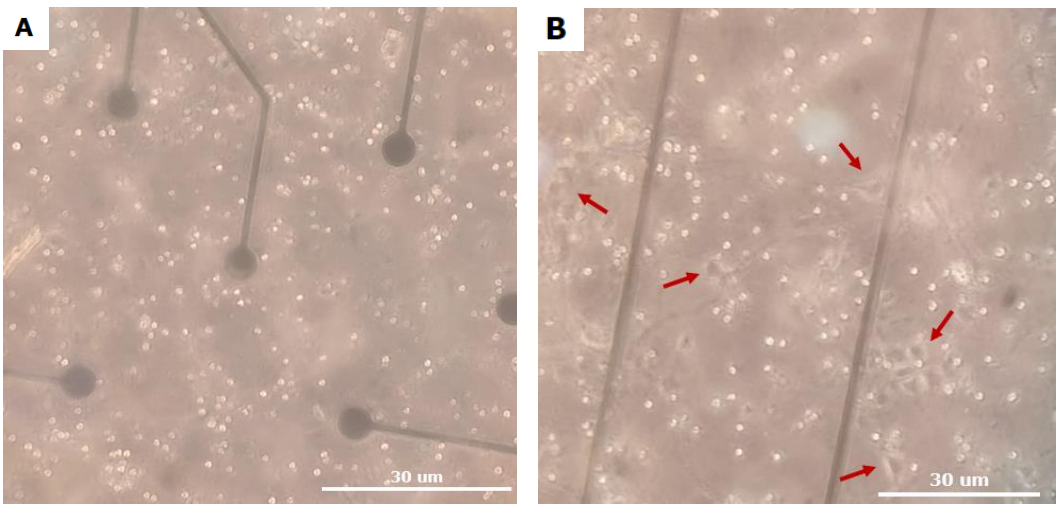

Figure 10. Appearance of dead neurons in the center of the MEA (A) and healthy cells in the periphery (B) indicated with red arrows. [Guerras, Àngels. 2023]

In this context, it is plausible that the close proximity of neurons to the electrodes, combined with an excessive cellular density within the central area, creates an unfavorable environment, compromising cell survival. However, it is worth noting that while the group of Neurochemistry is familiarized with MEA protocols using brain slices, cell culture performance is relatively a new endeavor, in such a way that the corresponding protocol is not yet properly standardized in the lab.

What's more, the existence of significant noise in the recorded signals was another obstacle we experienced during data recordings. This noise was mainly caused by the lack of specific tools in our setup to mitigate environmental artifacts and interference from the L5 metro, which largely contributed to the overall noise level in the recordings.

In front of these unideal conditions, we implemented several strategies to minimize vibrations and enhance the signal quality. Thus, considering that the electrical activity of neurons is inherently small in amplitude, it is desirable to maximize the SNR for clear and interpretable signals. For instance, we improved the setup by introducing foam and marble layers in the base of the setup, together with a 4-legged configuration, which potentially reduced the impact of noise coming from the underground station. To further reduce artifacts effects from the environment, we incorporated a structure mimicking the role of a Faraday cage by using aluminum foil , in such a way that it could shield the recording setup from the external electromagnetic interferences present in the laboratory. While these noise reduction measures were successfully adopted, it is crucial to emphasize that the noise level, although it decreased, it remained notable during the recordings, making proper identification of neuronal electrical activity a challenging process.

Overall, the presence of dead cells in the cultured MEAs and the high levels of noise posed significant challenges throughout the recordings, in such a way that we were unfortunately not able to detect significant electrical activity from the neurons. Despite repeating the process twice, we only identified potential neuronal activity during the stimulation phase from a single electrode. In Figure 11, we present the outcomes obtained from the MC_Rack program for our recorded signals. Notably, electrode #15 has no signal due to it being grounded, and electrode #33 is nonfunctional, rendering it unsuitable for further analysis. The y-scale axis is set at 100 μV, allowing us to visualize the impact of noisy signals, which exhibit a range of approximately 15 μV. Furthermore, Figure 12 is a zoomed-in from the electrode #84 during the stimulation stage. Despite the pronounced noisy and oscillatory nature of the signal, it is possible to intuit potential neuronal activity, suggesting that underlying neuronal responses may be present as a response of voltage pulses.

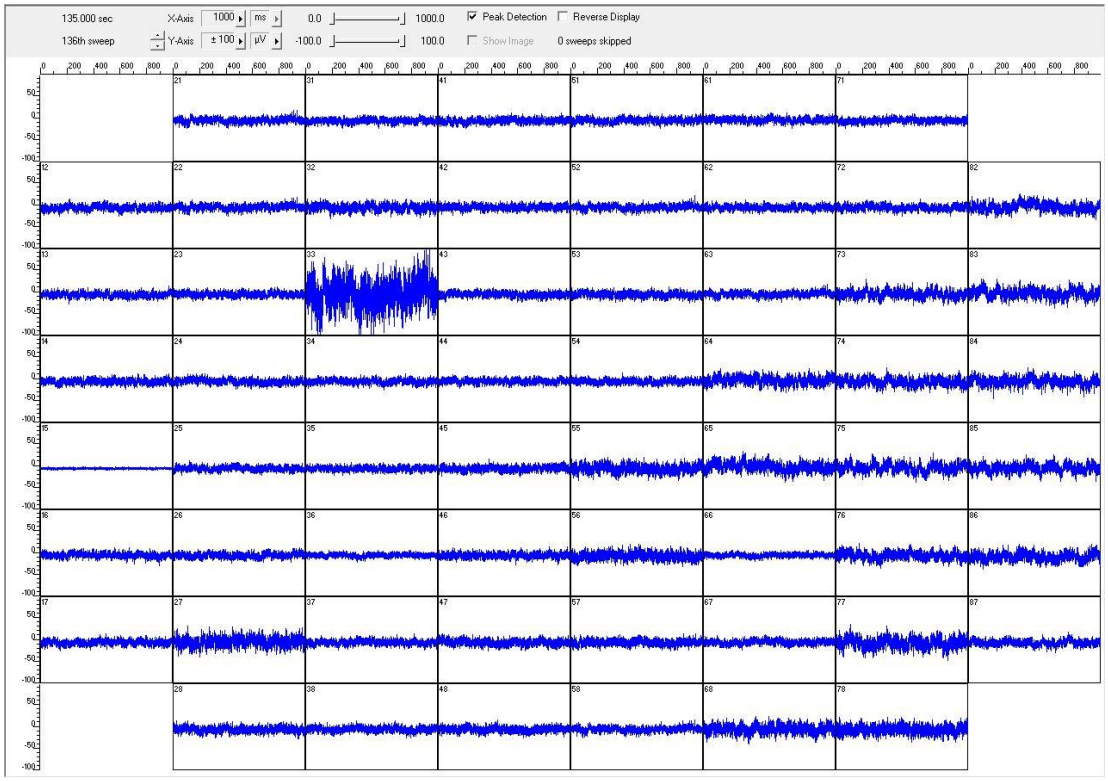

Figure 11. Display of the electrodes in MC_Rack software. With a x-axis corresponding to 1000 ms and y-axis to ± 100 µV, e15 is grounded and e33 is nonfunctional, while the rest contain significant noisy signals. [Guerras, Àngels. 2023]

