

Brain Organoid Reservoir Computing For Tactile Processing

By

SIEKANSKA, KAROLINA

MSc Biorobotics Dissertation



Department of Engineering Mathematics

UNIVERSITY OF BRISTOL

&

Department of Engineering Design and Mathematics

UNIVERSITY OF THE WEST OF ENGLAND

A MSc dissertation submitted to the University of Bristol

and the University of the West of England in accordance

with the requirements of the degree of MASTER OF

SCIENCE IN ROBOTICS in the Faculty of Engineering.

September 5, 2024

Declaration of own work

I declare that the work in this MSc dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

Karolina Siekanska 05/09/2024

Acknowledgement

I would like to thank my supervisors, Dr.Ben Ward-Cherrier and Tianyi Liu for making this project possible and providing their advice and support throughout the project. I would also like to thank FinalSpark for allowing me to use their neuroplatform, which made this project possible.

Abstract

Brain organoids can act as biological computers, as their organoid neural networks (ONNs) can be trained similarly to artificial neural networks (ANNs) using electrical stimuli, at a fraction of the energy usage and cost. When integrated with hardware such as multi-electrode arrays (MEAs), organoids can perform machine learning computations, with existing work in the field indicating feasibility of organoids to classify auditory inputs. This project details the evaluation of a brain organoid from a pre-existing biological neuroplatform for classification of tactile information from the NeuroTac sensor. Stimulation parameters, sequences of stimulation and other aspects of computation were investigated, and organoid output in the form of neuronal spiking activity was recorded using the FinalSpark Neuroplatform and assessed for significant changes above baseline. Multiple impulses show a cumulative effect on spiking activity and optimal setting of parameters is established, no other clear trend in parameters is established. Stimulation impulses show consistent activity above baseline, although show a large deviation from the mean, likely due to synchronicity of oscillations in the organoid. Sequences of stimulation show increased spiking activity, with a somewhat distinct spatiotemporal spiking distribution. The results indicate promising computational ability of the organoid but many limitations are discovered. This project could be carried forward to assess the organoid's classification ability to tactile information.

Github repository for code: <https://github.com/EMATM0055-2023/ematm0055-2023-katattacks35>

This project fits within the scope of ethics pre-approval process, as reviewed by my supervisor Dr Ben Ward-Cherrier and approved by the faculty ethics committee as application 12723.

Number of words in the dissertation: 6982 words.

Contents

	Page
1 Introduction	6
2 Key contributions	8
3 Literature Review	9
3.1 Abbreviations	9
3.2 Brain Organoids	9
3.3 Organoid Intelligence	10
3.4 Encoding and Decoding Spiking Activity	15
3.5 Summary	18
4 Research Methodology	19
4.1 FinalSpark Platform	19
4.2 Experiments	21
5 Results	27
5.1 Parameter Optimisation	27
5.2 Channel-specific spiking distribution	32
5.3 Consistency of Response	33
5.4 Spatiotemporal Sequences of Stimulation	35
6 Discussion and Conclusion	39
6.1 Research Summary	39
6.2 Discussion	41
6.3 Limitations	42
6.4 Conclusion	43

6.5 Future Work	43
A Appendix	44
A.1 Stimulation parameter settings:	44
References	51

1 Introduction

1.0.1 Motivation

The human brain has a greater processing capability than available silicon-based computing, and can process several tasks at once utilising a fraction of the energy and time that artificial computing networks require.[1] Brain organoids are 3D cultures of human brain cells and can effectively mimic the function of biological neural networks (BNNs). Organoid neural networks (ONNs) can be utilised to perform computational tasks using brain-machine interface technologies and act as biological computers in an emerging field known as organoid intelligence (OI). Preliminary research in the field suggests human brain organoids are capable of unsupervised learning to recognise auditory inputs. [2] The demonstration of organoid learning on par with or superior to artificial neural networks (ANNs) could lead to a more energy efficient and effective way to process data in robotics and artificial intelligence (AI).

Human brains outperform machines in several ways, including being able to perform both sequential and parallel processing, perform more decisions at simultaneously, process incomplete datasets, and have a storage capacity of about 2,500 terabytes. [1] In addition, brains consume far less energy than machines, with the USA's Frontier, the world's most powerful supercomputer since June 2022, using 21 megawatts to perform at 1 exaFlop, while the brain only uses 20 watts to operate at 1 exaFlop.[1] Brain organoids exhibit similar biocomputing advantages to the human brain and this can be achieved by interfacing organoids with hardware such as computers and sensors. ONNs can be trained with complex sensory inputs for machine learning techniques such as supervised and unsupervised learning.

Organoid-machine interface technologies that allow for communication between electronic sensors and organoids are crucial to develop understanding of OI. Pre-existing technologies have demonstrated human brain organoids are capable of unsupervised learning to recognise auditory inputs. [2]. This project proposes the evaluation of a brain organoid for computational tasks,

specifically to demonstrate the ability of the brain organoid to reliably classify tactile data gathered from the NeuroTac sensor, training the organoid using electrical stimulation.

In this project, we take a sensor with known outputs and create a system to translate this data to a brain organoid. Benefits of this approach using a low-cost neuromorphic chip and achieving direct communication from sensor to organoid. Challenges include forming a uniform way to translate data to the organoid, and analysing organoid neural activity, which can often be noisy and hard to decipher.

A key element of this project will be characterising how aspects of input stimulation impulses can produce a consistent and significant response in the organoid, in the form of increased neuronal spiking, which can be difficult to determine due to the novelty of organoid computing and therefore unpredictability of responses.

1.0.2 Aims

1. "To evaluate the reliability and significance of organoid output for computational tasks".
2. "To measure the significance of the organoid's ability to classify tactile data from the Neuro-Tac."

1.0.3 Objectives

1. "To program and characterise electrical stimulation parameters on the FinalSpark neuroplatform"
2. "To characterise functional connectivity, optimal stimulation parameters and consistency of response to impulse."
3. "To characterise response to spatiotemporal sequences of stimulation."
4. "To translate NeuroTac spiking activity into stimulation sequences and analyse organoid response."
5. "To train organoid on spatiotemporal sequences of stimulation and test consistency in response."

2 Key contributions

All experiments were carried out on the FinalSpark Neuroplatform, which is an online-accessible platform that allows for access to tailored software which allows for the programming of electrical impulses to human brain organoids through an MEA. MEA output in the form of neuronal spiking activity is also visible. (Further information about the neuroplatform can be found in the Literature Review and Research Methodology). Growth and maintenance of organoids, the integrated hardware and software is intellectual property of FinalSpark. FinalSpark software contains inbuilt functions and protocols for stimulation.

Code for running experiments was run on a Python notebook on Datalore. Aid to help familiarise the platform was provided by co-supervisor Tianyi Liu. Regular supervisory meetings with both Tianyi Liu and Ben Ward Cherrier aided the organisation and flow of research progress.

All experiment planning, code execution, analysis, and written and visual interpretations of data was my responsibility, as well as all the written aspects of the report.

References to existing literature are made to provide context, justify hypotheses and aid scientific explanations.

3 Literature Review

3.1 Abbreviations

- AER = address-event representation
- ANN = artificial neural network
- AI = artificial intelligence
- BNN = biological neural network
- BNP = biological neuroplatform
- CMOS = complementary metal oxide semiconductor
- EEG = electroencephalography
- iPSC = induced pluripotent stem cell
- MEA = multi-electrode array
- OI = organoid intelligence
- ONN = organoid neural network

3.2 Brain Organoids

Organoids are small masses of tissue grown from stem cells, and these can mimic behaviour of organs, e.g. a brain organoid will form similar neural networks and mimic function of a human brain.[3] Organoids are commonly grown from human induced pluripotent stem cells (hiPSCs) which can differentiate into a variety range of cell types. Direction of stem cell growth is controlled by changing the microenvironmental conditions the cells are grown in (such as varying the nutrient

media, growth factors, oxygen levels, etc). iPSCs are adult somatic cells induced into a stem cell state by providing key growth factors that allow them to de-differentiate.[4]

Brain organoids, which can be known as neurospheres, are typically formed of cortical tissue. The neurospheres used in this project (provided by FinalSpark) consist of around 10,000 neurons each and contain a diversity of cell types including GABAergic, Glutamatergic and Cholinergic neurons, in addition to glial cells such as astrocytes and oligodendrocytes, cell types which are all needed for a functional neural network. [5] [6] They are grown from hiPSC-derived neural stem cells (NSCs) and form forebrain-like tissue around $500\text{ }\mu\text{m}$ in size, these are depicted in Figure 3.1 .[7]