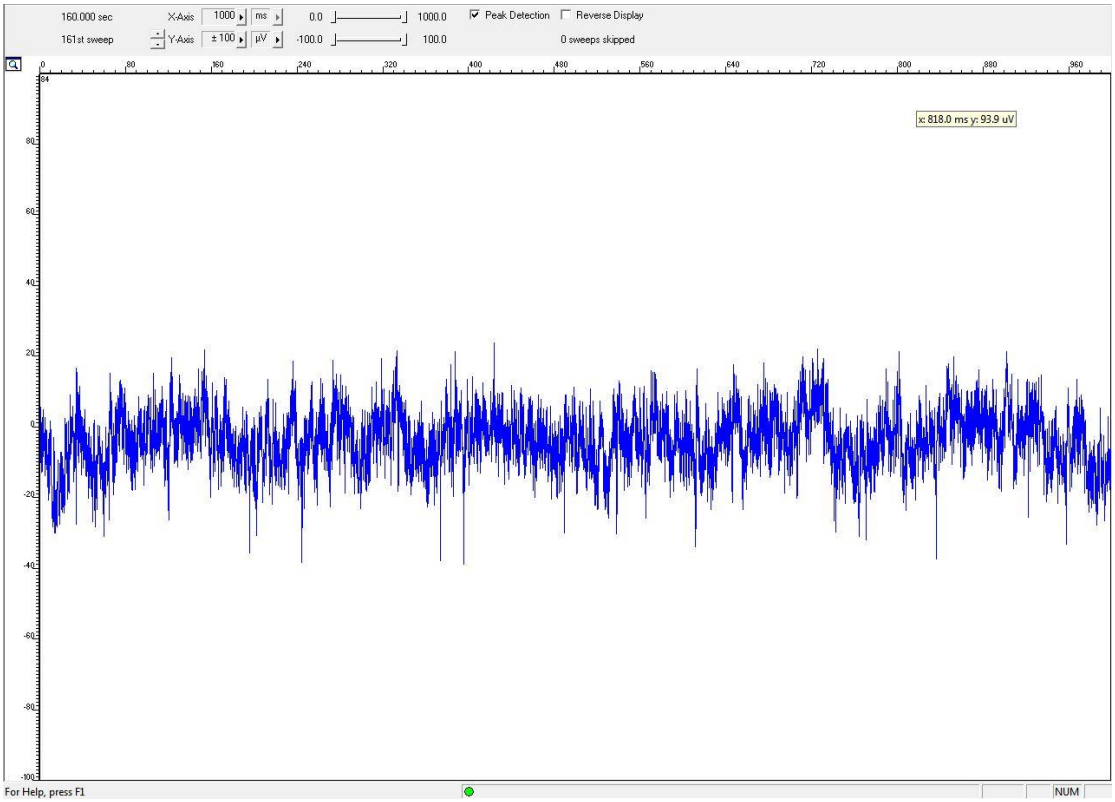

Figure 12. Zoom-in of e84 during the 10-min stimulation phase showing potential neuronal activity as small spikes of around 30-40 µV amplitude. [Guerras, Àngels. 2023]

Analysis of neural networks using SPYCODE.

Due to the lack of discernible electrical activity in the recordings from our neuronal primary cultures, we developed a contingency plan by reusing previously obtained data in the lab back in March 2019. Although the alternative files consisted of recordings from ex vivo sagittal brain slices from transgenic HD mouse models (female and male), which was different from our initial aim, it provided us with a new opportunity to use SPYCODE and grasp its potential within the established timeframe.

To put the new data into context, the compared populations included 2-month-old WT and knock-out (KO) mice lacking the RTP801 protein, which has been associated with motor-learning dysfunction in HD [48]. The cortex of the brain slices was examined by carefully positioning the cortical tissue on top of the electrodes in the MEAs, keeping the focus on the electrical activity occurring in this area.

The usage of these ex-vivo recordings from the HD mouse models served as a strategic alternative to overcome the challenges posed by our own recorded neuronal cultures, allowing us to delve into the computational tools and algorithms offered by SPYCODE.

SPYCODE was initially designed to analyze neuronal cultures rather than brain slices. It is important to acknowledge that the electrical activity recorded from brain slices tends to be lower compared to that recorded from cell cultures, resulting in a smaller SNR. However, we made efforts to adjust the methodology to the particular features of our data. By adapting the approach, we aimed to overcome these constraints and properly evaluate the recorded electrical activity in our experimental settings. To analyze the recorded data, we followed the methods outlined in section 4.2.

Filtering methods were used on the data. However, using a bandpass filter with a frequency range of [300 3000] Hz did not result in significant enhancements in signal quality. This might be explained by the existence of intrinsic noise in the recordings, as well as the specific properties of brain slices' electrical activity (i.e. small amplitude of signals).

Spike detection for electrode 12 in both WT and HD conditions is illustrated in Figure 13a and 13b respectively. While the algorithm recognizes spikes (peaks with amplitudes of around 20 μV or greater), it may also collect possible noise. However, the threshold value of 7*sd was purposefully set to test SPYCODE's capabilities by detecting a wide range of activity, with the goal of enabling burst detection and analysis, which would be minor if the threshold was decreased. The results suggest similarities in the electrical activity between the WT and HD conditions. However, it should be mentioned that signals can vary across different electrodes within the MEA, in such a way that the data from a single electrode does not fully represent the overall electrical activity of the corresponding condition.

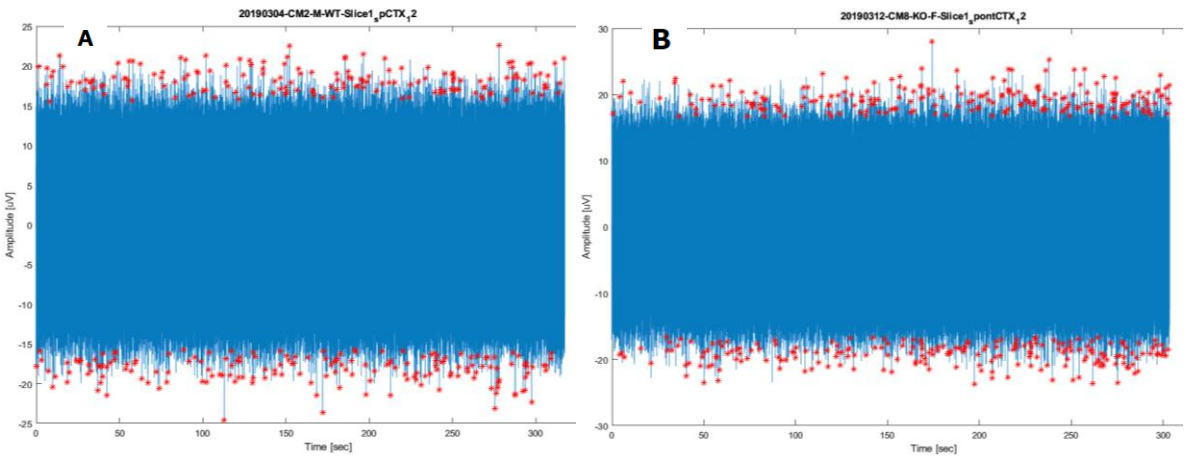

Figure 13. Signal and spike detection from electrode 12 for (A) WT, and (B) HD genotypes. [Guerras, Àngels. 2023]

At this point, measuring cell activity by MFR and MBR calculation reveals important information about the overall firing activity of the neural network. The results of the MFR and MBR analysis are presented in Figure 14, showing the distribution of average activity identified across all electrodes for both the WT and HD conditions. Based on the outcomes, it is observed that there is no discernible difference between the firing and burst rate between the two genotypes.

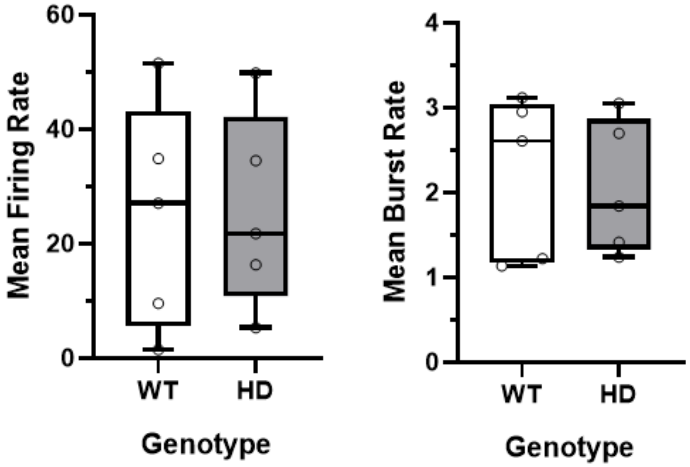

Figure 14. Neuronal activity is similar in both genotypes for n = 5 WT and 5 HD mice. (A) WT and HD don't show differences in the MFR (spikes/s). (B) Genetic mutation in HD samples does not seem to alter MBR (burst/min). All data is presented as the mean ± SEM and statistical analysis were performed with Student t-test (Appendix VI & VII), yielding (A) p-value = 0.9571 and (B) p-value = 0.7828. [Guerras, Àngels. 2023]

These data imply that RTP801 absence is not responsible for abnormal synaptic plasticity in the cortex of KO mice, which might be attributed to an analysis of total cortical electrical activity. While this protein has been reported to reduce cell density and activity in the motor cortex layer V (LV) [48], our study included data from all 60 electrodes in the MEA, rather than only those belonging to the LV. However, further data analysis is required to assess differences in other brain areas and also other features of neuronal activity that may differ between genotypes, such as network connectivity.

The results obtained from the ISI computation showed that the overall firing dynamics of neurons in the two groups (WT and KO) are similar. The corresponding plots are presented in Appendix VIII.

We faced difficulties throughout the functional connectivity study due to the algorithm's complexity and our limited experience with its core functions, as it was built by an external team. Hence, we encountered multiple errors that prevented us from adequately processing and interpreting our data.

Given such limitations, the outcomes for the functional connectivity study are confined to only one WT sample, i.e. the unique data for which we achieved relevant results. Nonetheless, we present the burst event connections observed across the cortical brain slice in Figure 15, which illustrates the correlations between different electrodes and reveals potential functional connections within the cortical network.

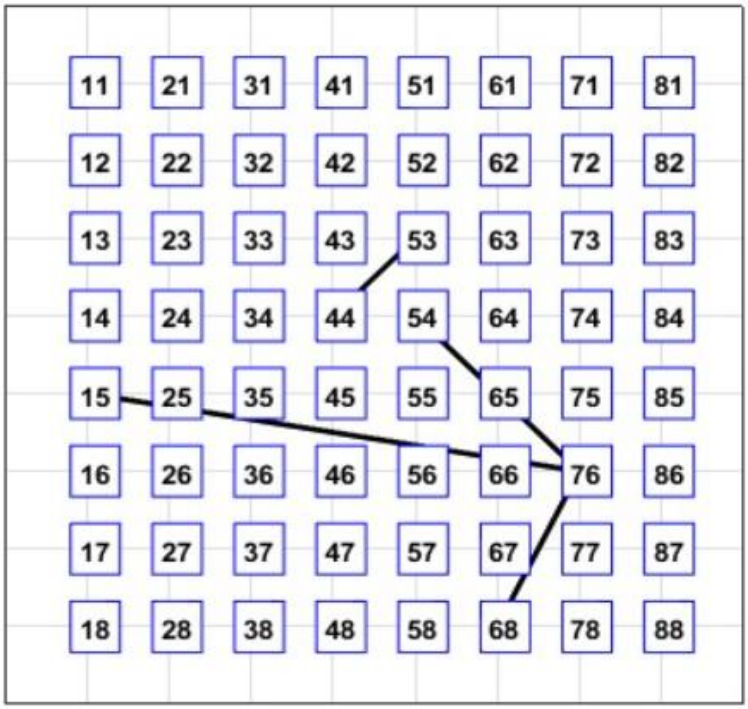

Figure 15. Functional connectivity for a WT sample based on burst event detection. [Guerras, Àngels. 2023]

This outcome reveals poor functional connectivity, occurring between two nodes. The first node comprises electrode 76, which exhibits strong correlations with electrodes 54, 15 and 68; while the second node involves electrodes 53 and 44.

Due to time constraints, we were unable to delve deeper into the errors found along this part of the analysis in the time limit of the project execution. Although the reported results are limited, we believe it is important to emphasize the potential of this approach for further investigation and implementation in future research. Thus, it is crucial to address these challenges to understand the complete possibilities offered by SPYCODE, including the acquisition of the correlation matrices to assist an intuitive description of the network's connections.

6. EXECUTION SCHEDULE

The execution plan gives an in-depth overview of the project monitoring and control to effectively complete the activities and thereby achieve the initial requirements and goals. This stage is critical because it contributes to the development of appropriate techniques and strategies to boost the efficiency of the final results.

This section contains three of the fundamental project execution items: Work Breakdown Structure, Gantt chart and PERT diagram, which are valuable tools for designing and tracking the project schedule.

6.1. Work Breakdown Structure

Work Breakdown Structure, or WBS, is a useful project management technique for determining the overall scope of a project. It is based on a systematic decomposition of the work into small, manageable tasks, typically known as work packages, that may be conveniently assigned for completion. Thus, WBS provides an accurate estimation of the time and assets required to accomplish the sub-tasks, allowing the project to be evaluated more efficiently [49].

The WBS is based on an ordered list of deliverables and the corresponding dictionary. The initial level includes the finished product, and the successive stages are broken down until they reach the indivisible jobs or work packages.

1. Study of circuits and neural networks using multi-electrode arrays in movement disorders

- 1.1. Start and definition of the project
  - 1.1.1. Project follow-up
  - 1.1.2. Review of literature and research
  - 1.1.3. Definition of objectives and initial hypothesis
- 1.2. Obtain cell culture
  - 1.2.1. Cell cultures
  - 1.2.2. Viral infection
  - 1.2.3. Cell fixation
  - 1.2.4. Culture characterization
  - 1.2.5. Cell quantification
- 1.3. Recording with MEAs
- 1.4. SPYCODE analysis
  - 1.4.1. Software learning
  - 1.4.2. Analysis of the results
- 1.5. Discussion
- 1.6. Project closement
  - 1.6.1. Delivery of the final report
  - 1.6.2. Elaboration of an oral presentation

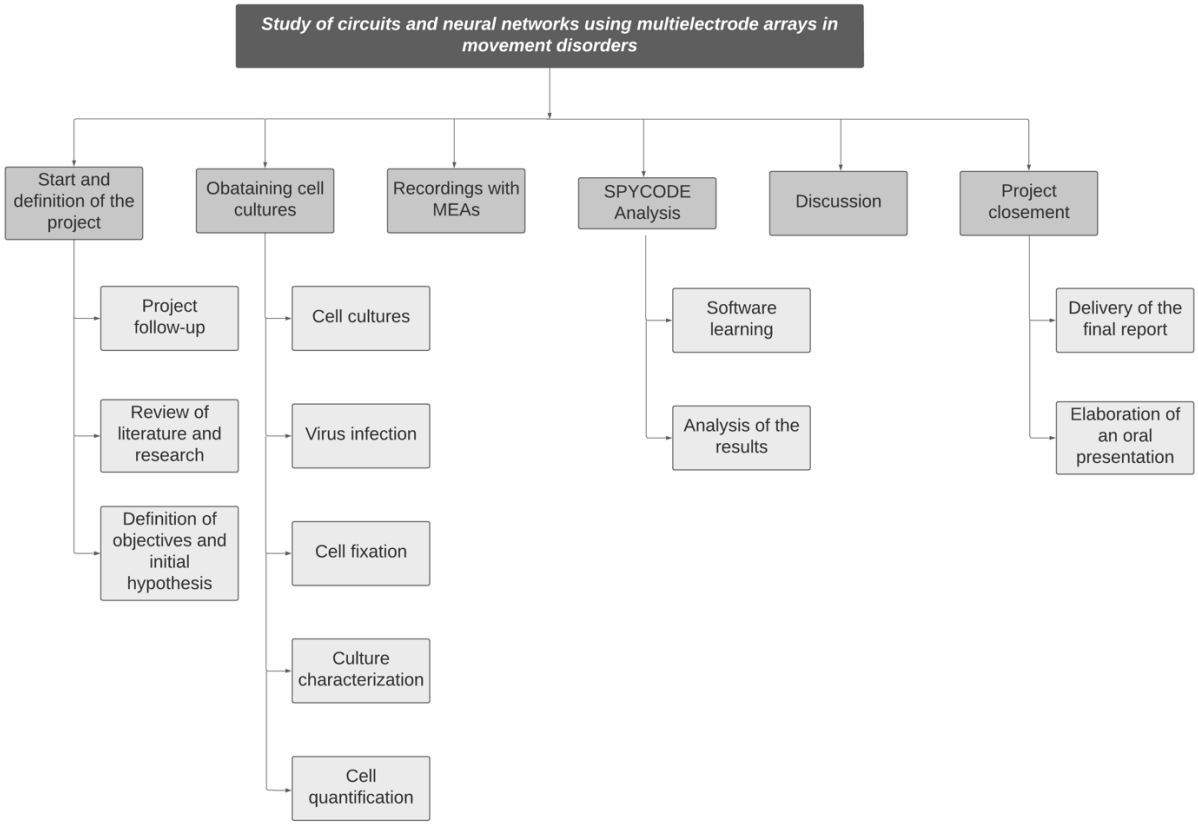

Figure 16. WBS of the project. [Guerras, Àngels. 2023]

Figure 16 depicts the WBS of this project. The first stage, corresponding to the *Study of circuits and neural networks using multielectrode arrays in movement disorders*, is divided into six different tasks: *Start and definition of the project, Acquisition of cell cultures, Recordings with MEAs, SPYCODE analysis, Discussion and Project closement,* and all of them include the related work packages.