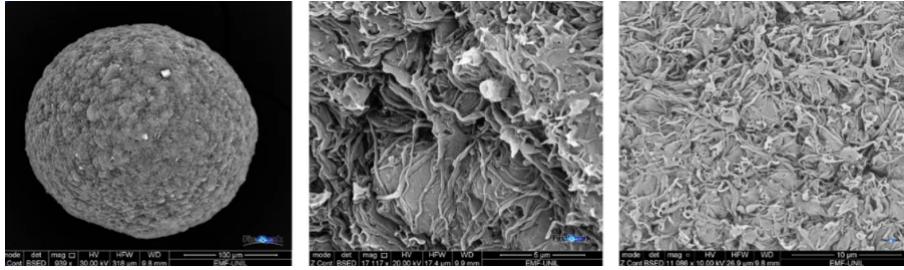


Figure 3.1: Electron micrograph of FinalSpark's neurosphere.[6]

3.3 Organoid Intelligence

Organoid intelligence (OI) is an emerging field which utilises 3D cultures of human brain cells and brain-machine interface technologies to form biological computers. These hybrid bio-electronic interfaces can advance our understanding of the physiology of cognition, learning, memory, neurological diseases. Current directions for OI field include scaling up current organoids into more complex and long-lasting structures enriched in cells and genes associated with learning, integrating brain organoids with next generation machine learning systems and to develop new models, algorithms and interface technologies to communicate with organoids to increase understanding of their computational potential, and to use them to process and store data.[8]

3.3.1 ONNs

Organoid neural networks present similar advantages of using human brains, as these mimic brain neural architectures and display functional connectivity. Neurons grown in brain organoids exhibit similar *in vivo* characteristics of human neurons, such as having a resting membrane potential, firing patterns and plasticity in some specific subtypes of neurons.[8]

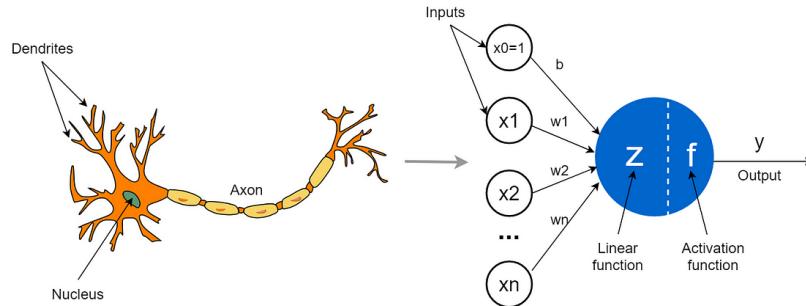


Figure 3.2: Comparison of biological to artificial neuron model.[9]

Artificial neural networks (ANNs) have predominantly been used for machine learning algorithms for training on datasets, such as classification of images for computer vision. OI proposes the training of actual biological neural networks in the same manner, by connecting brain organoids to a multi-electrode arrays (MEA) which have the ability to both stimulate the neurons and record their electrophysiological activity.

3.3.2 Reservoir Computing

Reservoir Computing (RC) is a framework derived from recurrent neural networks in which input signals are mapped to a higher dimensional computing space known as a kernel property through a reservoir structure. The reservoir structure is composed of singular units with are randomly connected to one another, depicted in Figure 3.3 and these connections remain constant throughout computation.

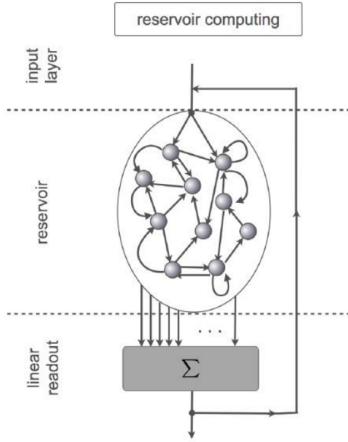


Figure 3.3: Reservoir Computing Framework.[10]

A readout function, which is a neural network layer able to transform the inputs using weights, is used to monitor the state of the reservoir and train it to the desired output. This function can be trained by examining the reservoir response to known inputs. [10]

3.3.3 Brain Organoid Reservoir Computing for Artificial Intelligence

A technique called Brainoware has been developed which utilises neuromorphic chips to train the brain organoid neural networks (ONNs) via unsupervised learning, utilising neuroplasticity to re-structure its functional connectivity.

Brainoware consists of a mature human brain organoid placed on a high density MEA which allows for the transmission and reception of electrical signals.[2]

Mature ONNs receive external electrical stimulation inputs and send outputs in the form of neuronal spiking activity, adjacent to action potentials in the neurons. These organoids act as adaptive living reservoirs and perform unsupervised learning to classify the inputs, which are perceived as spatiotemporal sequences of stimulation. [2]

Outputs from the ONNs can be processed by a readout function in the hardware which acts to map feature values or inputs to desired labels of data. The Brainoware system can further improve its performance by training stimulation inputs using the organoid's natural capability for synaptic plasticity. [2]

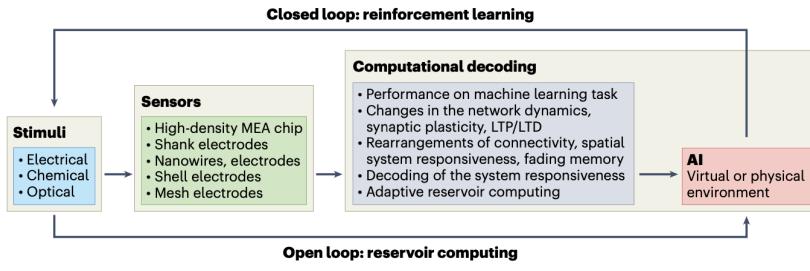


Figure 3.4: Reservoir computing and reinforcement learning model using organoids.[11]

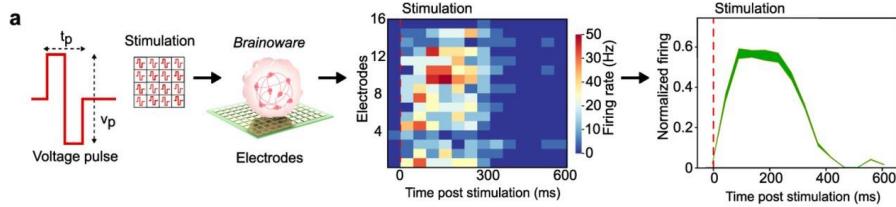


Figure 3.5: Foundations of Brainoware platform.[2]

Inputs of electrical stimulation were categorised by their pulse width (t_p) and magnitude of voltage (v_p). It was demonstrated that pulses of a longer duration and greater voltage induced a stronger spiking response and slower relaxation dynamics. This is depicted in Figure 3.6b. Factors such as non-linear response and fading memory (or short-term memory) were able to be controlled by regulating stimulation parameters. Brainoware evoked a non-linear response to pulse voltage which mirrored the response of a typical reservoir computing hardware such as a memristor. [2]

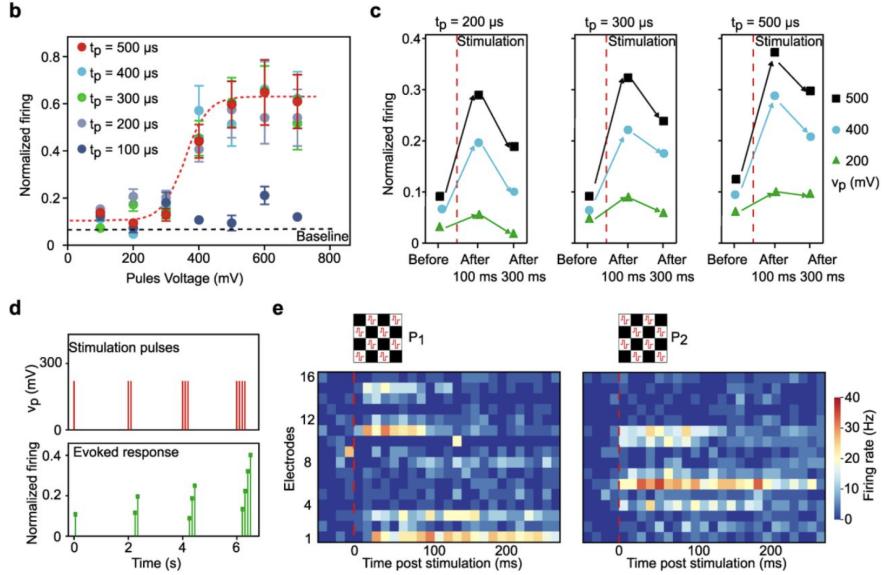


Figure 3.6: Analysis of stimulation effect on organoid output using Brainoware.[2]

Multiple individual trains of impulses at short intervals of 50ms showed accumulation of organoid spiking, and delay of relaxation. This also mimicked the response of a memristor. [2] Two different spatial patterns of stimulation, depicting as P1 and P2 in Figure 3.6e., evoked distinct distributions of activity and indicated spatial information processing.

The Brainoware platform was also tested on a 2D Hénon map, a typical non-linear dynamic system with chaotic behaviour. This 2D map was converted into 1D heatmap, which was used to determine the spatiotemporal sequences of bipolar voltage pulses of input to the organoid. A regression score of 0.356 on day 1 increased to 0.812 after four training epochs. Comparison with an ANN solving the Hénon map demonstrated that Brainoware significantly outperformed the ANN. An ANN with a long short-term memory (LSTM) unit did slightly outperform Brainoware, however it had been trained for 50 epochs, while Brainoware had trained for 4 epochs. This demonstrated Brainoware could decrease training times by greater than 90% [2]

Organoids treated with calmodulin-dependent protein kinase II (CAMKII) inhibitor, which blocks activity-dependent synaptic plasticity, only improved their regression score from 0.31 on day 1 to 0.385 after 4 training epochs. This demonstrated that learning activity of Brainoware was dependent on neural synaptic plasticity. [2]

3.4 Encoding and Decoding Spiking Activity

3.4.1 Neuromorphic Hardware

Neuromorphic models replicate the behaviour of the human brain and nervous system, which can aid their computing processivity and energy efficiency. This has been applied to many areas, such as computing with artificial neural networks, and robotics and prosthetics, where neuromorphic sensors improve tactile sensing and mimic human touch.

Neuro-inspired hardware typically utilises oxide-based memristors, which are non-linear two terminal electrical components that link electric charge with magnetic flux,[12] spintronic memories (utilising the intrinsic spin and magnetic moment of electrons), threshold switches and transistors.[13]

Examples of neuromorphic hardware include Georgia Tech's programmable neural array which uses a field-effect transistor to change electrical conductivity by altering the amount of voltage applied. This array can be used to amplify or switch electronic signals and effectively mimics channel-ion characteristics of neurons in the brain. [14]

Massachusetts Institute of Technology (MIT) researchers in 2011 created a computer chip which mimics neuronal synapse communication using 400 transistors and complementary metal oxide semiconductor (CMOS) manufacturing techniques.[15]

NeuroTac

An example of a neuromorphic sensor includes the NeuroTac, which utilises an event-based camera to process dynamic changes in brightness, and converts these changes to events in the address-event representation (AER) format. These events are analogues to biological spike trains. The sensor features a compliant dome-shaped outer membrane inspired by human fingertip structures, which is filled with silicone gel and covered with an acrylic lens. Inside the membrane, white-tipped pins displace upon contact and an LED ring illuminates the internal surface to capture this movement.[16]

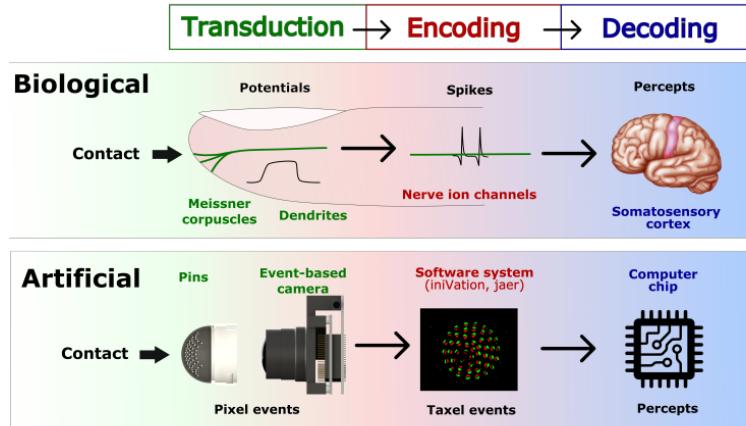


Figure 3.7: Comparision of biological tactile system compared to artificial NeuroTac system.[16]

The NeuroTac sensor processes pixel events produced by the camera, initially filtering them, then pooling them into a single taxel event. This taxel event, which represents a collective response from a group of pixels, is akin to a spike from an artificial mechanoreceptor. The process is dynamic, with the sensor updating the positions of these taxel events to reflect the ongoing tactile interactions. [16]

The sensor can identify both artificial and natural textures using a K-nearest neighbour (KNN) algorithm. Temporal methods showed the highest accuracy in classification tasks. [16]

3.4.2 FinalSpark's Biological Neuroplatform BNP

FinalSpark's BNP is formed of neurospheres integrated with MEAs, which are used to send electrical signals to the organoid via a digital-to-analog converter (DAC) and receive signals via an analog-to-digital converter (ADC). Each electrode is only connected to a few neurons, so the platform is used to trigger functional changes in neuronal behaviour as opposed to studying the activity of every single neuron in the organoid. [5]

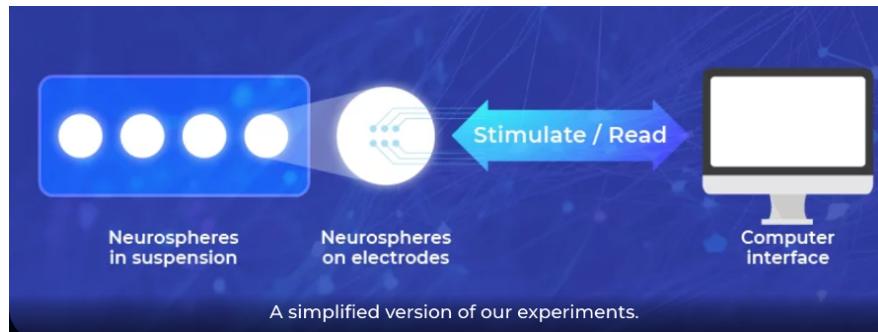


Figure 3.8: Basis of FinalSpark's BNP. [17]

The platform's benefits include reliable neurosphere production and preservation, reliable microfluidics to conduct measurements and micro-environmental conditions are maintained. These include parameters such as optimal temperature, carbon dioxide, oxygen, nitrogen, humidity, pH, gas bubbles, medium flows, and medium levels. BNP is integrated with Python scripting to enable seamless reading of neuron signals and neuron stimulations.[17]

BNP collects large amounts of neurophysiological in vitro data, performed under controlled and repeatable conditions and FinalSpark's provides a recorded 24/7 livestream of this data.[17]

Process of collecting data on the BNP:

1. Neurosphere is placed on the electrodes of an MEA.
2. Neurosphere is stimulated using a computer system that reads electrophysiological signals.
3. Environmental conditions of the neurosphere such as microfluidics, temperature and carbon dioxide are maintained, keeping the neurosphere alive for several weeks or months.

Experiments conducted show neurospheres can live up to 120 days in these conditions. The neurospheres respond very quickly to each stimulation (less than 20 milliseconds) and responses are very reliable for 90% of stimulations. [17]

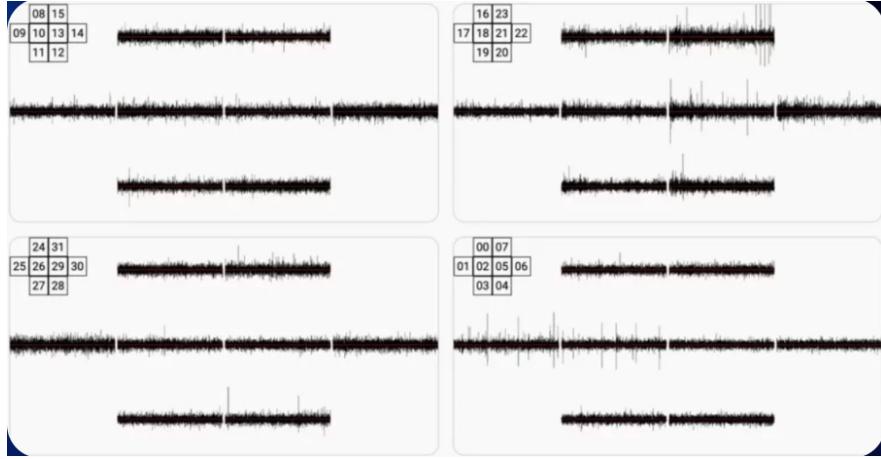


Figure 3.9: Multielectrode array (MEA) output of neuronal activity on FinalSpark’s BNP.[17]

3.5 Summary

ONNs show promising computational potential, mimicking or even surpassing accuracy in classification tasks as demonstrated in the Brainoware study. Key trends are identified in the stimulation parameters and patterns, such as increasing pulse voltage and duration evoking stronger spiking responses in the organoid. Spatial patterns of stimulation were also able to elicit a distinct response. These trends show feasible implementation of computational ability of the organoid, which should be able to be replicated on a different organoid, such as organoids on the FinalSpark Neuroplatform.