At this point, the WBS dictionary has been expanded to provide precise details regarding each task, including the work package description and its associated control account, and pertinent deadlines.

Table 7. WBS dictionary for "Project follow-up"

WBS ID	Name	Control code
1.1.1.	*Project follow-up*	*1.1.*
Description	The activity consists of the monitoring of information collected over the course of the project in order to ensure its appropriate execution. The project's written report will show the project's ongoing control.	
Duration	23 weeks (all the course of the project)	

Table 8. WBS dictionary for "Review of the literature and research"

WBS ID	Name	Control code
1.1.2.	*Review of literature and research*	*1.1.*
Description	Conduct a thorough investigation into neurology, particularly neurodegenerative disorders related with movement dysfunction, in order to get in touch with each stage, with specific attention paid to the electronics underpinning the measuring instrument and software. Self-documentation process on current tools and trends to take advantage of free-use and reliable information.	
Duration	1 week	

Table 9. WBS dictionary for "Definition of objectives and initial hypothesis"

WBS ID	Name	Control code
1.1.2.	*Definition of objectives and initial hypothesis*	*1.1.*
Description	The formulation of a research question, together with a supporting hypothesis and objectives, is a critical stage in ensuring proper project execution while delivering relevant data for application in practice. Objectives that are well-defined and detailed are more likely to lead us in making decisions regarding research design and, thereafter, what data will be collected and evaluated. This procedure is carried out with the research team to assign particular responsibilities to the various members and arrive at unified conclusions.	
Duration	1 week	

Table 10. WBS dictionary for "Cell cultures"

WBS ID	Name	Control code
1.2.1.	*Cell cultures*	*1.2.*
Description	Cell culture to grow and maintain living cells outside of their natural environment with the appropriate medium supplementation. Cells are seeded both in MEAs and P24 plates, for additional investigation. Following the cell culture procedure is critical for generating precise and reproducible findings, guaranteeing cell quality, and maintaining a safe laboratory environment. It is important to consider that plate coating must be prepared with anteriority and the whole procedure must be carried out inside the laminar flow cabinet.	
Duration	5 weeks	

Table 11. WBS dictionary for "Viral infection"

WBS ID	Name	Control code
1.2.2.	*Viral infection*	*1.2.*
Description	We seek to target and reduce the expression of the VP13A gene by introducing the sh-VPS13A molecule into cultured cells. This phase will allow us to compare the effects of this downregulation on different cell processes when compared to a control group of cells. Again, as it implies the manipulation of biological material, this step must be conducted inside the laminar flow cabinet, under sterile conditions.	
Duration	5 weeks	

Table 12. WBS dictionary for "Cell fixation"

WBS ID	Name	Control code
1.2.3.	*Cell fixation*	*1.2.*
Description	Fixing cells in the P24 plates is an important step to maintain cellular morphology and the integrity of the cellular components for further examination. Fixation will allow us to describe the cell population in our cultures and, as a result, uncover relevant neuronal and glial cell mechanisms. Fixation is conducted at 15DiV after the cell culture and as indicated in the protocol, the process must be conducted under a safety cabin for cytotoxic products.	
Duration	5 weeks	

Table 13. WBS dictionary for "Culture characterization"

WBS ID	Name	Control code
1.2.4.	*Culture characterization*	*1.2.*
Description	Realization of an ICC technique to determine whether the cellular population of the cultures is homogeneous or heterogeneous with regard to particular markers for microglia, neurons, and astrocytes. It is important to follow the protocol for ICC, as well as the manufacturer guidelines for the posterior visualization in the microscope.	
Duration	2 weeks	

Table 14. WBS dictionary for "Cell quantification"

WBS ID	Name	Control code
1.2.5.	*Cell quantification*	*1.2.*
Description	It entails using ImageJ software to determine the number of neurons in a culture and assess possible differences between control and KD groups.	
Duration	1 week	

Table 15. WBS dictionary for "Recordings with MEAs"

WBS ID	Name	Control code
1.3.	*Recordings with MEAs*	*1.3.*
Description	The procedure entails collecting data on neuronal electrical activity under multiple conditions, including spontaneous activity and stimulation. It is extremely necessary to follow the guidelines provided by the manufacturer and make sure that the setup is correctly configured. Recordings must take place after 15DiV and using the software described by the manufacturer.	
Duration	4 weeks	

Table 16. WBS dictionary for "Software learning"

WBS ID	Name	Control code
1.4.1.	*Software learning*	*1.4.*
Description	Training with SPYCODE is essential before reviewing results to become acquainted with the software and its features. This process consists of comprehending and training with previously collected data from prior investigations.	
Duration	2 weeks	

Table 17. WBS dictionary for "Analysis of the results"

WBS ID	Name	Control code
1.4.2.	*Analysis of the results*	*1.4.*
Description	Using SPYCODE software, signal processing techniques such as spike and burst detection and network analysis are used to evaluate electrical activity patterns.	
Duration	2 weeks	

Table 18. WBS dictionary for "Discussion"

WBS ID	Name	Control code
1.5.	*Discussion*	*1.5.*
Description	The discussion of the results involves interpreting the outcomes obtained from the data analysis and discussing their implications for understanding neural function and behavior in the context of movement disorders. This process must be conducted as a team, with the Group of Neurochemistry.	
Duration	1 week	

Table 19. WBS dictionary for "Delivery of the final report"

WBS ID	Name	Control code
1.6.1.	*Delivery of the final report*	*1.6.*
Description	Document including features of project management, monitoring, and development, as well as their pertinent subsections.	
Duration	1 week	

Table 20. WBS dictionary for "Elaboration of an oral presentation"

WBS ID	Name	Control code
1.6.2.	*Elaboration of an oral presentation*	*1.6.*
Description	Planning and creation of a PowerPoint presentation that includes a project description. This work package also covers the writing of the presentation script.	
Duration	1 week	

6.2. PERT chart

The PERT chart, which stands for Program Evaluation and Review Technique, is a project management tool used to plan and organize project activities [50]. Next, we illustrate the PERT diagram based on the corresponding precedence matrix, which establishes the dependencies between different project tasks and respective estimated timings in weeks. Note that task and activity are referred to as synonym concepts.

The precedence matrix, shown in Table 21, assigns a number from 1 to 14 to each activity. The table indicates which activities must be completed before proceeding to the next, as well as the expected time necessary to finish them.

Table 21. Table of task precedencies

Activity	Name	Duration (wk)	Precedent
Start and definition of the project	1	0	-
Review of literature and research	2	1	1
Definition of objectives and initial hypothesis	3	1	2
Cell cultures	4	5	3
Viral infection	5	5	4
Cell fixation	6	5	5
Culture characterization	7	2	6
Cell quantification	8	1	7
Recordings with MEAs	9	4	5
Software learning	10	2	3
Analysis of the results	11	2	9, 10
Discussion	12	1	8, 11
Delivery of the final report	13	1	12
Elaboration of an oral presentation	14	1	13

Considering the table of presences and the estimated timings, the PERT chart for our project was created to provide a clear overview of the project's timeline. According to Figure 17, tasks are represented by lines relating two nodes. Thus, the nodes visually represent the flow and dependencies between activities, while indicating the early time, i.e. minimum time required to perform a task, and the last time, meaning the latest time that the activity can finish without causing a delay to the project. Finally, the critical path of our project corresponds to those tasks highlighted in red, while those in gray allow for temporary slack.