Neuromorphic hardware, such the NeuroTac sensor, mimics biological matter from its hardware design to internal processing. The NeuroTac processes external stimuli in a similar way to neurons, by pooling individual pixel events into a single taxel event not unlike action potentials pooling into a spike train. This neuromorphic design provides a good basis for translating information to an organoid, as a taxel event or sequence of taxel events could be translated into a sequence of electrical stimulations. Classification of tactile information by organoids would provide a novel approach to develop understanding of and assess their computational ability.

4 Research Methodology

4.1 FinalSpark Platform

All experiments will be carried out on FinalSpark's Biological Neuroplatform, which has an integrated software used to trigger electrical impulses to stimulate organoid activity on MEAs.

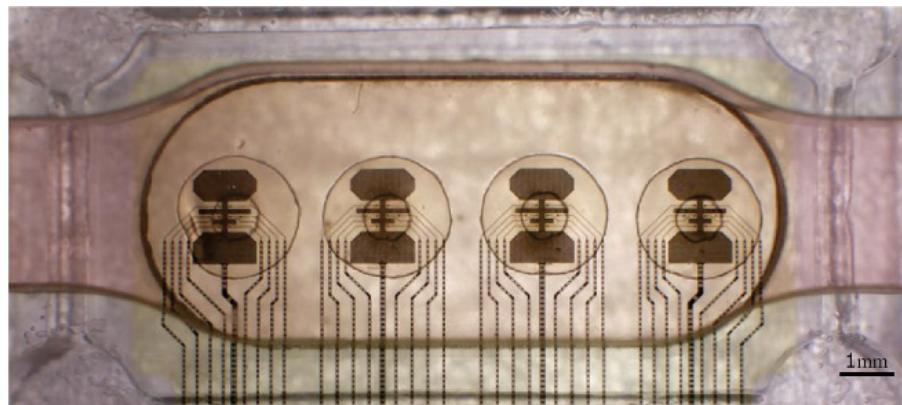


Figure 4.1: FinalSpark's hardware, depicting four organoids in one MEA.[18]

Each MEA has 32 electrodes/channels, each 8 connected to one organoid, totalling 4 organoids in one MEA. In this project, all of the experimentation will be carried out using one MEA, and most focusing on the activity of one organoid.

FinalSpark's python software as shown in 4.2 generates programmable electrical impulses via the trigger generator, which elicits spiking activity in the organoids that is subsequently recorded on the software.

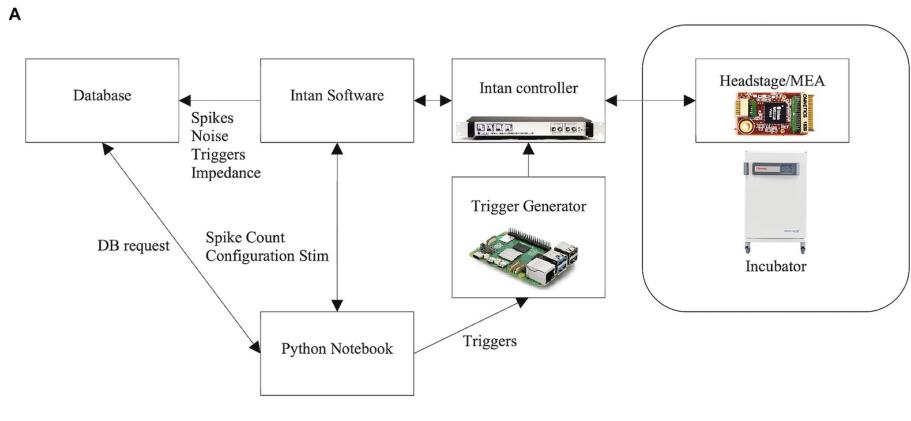


Figure 4.2: FinalSpark’s software architecture.[18]

The neuroplatform records stimulation trigger times and electrical activity of neurons from the organoid (at a sampling rate of 30kHz) 24/7 using a time-series database called InfluxDB. To minimise stored data, significant events such as threshold crossings which are likely due to action potentials (spikes) are recorded. [18] The MEA is monitored every $30\mu s$ to determine if a threshold T is crossed, determined by Equation 4.1.

$$T = 6 * Mdn(\sigma_i) \quad (4.1)$$

Where σ_i is the standard deviation over a set of i voltage values and $Mdn()$ represents the median function computed over 101 consecutive i values. The median function acts as a filter and reduces sensitivity to outliers and anomalies. A multiplier of 6 achieves reliable spike detection. [18]

4.2 Experiments

4.2.1 Protocol

The general experimental protocol for following will consist of programming stimulation parameters, running the stimulation code and retrieving spike data. Pseudocode for the three elements of experiment protocol can be found in the Appendix, as well as attributes of the stimulation impulses that can be controlled. This will primarily consist of retrieving number of spike events over a few hundred milliseconds. This allows for measurement of immediate response to the stimulation impulse and this is the time period across which direct results are expected based on previous organoid computing approaches.

The number of trials for each and every stimulation experiment is 10 unless stated otherwise. This allows for appropriate assessment of significance.

The organoid in this project is connected to 8 electrodes (also referred to as channels) which are depicted as channels 56-63, which will be referenced throughout the project.

Experiments were performed predominantly on channel 61 as this channel was determined to have the most increase in activity above baseline.

All hypotheses are predicted based on existing work in the field, predominantly the findings from the Brainoware study. [2]

4.2.2 Parameter Optimisation

The Intan Software allows for the optimisation of parameters including amplitude and duration of each phase of a stimulation signal. In this project, bi-phasic impulses with positive-first current charge will be used.

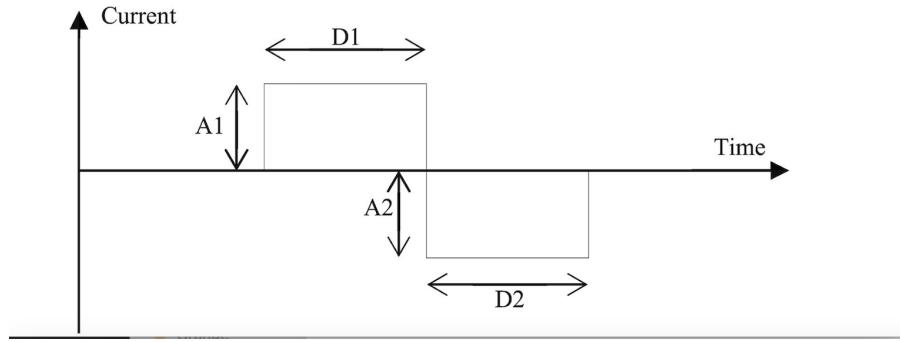


Figure 4.3: FinalSpark's stimulation parameter settings.[18]

The parameter optimisation phase will entail stimulating one organoid with varying parameters including pulse amplitude, pulse duration, and exploration of effect of number of impulses and interval between them. The spiking activity of the organoid, particularly the 'spike events' will be recorded 300ms after each impulse. This is where the most activity is expected to occur directly following the impulse based on the Brainoware study[2]. Spike events record the maximum value of voltage during each 3ms spike recording.

The following will be tested:

1. Total pulse amplitudes (A_1+A_2 in figure 4.3) of in the range of $2\mu\text{A}$ - $20\mu\text{A}$.
2. Total pulse durations (D_1+D_2 in figure 4.3) in the range of $200\mu\text{s}$ - $1200\mu\text{s}$.
3. Number of stimulation impulses in the range 1-10.
4. Interval duration between 10 impulses in the range of 0.01s - 1s .

The methods for running the experiments are found in the Appendix, with parts 1 and 2 described by Algorithm 1 and parts 3 and 4 described by Algorithm 2. Retrieval of spike data is described by Algorithm 3 in the Appendix.

The goal of this phase is to identify trends in the data and to determine which parameter settings provide the highest spike output in the organoid, which can then be used in sequences of stimulation to provide a significant response in the organoid.

Hypothesis:

Average spiking activity will increase with increasing pulse amplitude. Longer pulse durations will also increase spiking activity until a point where they plateau or decrease the activity. The greater the number of impulses, the greater the number of spike events with a cumulative effect between impulses. Shorter interval durations between multiple impulses should increase spiking activity.

4.2.3 Channel-specific spiking distribution

Stimulation of different channels within the organoid should produce different outputs of spatiotemporal activity. Using the optimised parameter settings, different electrode channels should elicit a different pattern of activity.

In this phase, each channel will be tested with the optimised stimulation settings and its output for difference in activity, also to explore any potential functional connectivity between the channels.

Method:

1. Set stimulation parameters to optimal settings from previous experiment.
2. Set target electrode to channel 56.
3. Run 10 trials, with 20 seconds in between each set of impulses.
4. Record start and stop times of the experiment.
5. Use the stop time as a guideline to determine trigger information of the final impulse in the set of impulses.
6. Determine and record spike events 100ms before and 300ms after the final impulse.
7. Repeat steps 1-6 for channels 57-63.

Hypothesis:

Stimulation of each different channel in the organoid will show a distinct distribution of spatiotemporal spiking activity, with most activity occurring 100ms post impulse.

4.2.4 Consistency of Response

The optimised parameter will be tested continually over a period of time and the spike events will be recorded 300ms post each set of impulses.

The purpose of this experiment is to determine how consistent the spiking response remains over time during constant stimulation, which is important as this is effectively the input signal used for computation processes in our organoid 'biocomputer'. The ability to send input signals which elicit the expected spiking response each time allows for the biocomputation to be repeatable and reliable.

The parameters will be tested for over a 20 minute period and spike events recorded.

Method:

1. Set stimulation parameters to optimal settings from previous experiment.
2. Set target electrode to channel 61.
3. Stimulate 10 times in one trial.
4. Record start and stop times of each trial.
5. Repeatedly run trials with 20 seconds between each trial and stop at 20 minutes of stimulation.
6. Use the stop times of each trial as a guideline to determine trigger information of the final impulse in the set of impulses.
7. Determine and record spike events 300ms after the final impulse.
8. Repeat steps 6 and 7 for each trial in the experiment.

Hypothesis:

Immediate response to stimulation may wane over the course over the 30 minute period of constant stimulation, but will still show a significant increase in spiking activity above baseline.

4.2.5 Spatiotemporal Sequences of Stimulation

In this phase, two different patterns of stimulation will be tested in one organoid.

In the initial phase, each pattern will consist of three different channels that are stimulated at the same time with the optimised parameters and the spatiotemporal spiking activity will be analysed, depicting 100ms before and 300ms after each set of impulses.

The effect of stimulating different channels at different times will then be tested. The incorporation of data from the NeuroTac shall be translated to depict the activity.

Pattern 1 consists of channels 56, 59 and 61. Pattern 2 consists of channels 58, 60 and 62.

Method:

1. Set stimulation parameters to optimal settings from previous experiment.
2. Set target electrodes to 56, 59 and 61.
3. Run stimulation using the optimal parameters.
4. Record start and stop times.
5. Repeat steps 3 and 4, 10 times.
6. Use the stop time of each trial as a guideline to determine trigger information of the final impulse in the set of impulses.
7. Determine and record spike events 100ms before and 300ms after the final impulse.
8. Repeat steps 6 and 7 for each trial in the experiment.
9. Repeat steps 1-8, setting the target electrodes to 58, 59 and 62.

Hypothesis:

Most spiking activity will be seen 100ms post impulse. Each pattern of stimulation will show a distinct distribution in spiking activity. Stimulation of three channels simultaneously will show increased spiking activity overall when compared to stimulation of only one channel.

4.2.6 Metrics

For each experiment, metrics used include calculations of a mean average, range and standard deviation.

Standard deviation and range are used to measure accuracy of the spiking response. Average spike events within one standard deviation show the difference in readings that lie within 60% of the mean or 'true' value. The lower the standard deviation, the more accurate the results are to this true value. The range is used to determine the boundaries within which the spiking response appears, the smaller this value the more concise these values are and the reliable and predictable the organoid's output is. Some limitations of using the mean and range is that outliers or anomalies can drastically change this value, and limit or misrepresent trends in the data. Standard deviation may also be affected by outliers and assumes the data is normally distributed.

5 Results

5.1 Parameter Optimisation

5.1.1 Pulse amplitude and pulse duration

Pulse amplitudes of $2\mu\text{A}$ - $20\mu\text{A}$ and pulse durations of $200\mu\text{s}$ - $1200\mu\text{s}$ were tested to find an optimum set of values which produce the most spiking activity above baseline.

Figure 5.1 suggests that lower pulse durations generally shower greater spiking activity immediately post impulse, showing decline past $600\mu\text{s}$. Lower amplitudes surprisingly showed greater levels of activity, but also with a greater margin of error, particularly at $3\mu\text{A}$. Greater amplitudes shower fairly low activity, and almost all parameters consistently performed below baseline. The results indicate that the optimal parameter is $10\mu\text{A}$ $600\mu\text{s}$, wherein the most average spiking activity is seen.

The low spiking activity at high amplitudes could be explained by declining of organoid activity or differing organoid activity during different hour or time of day. Optimal spiking at $10\mu\text{A}$ $600\mu\text{s}$ could be due to phase-matching of neuronal oscillatory activity at this frequency.

These results are surprising as they contradict the hypothesis of greater amplitudes increasing spiking, which is only true up until $10\mu\text{A}$. This could be due to too much stimulation being blocked or downregulated by the neuron cells. The pulse duration seems to show a trend of lower durations producing most activity, which is contradictory to the hypothesis.

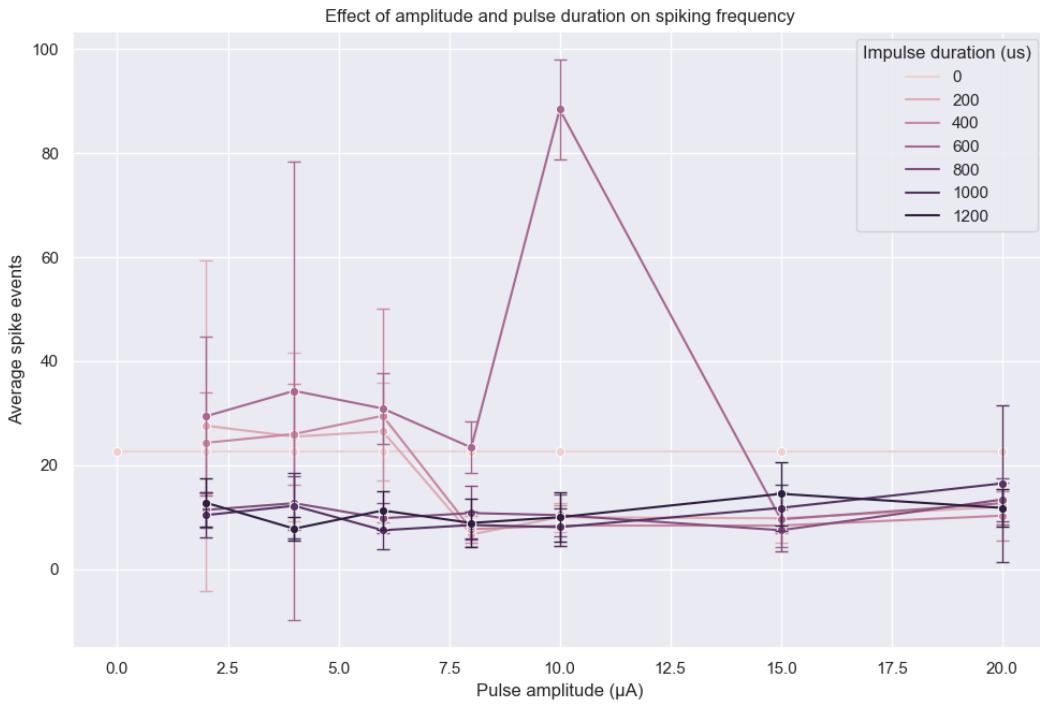


Figure 5.1: Effect of pulse amplitude and pulse duration on spiking frequency 300ms post stimulation. Pulse amplitude represents the total amplitude of the biphasic impulse (A_1+A_2) and pulse duration represents total pulse duration (T_1+T_2). Datapoints are mean averages (trials, $n=10$), with error bars indicating standard deviation. Baseline activity is indicated by horizontal line at impulse duration 0.

Overall, the parameters show little correlation or trends, perhaps due to random activity in the organoid. However, the clear optimal parameter is at $10\mu\text{A}$ $600\mu\text{s}$.

5.1.2 Impulse frequency and interval duration

Multiple stimulation impulses were tested in the range 1-10 and interval duration between 10 impulses in the range of 0.01s - 1s were tested to determine how this affected mean spike events post impulse.