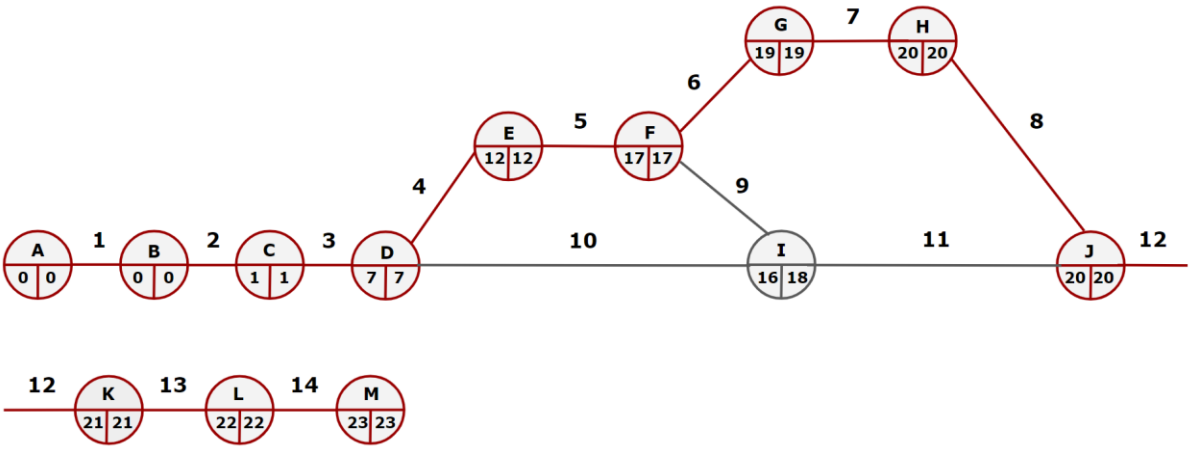

Figure 17. PERT-CPM diagram. [Guerras, Àngels. 2023]

6.3. GANTT diagram

Ultimately, the work execution schedule has been visually portrayed with a Gantt chart, which depicts the project plan over time, indicating the start and finish dates of each activity. This enables for continuous control of the tasks and facilitates the temporary scheduling and monitoring of the project. The X-axis represents time (in weeks) while the ordinates indicate the name of each activity.

The GANTT created for this project is shown in Figure 18. As it can be seen, the workflow is defined into 23 weeks of work, beginning with the definition of the project in mid-January, and closing with the delivery of the final report on June 6th and the presentation of the final results on June 15th. The tasks forming the critical path appear in red, while the activities that can accept delays are displayed in blue, with their slack times in gray.

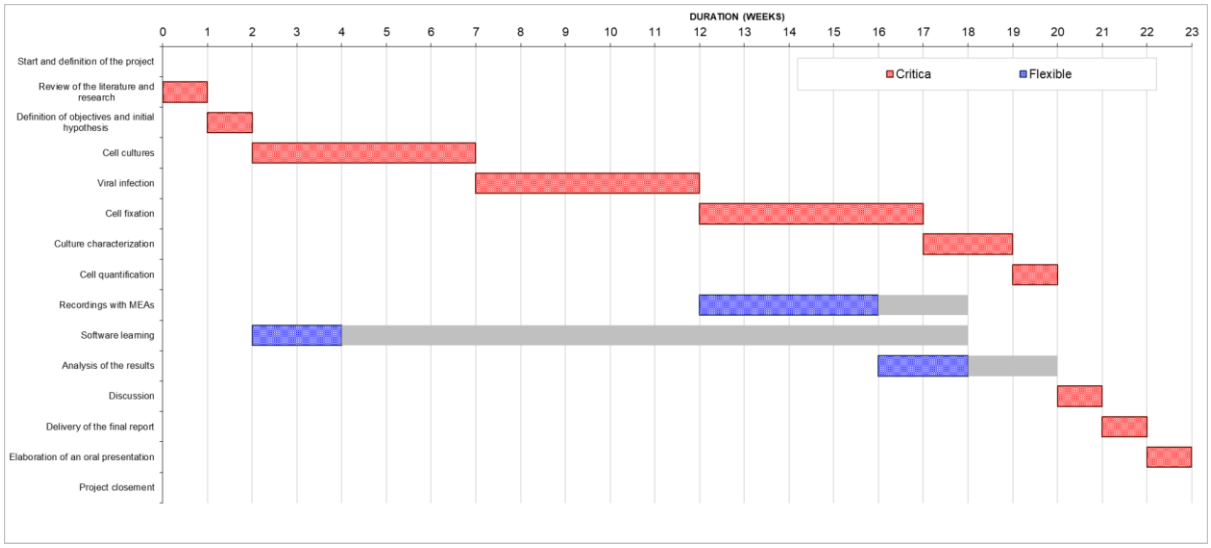

Figure 18. GANTT diagram. [Guerras, Àngels. 2023]

7. TECHNICAL VIABILITY

The following section of the report includes a technical feasibility evaluation of the whole project in order to effectively meet the objectives and requirements utilizing existing and available technology. Thus, managing complex projects poses unique issues that must be identified and handled in order to assure project feasibility and high-quality results.

The internal and external factors that may impact the outcomes of our study have been examined through the SWOT analysis in Table 22, by identifying the associated strengths, weaknesses, opportunities, and threats.

Table 22. SWOT analysis

Strengths	**Weaknesses**
- High skilled team with competences in neuroscience. - Possibility to study neural networks involved in movement disorders. - Well-established techniques for MEAs utilization. - Ability to learn and adapt.	- Limited competences to process big volumes of data. - Complex and technically challenging process that requires specialized equipment. - Additional sources of error, including signal noise. - Lack of a standardized protocol for cell culture in MEAs
Opportunities	**Threats**
- Market potential and growing interest for data analysis in neuroscience. - Possibility of providing insights into the disease's fundamental causes. - Posterior pharmaceutical research to develop new treatments. - Affordable to implement in laboratories	- Regulatory and ethical considerations involved in animal testing. - The results of the research project may not be conclusive. - Development of new technology. - Competitors working on similar directions. - Scarcity of cell cultures. - Limited literature about some diseases; for instance, ChAc.

The first row of Table 22 contemplates the project's internal strengths and weaknesses. One of the main valuable advantages is the team's extensive neuroscience knowledge. Having particular expertise in this area can lead to more efficient investigation and an improved understanding of the brain processes that trigger movement disorders. This internal strength is inherently linked to the fact that neuroscience continues to develop and adapt to emerging technology, requiring the team to be able to learn and benefit

from novel opportunities. Furthermore, because the usage of MEAs can be complicated, the protocols supplied by MCS assume a considerable strength to boost the quality and accuracy of the data.

Analyzing massive volumes of electrical signals generated by MEAs is regarded as an internal project weakness. Thus, advanced data processing and interpretation abilities are necessary to ensure relevant insights. Furthermore, working with MEAs involves complicated and technically demanding processes to establish the correct operating setup, and a lack of essential expertise or equipment may impair the quality and reliability of findings. Regarding this assumption, MEAs are particularly susceptible to vibrations from the environment (e.g. Metro L5) and mechanical disturbances (e.g. artifacts). Not having the appropriate setup for stabilizing and counteracting noise, may result in lower SNR and alteration of electrical patterns in neural networks. Despite our efforts to mitigate the influence of external vibrations by incorporating a layer of insulating materials (i.e. marble and foam) and a four-legged configuration, dealing with noisy data remains an ongoing challenge. Another internal weakness for the project's evolution is the lack of a clear and well-defined protocol for cell growing in MEAs in the lab. Researchers from the Group of Neurochemistry are working to establish the methodology that yields the best results in terms of cell density for optimal survival.

Similarly, opportunities and threats appear in the second row of the SWOT as external features of the project. Data analysis is gaining popularity in neuroscience as a means of extracting insights from neural activity in the context of movement and related diseases. Thus, electric activity and connections from brain networks can be uncovered in order to deeper comprehend the underlying cellular and molecular processes associated with the disease, and consequently, aid towards the development of novel effective therapies. Finally, because of the deployment of available equipment and technologies, the project is affordable and cost-effective for laboratory implementation, encouraging the adoption and integration of current approaches into academic institutions and laboratories.

Threats may hinder project development, thus they must be detected and addressed in advance with appropriate strategies. The regulatory and ethical constraints involved with animal experimentation imposed by legal bodies are a significant external limitation, demanding regulatory standards to be met for guaranteeing appropriate animal practice. It is convenient to consider scarcity of cell cultures due to delayed mouse pregnancy as an external limitation. The unavailability of an adequate number of cell cultures derived from female mice may result in insufficient data collection for the project, leading to successive delays in the timeline. Furthermore, due to the complexity of monitoring neural activity, there is an existent possibility of arriving at inconclusive conclusions, necessitating additional investigation and investment of time and money. Ultimately, the scarcity of existing research on rare movement disorders, such as ChAc, poses a substantial challenge that may impede the comparison and validation of our findings against existing scientific literature. However, competitors working on comparable research lines are viewed as an external threat since they could restrict the project's access to necessary support from funders and stakeholders.

8. ECONOMIC VIABILITY

In order to achieve the desired outcomes for the project, an evaluation of the economic aspects is performed, considering its potential to be feasible and sustainable within the described market [51].

At this point, we will go over the fundamental concepts behind the economic viability of bringing the project conception into practice, which requires an estimation of the costs [52]. The budget presented in Table 23 provides an accurate assessment of all cost items deemed necessary during the course of this work, divided into sections to guarantee a thorough and comprehensive financial framework. Note that all materials are alphabetically arranged to facilitate their search.

The budget for laboratory research may be separated into fixed and variable costs, which refer to expenses that do not change during the project lifetime and those that are proportional to the amount of work or resources employed, respectively [52]. On the one hand, fixed costs hold constant over the entire activity, such as infrastructure and equipment, administrative and research salaries or even license fees. Please note that, despite the expenses associated with building acquisition, as well as electricity and waste management are not explicitly accounted for in the budget, they are essential for project operation. Similarly, the equipment acquired previously for other research purposes (i.e. incubator, microscopes, etc.) has been considered based on the relative usage of their lifetime relevant to the project, so their cost is reduced. On the other hand, examples of variable costs for the project include laboratory reagents and instrumentation, as well as biological samples and one-use material. [53]. These reagents and laboratory materials were not purchased specifically for this project but for general laboratory functioning. As a consequence, the actual budget of this project was very hard to estimate. Nevertheless, taking into account all these considerations, an estimation of 3600,00€ appears as a reasonable approach to the cost of equipment use and expendable materials of the project.

The human resources allocated to the project, which is a part of the degree program, are ultimately included in the overall budget. This cost is based on the time spent on laboratory work, conducting literature research, and writing the final report, amounting a total of 520 hours. The theoretical salary for an undergraduate Biomedical Engineering student is set at 12€/hour [54], in such a way that human resources are estimated at 6.240 €. Furthermore, it is noted that investigators and PhD students have participated in the project with their expertise and knowledge. Thus, their time contributions are anticipated to be 25€ and 85€ per hour respectively, expanding the total cost for human resources.

Other investigators and laboratory personnel are acknowledged to have cooperated. Although their time efforts have not been quantified in terms of financial compensation, they are recognized and appreciated for the project execution and the overall outcomes.

Table 23. Budget of the project

Item	Company
Laboratory Reagents and biological samples	
AF555 goat anti-mouse IgG Ref: A21424	Thermo Fisher Scientific, USA
AF647 goat anti-rabbit IgG Ref: A-21245	Thermo Fisher Scientific, USA
B-27 Serum-Free Supplement	Thermo Fisher Scientific, USA
Bovine Serum Albumin (BSA) Ref. 17504044-10 ml	Sigma-Aldrich, USA
Fetal Bovine Serum (FBS) Ref. 11550356-500ml	Thermo Fisher Scientific, USA
Fluoromount-G^{R} Mounting Medium hermo Fisher Scientific, USA	bioNova cientifica s.l., Spain
GlutaMAX Media & Supplement Ref. 35050061	Thermo Fisher Scientific, USA
Hoechst Solution. Ref. H3570-10ml	Thermo Fisher Scientific, USA
Mouse Anti-NeuN (ref: MA8377) Ref. MAB377-500ug	Sigma-Aldrich, USA
Mouse monoclonal Anti-Glial Fibrillary Acidic Protein Ref: G3893-100ul	Sigma-Aldrich, USA
Neurobasal ^{TM} medium Ref. 10888022-500ml	Thermo Fisher Scientific, USA
Normal Goat Serum Blocking Solution. Ref. S-1000-20	Vector Laboratories Ltd., UK
Paraformaldehyde Solution 4% Ref. J61899.AK 250ml	Thermo Fisher Scientific, UK
Phosphate Buffered Saline PBS 10x solution. GIBCO 70011044-500ml	Fisher Scientific, USA
Pregnant mice	Animal House of the School of Medicine
Poly-D-Lysine hydrobromide. Ref: P0899-50 mg	Sigma-Aldrich, USA
Poly-L-Lysine. Ref: P-4832-50ml	Sigma-Aldrich, USA
Rabbit Anti Iba1. Ref: 019-19741	Wako Chemicals, USA

Saponin. Ref: A18820.22	Thermo Fisher Scientific, USA
Sodium-Azide 0.03% Solution. RTC 000068-1L 0.305%	Sigma-Aldrich, Madrid, Spain
TergAzyme Enzyme Active Powdered Detergent. Ref: 1325	Thermo Fisher Scientific, USA
Trypsin. Ref: R002100-100ML	Thermo Fisher Scientific, USA
Viral particles (VPS13A shRNA encapsulated)	Vector Builder, USA
Cost	***2.500,00 €***

Single-use Lab Material	
10 ul pipette tips. Ref: 06-360-2008	Nerbe Plus GmbH, Germany
20 ul pipette tips. Ref: 07-112-0000	Nerbe Plus GmbH, Germany
100 ul pipette tips. Ref: 07-122-0073	Nerbe Plus GmbH, Germany
200 ul pipette tips. Ref: 07-122-0073	Nerbe Plus GmbH, Germany
1000 ul pipette tips. Ref: 07-132-0095	Nerbe Plus GmbH, Germany
5 ml pipette tips. Ref: 1001850	Gilson, Spain
10 ml serological tips. Ref: 12-461-9108	Nerbe Plus GmbH, Germany
25 ml serological tips. Ref: 12-481-9102	Nerbe Plus GmbH, Germany
Cover glasses 12mm. Ref: KTVD10012Y1A01	Knittel Glasbearbeitungs GmbH, Germany
Eppendorfs. Ref: 04-212-3000	Nerbe Plus GmbH, Germany
Falcons 50 ml. Ref: 02-572-3001	Nerbe Plus GmbH, Germany
Falcons 15 ml. Ref: 02-502-3001	Nerbe Plus GmbH, Germany
Gloves. Ref 101605NM	Sudelab s.l., Spain
P24 plates. Ref: CCP-24H	TPP Techno Plastic Product, Switzerland