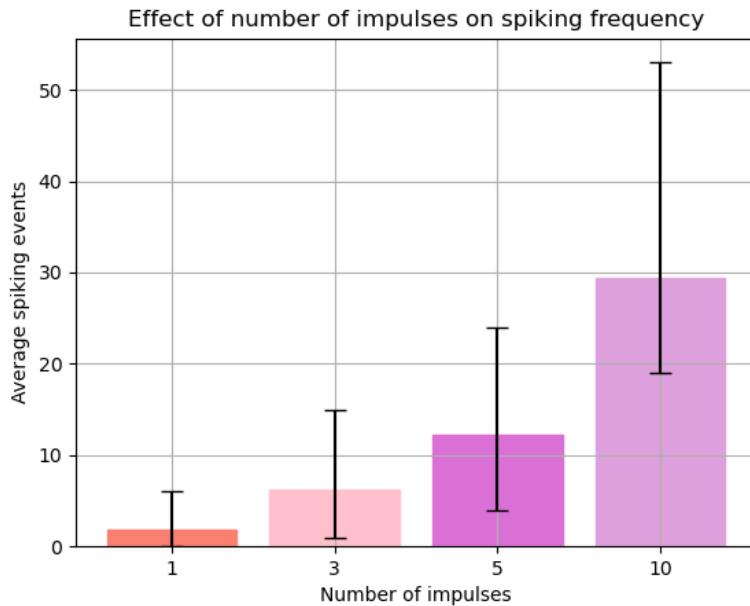


Figure 5.2: Effect of differing number of impulses on spiking frequency 300ms post stimulation. Datapoints are mean averages (trials, n=5), with error bars indicating standard deviation.

Figure 5.2 shows a strong correlation between number of stimulation impulses and average spiking activity in the organoid, with increasing impulses showing increasing spiking. The greater the number of impulses however also showed greater range and deviation from the mean, although generally the activity and average increased significantly.

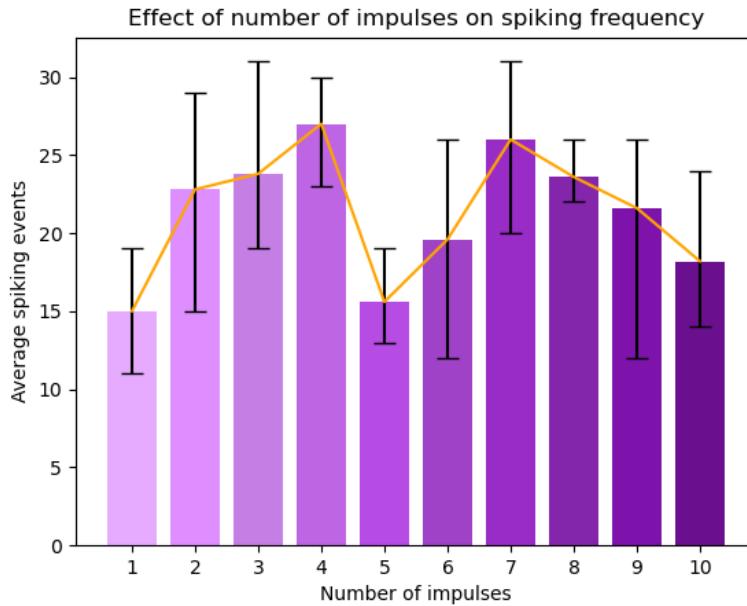


Figure 5.3: Effect of each impulse in a set of 10 impulses on spiking frequency 300ms post stimulation. Impulses settings were $10\mu\text{A}$ $600\mu\text{s}$. Datapoints are mean averages (trials, $n=10$), with error bars indicating standard deviation.

Figure 5.3 depicts the cumulative effect of increasing impulses in a set of 10 impulses. The first four impulses on average show a trend of increasing activity with a large decline at impulses 5 and 6. This suggests that there is a limit on the number of impulses that can produce a cumulative effect on spiking. An explanation for this could be that the neurons in the organoid may reach peak excitation and may have a forced relaxation / downregulation of excitation to not overstimulate the cells.

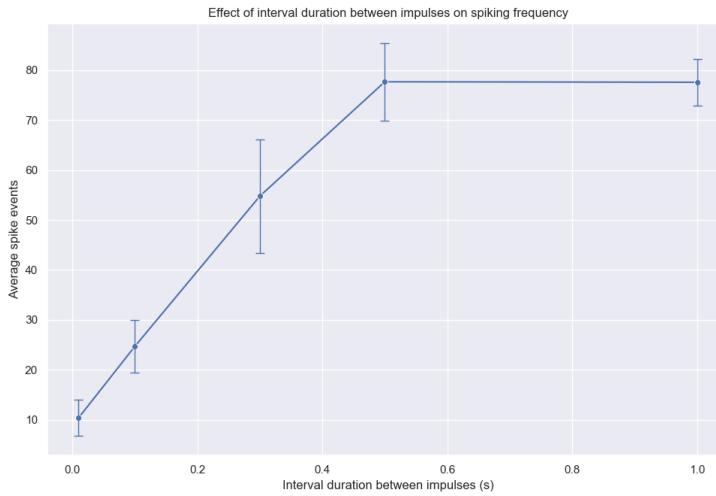


Figure 5.4: Effect of timing between multiple impulses on spiking frequency 300ms post stimulation. 10 impulses were stimulated at $10\mu\text{A}$ $600\mu\text{s}$. Datapoints are mean averages (trials, $n=10$), with error bars indicating standard deviation.

Figure 5.4 indicates that intervals of 0.5 seconds or greater produced the most spiking activity, and less frequent intervals produced the overall lowest activity. Intervals of 0.3 seconds produced the greatest margin of error, but overall the results were consistent and showed a trend in activity wherein activity peaked at 0.5 seconds and plateaued past that point. Although this shows the opposite of the predicted hypothesis, a possible explanation could be that 0.5 seconds is the optimum as it matches the frequency of the organoid's oscillatory activity, and further accumulates and peaks the spiking activity. Another explanation could be that the neurplatform software may be limited in sending out signals at shorter intervals, as intervals of 0.01 seconds show the least activity for example. The impulses may not be sent out properly to have a cumulative effect on spiking activity.

5.1.3 Summary

This results determine the optimal parameters settings as $10\mu\text{A}$ $600\mu\text{s}$, 10 impulses with a 0.5 second interval.

5.2 Channel-specific spiking distribution

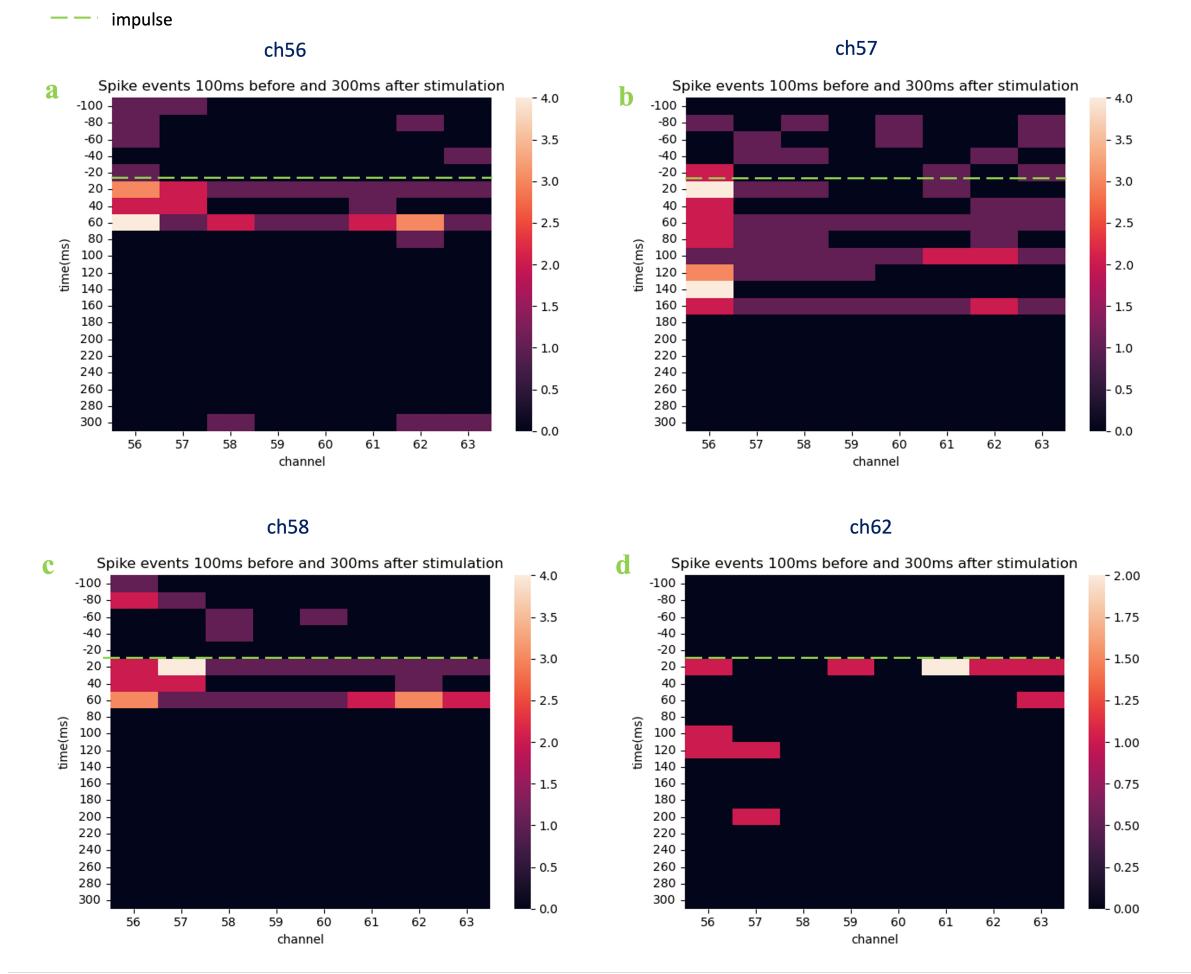


Figure 5.5: Spatiotemporal distributions of spiking events 100ms before and 300ms after stimulation of channels 56, 57, 58 and 62 of the organoid individually using the optimal parameters determined ($10\mu\text{A}$ $600\mu\text{s}$, 10 impulses with 0.5 second intervals).

Figure 5.5 shows that most activity occurs within 100ms post impulse, which depicts that the impulse directly impacts and increases spiking in the organoid.

There is a distinct distribution of spiking activity post impulse when stimulating different channels in the organoid. Depicted in Figure 5.5.a and 5.5.b, stimulating channel 56 produces more spiking in channels 57, 61 and 62, while stimulating channel 57 produces most spiking in channel 56. This suggests that there is some functional connectivity between these parts of the organoid, and

that nearby channels may be more affected by the stimulated channel (as channel 56 shows more activity when 57 is stimulated, for example). One explanation for this is that, since the electrodes are connected at random points of the organoid, the stimulated channel may be well connected with a nearby network of neurons, and the activity peaks at a nearby channel.

5.3 Consistency of Response

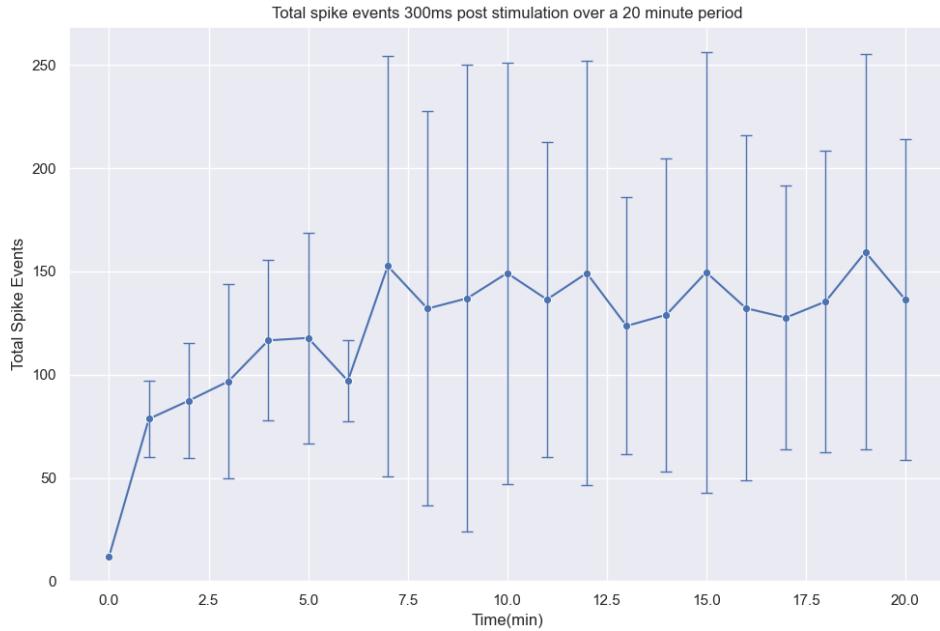


Figure 5.6: Total spiking activity 300ms post stimulation of using the optimal parameters determined ($10\mu\text{A}$ $600\mu\text{s}$, 10 impulses with 0.5 second intervals) over a 20 minute period. Each data-point represents average spike events post stimulation in one minute, error bars represent standard deviation.

Average spiking response post stimulation over the 20 minute period shows a consistent spiking above baseline, with a gradual increase in average events. The first five minutes of impulses show the most consistent response to stimulation, with the least deviation from the mean, as shown in Figure 5.6. After this point, the responses show a large deviation from the mean, which suggests an inconsistency in the response. However, the responses are still consistently above baseline.

Variation in response in the first 5 minutes could be explained by natural fluctuation in baseline activity in the organoid, post this point it is more likely that peaks in spiking are accompanied in troughs, possibly due natural synchronicity of neural activity.

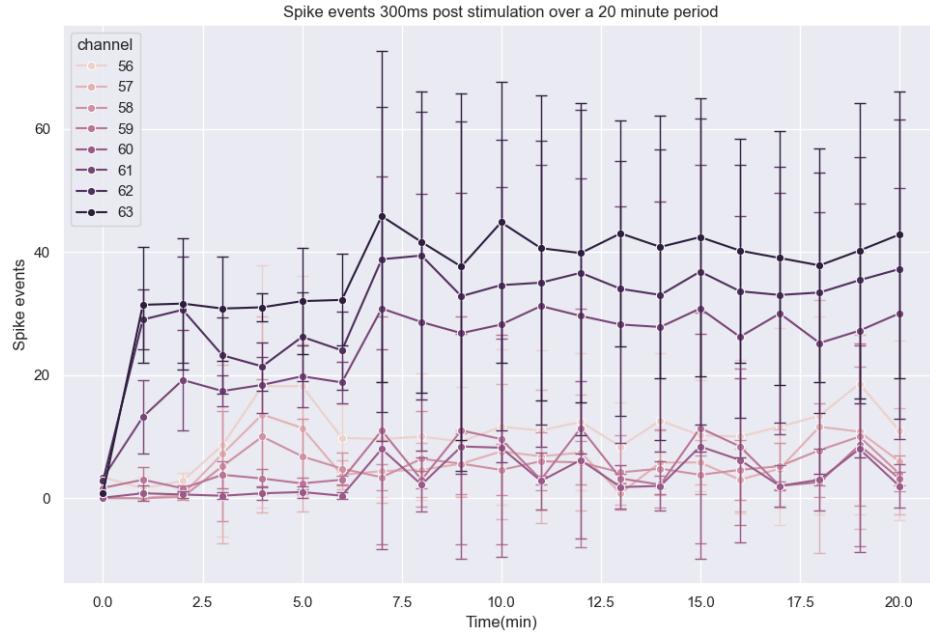


Figure 5.7: Average spiking activity 300ms post stimulation of using the optimal parameters determined ($10\mu\text{A}$ $600\mu\text{s}$, 10 impulses with 0.5 second intervals) over a 20 minute period. Each datapoint represents average spike events post stimulation in one minute.

Figure 5.7 depicts the changes in spiking response in each channel of the organoid, depicting distribution of activity across the organoid. Channels 57-60 do not show much increase above their baseline, while channels 61-63 showing the biggest increase in activity. However channels 61-63 also show the greatest deviation from the mean and largest range in response. This figure, along with figure 5.6 depict that the greater the spiking response, the greatest deviation and inconsistency in activity.

5.4 Spatiotemporal Sequences of Stimulation

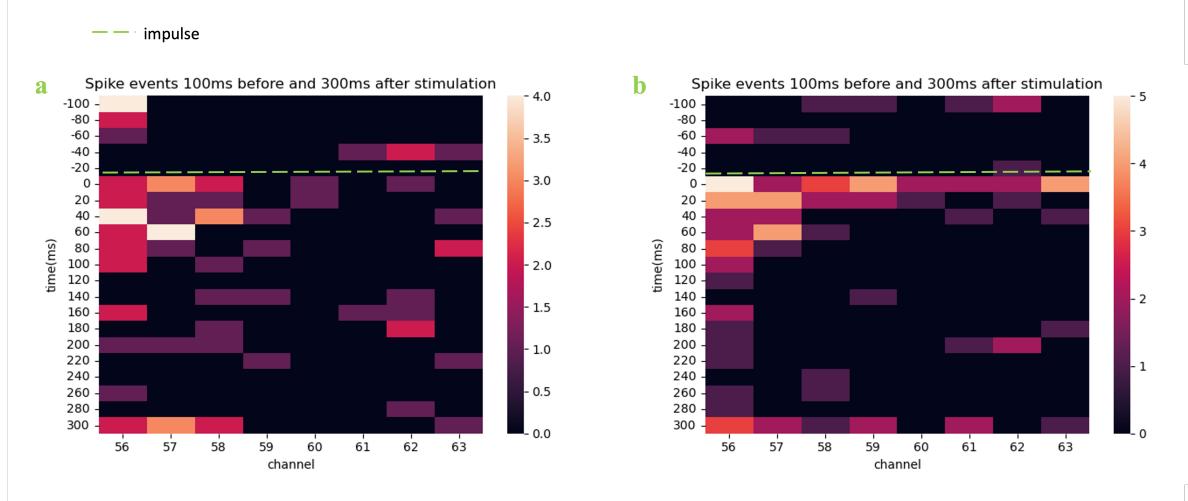


Figure 5.8: Effect of a sequence of stimulation on spiking frequency 100ms before and 300ms post stimulation using the optimal parameters determined ($10\mu\text{A}$ $600\mu\text{s}$, 10 impulses with 0.5 second intervals). a) pattern 1 - stimulation of channels 56, 59 and 61 simultaneously. b) pattern 2 - stimulation of channels 58, 60 and 62 simultaneously.