StarFrost. Ref: KTVS111711FKB01	Knittel Glasbearbeitungs GmbH, Germany
Cost	***500,00 €***
Lab Equipment	
60MEA200/30 iR-Ti	Multichannel Systems, Germany
Centrifugator Gyrozen 1248R	CONTROLTECNICA, Spain
Foam	Servei Estació, Spain
Incubator. Ref: 001277	Astec, Japan
Inverted fluorescence microscope	ZEISS, Germany
MEA 1060-BC-PA	Multichannel System, Germany
MACROMAN ™ Motorized Pipettor Controller, 0.1 - 100 ml.	Gilson, Spain
Neubauer Chamber	Marienfeld, Germany
Olympus CX21 Optical microscope	Olympus, Japan
Pipetman P2, 0.2 - 2 ul	Gilson, Spain
Pipetman P10, 1 - 10 ul	Gilson, Spain
Pipetman P20, 2 - 20 ul	Gilson, Spain
Pipetman P100, 10 - 100 ul	Gilson, Spain
Pipetman P200, 20 - 200 ul	Gilson, Spain
Pipetman P1000, 100 - 1000 ul	Gilson, Spain
STG 4004 4 Channel	Multichannel System, Germany
TC02 Controller	Multichannel System, Germany
USB-ME64-System	Multichannel System, Germany
Cost	***500,00 €***

Other materials	
MATLAB license	MathWorks Inc., USA
PC computer with Windows license	Windows Corporation, USA
Cost	***100,00 €***

Human Resources	
Undergraduate Biomedical Engineer: 520h of work	6.240
PhD investigator 50h of work	1.250
Director investigator 10h of work	850
Cost	***8.350,00 €***

TOTAL	***11.950,00 €***

9. LEGAL ASPECTS

In this section, we describe the set of regulations and protocols that must be rigorously followed to ensure the safety and validity of the results obtained from laboratory research.

On the one hand, laboratory activity is governed by a number of regulations, including the Directive 1/2003 on the general framework of research in Catalan universities, which supports the progress of knowledge via training, research, and technical innovation [55]; as well as law 14/2007, 3rd July, on Biomedical Research, that is a regulatory framework to ensure the optimal creation of novel biomedical challenges as well as the integrity of individuals participating in the investigative action [56].

Concerning the work carried out in laboratories from the Universitat de Barcelona, investigators must adhere to the instructions issued by the Office of Safety, Health and Environment (OSSMA) [57]. The purpose of the entity is to establish its own internal policies and guidelines, furnishing the University of Barcelona with a technical structure that controls and promotes the integration of occupational risk prevention and environmental protection at the UB. OSSMA rules are in compliance with the Spanish regulation, including the RD 656/2017 about chemical products storage and their complementary technical instructions of use in laboratories. [58] Projects involving research require a very comprehensive examination of their own methodologies, with a particular focus on their ethical consequences. As a result, the approval of the Universitat de Barcelona's Bioethical Committee (CBUB) is a legally mandated requirement before undertaking any research. Thus, the CBUB must work in a coordinated manner with those commissions and committees of the UB related to its functions, such as the Research Integrity Committee, the Animal Experimentation Ethics Committee, the Biosecurity, or the Data Protection Delegation, among others [59].

The law requires that any research involving animals must be authorized by the competent authority. In this context, all animal procedures were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the local animal care committee of the Universitat de Barcelona (226/17 and 225/17) and Generalitat de Catalunya (17/9837, 404/18), in accordance with the Spanish RD 53/2013 and the 2010/63/EU Directive of the European Commission.

It ought to note that the commercialization of MCS products requires compliance with a variety of requirements, including safety, performance, and environmental regulations, in order to ensure the safety and effectiveness of their products. MCS MEAs are in conformity with the following applicable European regulations and directives [60].

Table 24. Regulations applied to MCS products.

2014/35/EU	Low Voltage directive (LVD)
2014/30/EU	Electromagnetic Compatibility directive (EMC)
2012/19/EU	Waste Electrical and Electronic Equipment directive (WEEE)

2011/65/EU and 2015/863/EU	Restriction on the use of certain Hazardous Substances directive (RoHS)

Additionally, MCS electrical equipment, such as Stimulus Generators (STGs), complies with the relevant harmonized Union legislation, as evidenced by the implementation of the standards listed in Table 25.

Table 25. Regulations applied to MCS electrical equipment.

UNE-EN 61010-1:2011 [61]	Safety requirements for electrical equipment for measurement, control, and laboratory use; General Requirements.
UNE-EN 61326-1:2013 [62]	Electrical equipment for measurement, control, and laboratory use; EMC requirements

10. CONCLUSIONS

This project aimed to explore the electrical activity of neurons using MEAs and advanced processing algorithms, with a specific focus on the neurodegenerative disease of ChAc. Although all the initial objectives were not successfully met, such as establishing a standardized protocol for culturing primary neurons on MEAs, we made significant efforts to collect neuronal activity from primary cultures and recognize the intrinsic brain processes underlying moment-related diseases.

In the context of ChAc, we were able to describe neuronal cultures and evaluate the viability of cells with VPS13A depletion. As no significant differences in cell survival were identified between control and VPS13A KD neurons, this study indicates that the lack of VPS13A in neurons may be partially compensated by other proteins, which would contribute to explaining the late onset of the disease symptoms in ChAc patients. However, the lack of a well-defined procedure for cell culture in MEAs made recording and interpreting electrical signals from both control and VPS13A KD primary cultures a quite challenging process, which prevented us from fully exploring the specific neuronal activity patterns associated with ChAc. Hence, the implementation of a backup plan using recordings from ex-vivo brain slices allowed us to delve deeper into the capabilities offered by SPYCODE software.

We have acknowledged the multidisciplinary character of biomedical research in general. Teamwork between engineering approaches and neuroscience can result in novel solutions and methodologies that offer us a more thorough knowledge of brain complexity and the intricate mechanisms that underpin neurodegenerative disorders.

10.1. Future perspectives

Neuronal signal collecting becomes more important and necessary for further development in recognizing brain processes underlying movement disorders. Thus, this project laid the groundwork for future investigations that hold promise for novel advances in this context.

At this point, future work should focus on diving deeper into the network connectivity algorithms offered by SPYCODE, in such a way that we could refine our data analysis and assess intricate interactions within neural networks. Exploring these methods in depth would allow us to design longitudinal studies that examine changes in brain circuits over time associated with ChAc, facilitating the comprehension of the dynamics of neuronal activity to determine temporal patterns and identify biomarkers for novel treatment strategies. Additionally, the validation of our findings with existing research is essential to establish the reliability and significance of our results across the scientific community.

Future work could involve the integration of data from multiple modalities, i.e. genetic analysis, imaging techniques, electrophysiological recordings, and animal behavioral studies, to obtain a broader perspective of mechanisms driving neurodegenerative diseases to finally fill the gap between preclinical studies and clinical applications.

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APPENDICES

APPENDIX I. Primary cell culture protocol

1. Dissect cortex with the aid of a magnifying glass.
2. Pass the zones to a falcon and aspirate the solution.
3. Add 2 ml of trypsin and leave it in the bath at 37ºC for the minutes equivalent to the embryonic day (18.5).
4. Remove trypsin with p1000 and add 2 ml supplemental NB + 100 ul FBS. Move and invert smoothly.
5. Remove SN with p1000 and wash with 2 ml supplemental NB.
6. Remove SN with p1000 and wash with 1 ml supplemental NB.
7. Move up and down 20 times with p1000 with a cut tip.
8. Move up and down 10 times with p1000 without a cut tip.
9. Centrifuge 5 min at 750 rpm.
10. Remove SN and resuspend in 1 mL supplemental NB. Redo up and down 20 times with p1000 without cutting.
11. Count on the Neubauer chamber and sow in P24 plates and MEAs.

APPENDIX II. Cell fixation protocol

1. Perform 1 wash with 1x PBS at room temperature.
2. Incubate 15 min with 1 ml PFA 4%
3. Perform 3 washes with 1x PBS at room temperature.
4. Add 1 ml PBS + 0.02% Azide.
5. Wrap plates in parafilm and store in the fridge.

APPENDIX III. ICC protocol primary cultures protocol

1. Prepare and temper cell culture.
2. Wash with PBS 1x (10 µl/glass) and blot excess (x3)
3. Permeabilize with 1x PBS - 1% BSA - 0.1% Saponin for 10 min at RT
4. Wash with PBS 1x (10 µl/glass)
5. Block with 1x PBS - 15% NGS for 30 min at RT
6. Wash with PBS 1x (10 µl/glass)
7. Incubate with 10 µl O/N primary antibody at 4ºC. Dilute the primary in PBS 1x - 5% NGS
8. Wash with PBS 1x (10 µl/glass)
9. Incubate with 10 µl secondary antibody 1:100 for 1h at Rt. Dilute secondary in 1x PBS - 5% NGS
10. Wash with PBS 1x (10 µl/glass)
11. Mount with 10 µl Fluoromount-G
12. Let dry for at least 2 hours and store at 4ºC

APPENDIX IV. Protocol for NeuN quantification using ImageJ software.

1. **File → Open**

2. **Analyze → Set Scale**

Before using this command, use the straight-line tool to measure a known distance in a calibration image from the microscope at 40x magnification. The Set Scale dialog will open enabling adjustments to the measured distance and the unit of measurement, which must be set to millimeters (mm). Then, select the option "Global" and click OK.

3. **Process → Smooth**

Use this filter to remove noise.

4. **Process → Enhance contrast to 0.7% → Normalized**

With this step, we may achieve more control of the brightness and contrast adjustment. Normalized option helps to establish the same intensity threshold between images with different intensity levels.

5. **Image → Adjust → Threshold → Apply**

To analyze the image, a color binarization is applied, that is the B&W mode. This step involves setting a threshold (around 40) that indicates whether each pixel is considered black or white. Adjusting the threshold value is crucial to obtain optimal results in differentiating the foreground, which represents the stained nuclei, from the background, while ensuring that the particles of interest are not inadvertently excluded, in such a way that depending on how the threshold is adjusted, the particle size changes.

6. **Process → Binary → Convert to mask**

This option converts the image to an inverting black and white (particles are black while background appears in white).

7. **Process → Binary → Fill holes**

This command fills white spots found inside particles.

8. **Process → Binary → Watershed**

Watershed segmentation is a way of automatically separating or cutting apart particles that touch. When two particles are very close, they can be seen as an only one, but using this option the program interprets the object as two particles instead of one.

9. **Analyze → Analyze Particles**

Size is set at 0.00001 - Infinity mm^2, and circularity at 0.1 - 1. Select “display results” and “Summarize” with the parameters you are interested in.

10. **File → Save**

Save the results for posterior quantification with GraphPad.

APPENDIX V. Detailed results of NeuN statistical analysis using GraphPad Prism 9.5.1

Table Analyzed	**Per animal**
Column B	shVPS13A
vs.	vs,
Column A	shctrl
Unpaired t test	
P value	0,7044
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0,4076, df=4
How big is the difference?	
Mean of column A	14,10
Mean of column B	12,09
Difference between means (B - A) ± SEM	-2,007 ± 4,923
95% confidence interval	-15,67 to 11,66
R squared (eta squared)	0,03988
F test to compare variances	
F, DFn, Dfd	1,338, 2, 2
P value	0,8553
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	3
Sample size, column B	3

APPENDIX VI. Detailed results of MFR statistical analysis using GraphPad Prism 9.5.1

Table Analyzed	MFR
Column B	HD
vs.	vs,
Column A	WT
Unpaired t test	
P value	0,9571
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0,05547, df=8
How big is the difference?	
Mean of column A	24,90
Mean of column B	25,55
Difference between means (B - A) ± SEM	0,6532 ± 11,78
95% confidence interval	-26,50 to 27,81
R squared (eta squared)	0,0003844
F test to compare variances	
F, DFn, Dfd	1,355, 4, 4
P value	0,7753
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	5
Sample size, column B	5

APPENDIX VII. Detailed results of MBR statistical analysis using GraphPad Prism 9.5.1

Table Analyzed	MBR
Column B	HD
vs.	vs,
Column A	WT
Unpaired t test	
P value	0,7828
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0,2851, df=8
How big is the difference?	
Mean of column A	2,207
Mean of column B	2,048
Difference between means (B - A) ± SEM	-0,1587 ± 0,5565
95% confidence interval	-1,442 to 1,125
R squared (eta squared)	0,01006
F test to compare variances	
F, DFn, Dfd	1,451, 4, 4
P value	0,7272
P value summary	ns
Significantly different (P < 0.05)?	No
Data analyzed	
Sample size, column A	5
Sample size, column B	5

APPENDIX VIII. Results of the ISI analysis

The inter-spike interval (ISI) logarithmic histogram is similar for both the control and KD samples, suggesting that there is no significant difference in the distribution of time intervals between consecutive spikes.

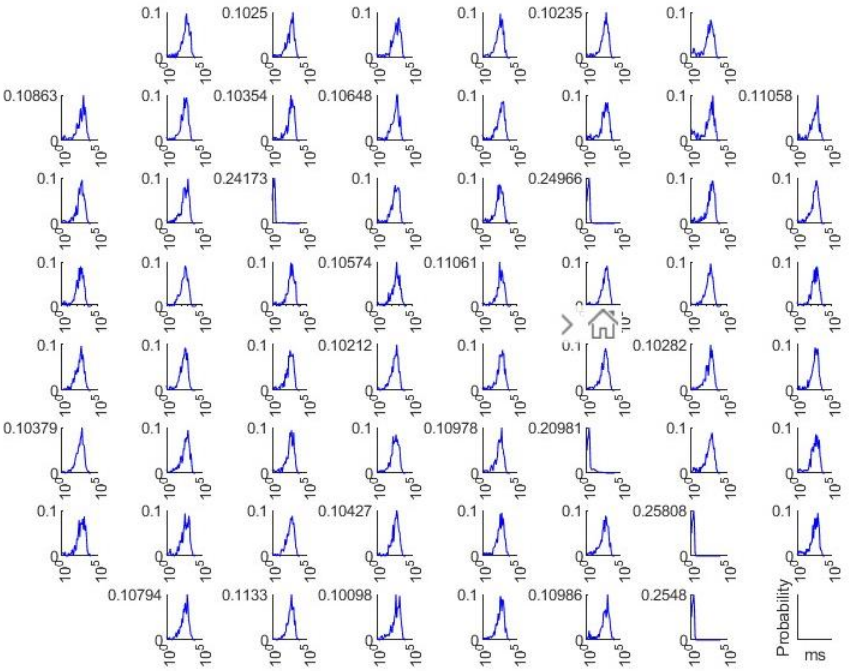

Figure 1. ISI histogram from one KO sample

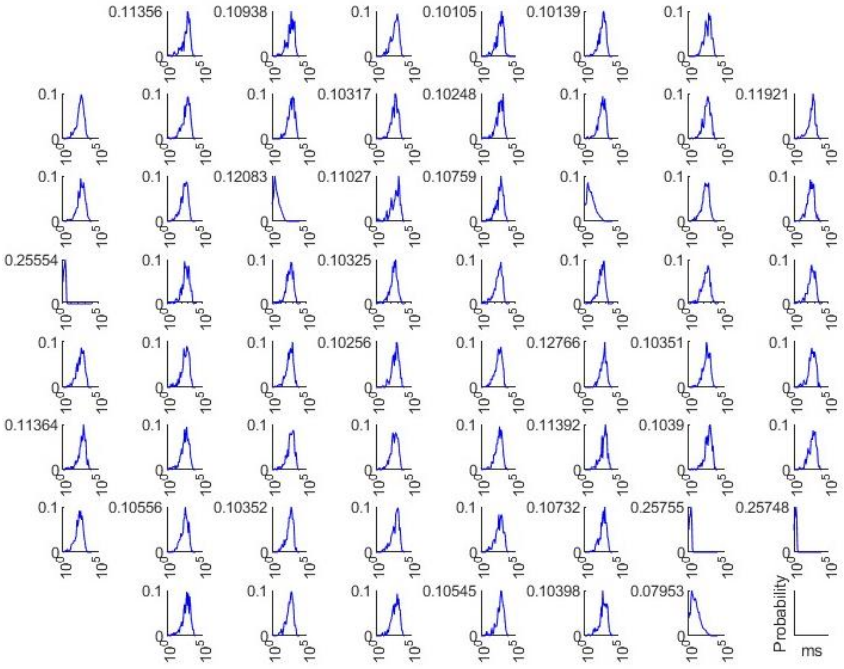

Figure 2. ISI histogram from one WT sample