There is some distinct distribution of activity between the two different patterns, with pattern 2 showing an increase in spiking in all channels of the organoid immediately post impulse, while pattern 1 concentrates activity in channels 56-58, as depicted in Figure 5.8. There is some similarity between the two pattern outputs, such as channel 56 showing the most activity overall and channels 60+ showing least activity.

Most spiking activity can once again be seen within 100ms post impulse, with a sharp decline in activity past this point. There are a lot of areas of minimal to no activity - this is likely due to differences in baseline activity of the different channels/areas of the organoid, and also likely due to the oscillatory activity of the neuron network being longer than 20ms.

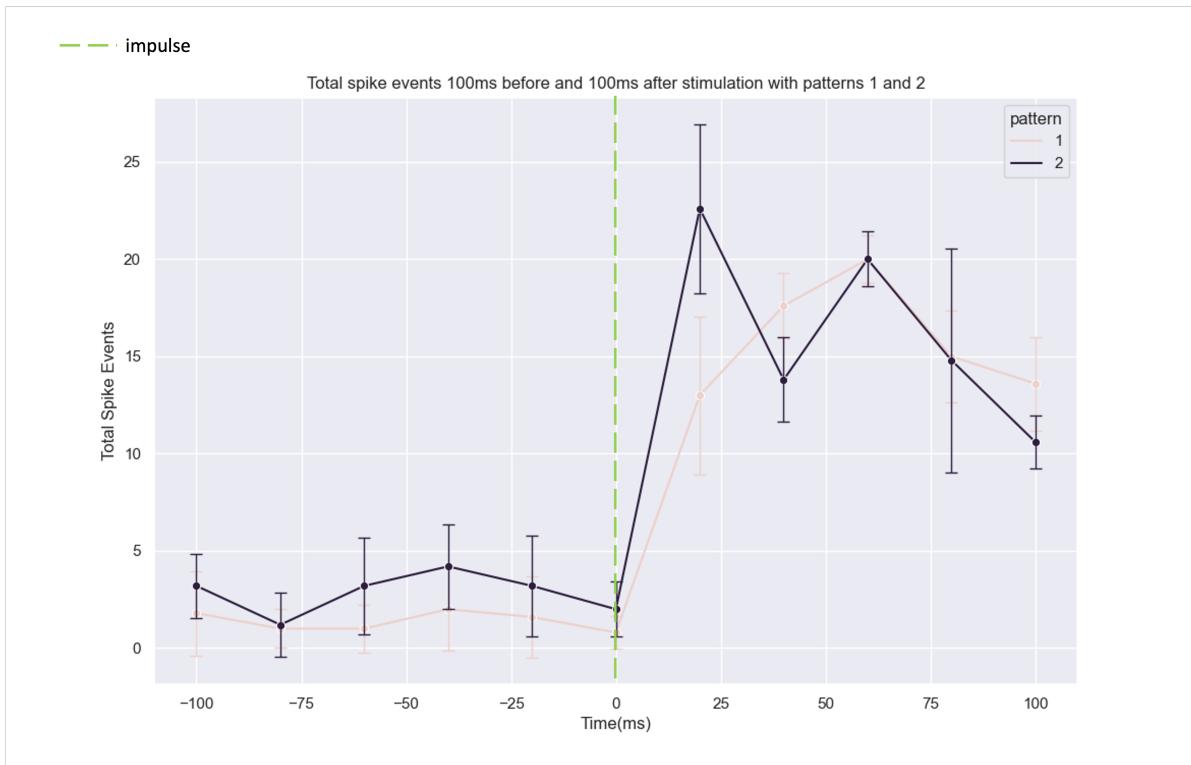


Figure 5.9: Effect of a sequence of stimulation on spiking frequency 100ms before and 100ms post stimulation using the optimal parameters determined ($10\mu\text{A}$ $600\mu\text{s}$, 10 impulses with 0.5 second intervals). Comparison of total spike events in the organoid post stimulation of channels 56, 59 and 61 simultaneously (pattern 1) and stimulation of channels 58, 60 and 62 simultaneously (pattern 2). Datapoints are mean averages (trials, $n=10$), with error bars indicating standard deviation.

Figure 5.9 depicts how total spiking increases, with spiking 100ms post stimulation of both patterns increasing spiking activity 4 fold. Pattern 2 elicits the greatest spike response within 25ms of impulse stimulation, while pattern 1 shows the largest spike around 60ms post impulse. The timing of the largest spike could therefore be useful when interpreting organoid output during classification tasks.

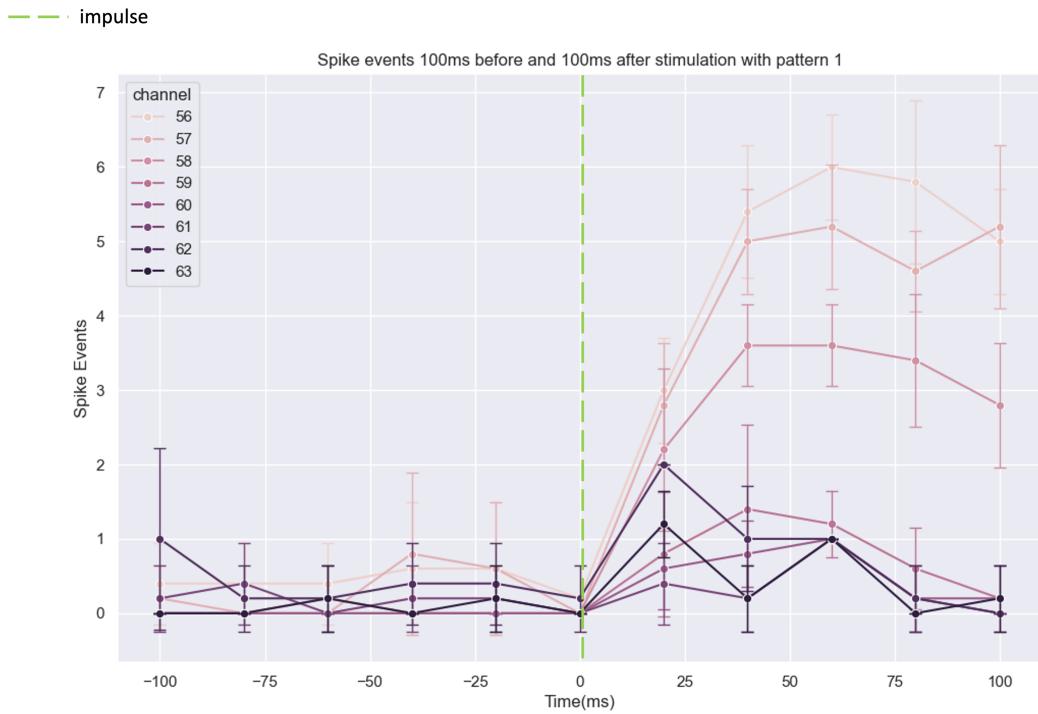


Figure 5.10: Effect of a sequence of stimulation on spiking frequency 100ms before and 100ms post stimulation using the optimal parameters determined ($10\mu\text{A}$ $600\mu\text{s}$, 10 impulses with 0.5 second intervals). Post stimulation of channels 56, 59 and 61 simultaneously (pattern 1). Datapoints are mean averages (trials, $n=10$), with error bars indicating standard deviation.

Figures 5.10 and 5.11 depict over 50% increase in spiking activity from 100ms before to 100ms after stimulation, specifically in channels 57-58. Distinct differences between the two include pattern 2 increasing average spiking activity in channels 60-62, while pattern 2 shows increased activity at channel 58. While standard deviation is small and shows consistency in response, spike events before and post simulation are low, with no more than 7 spikes observed in each channel 100ms post impulse. This is likely due to declining of neuronal function due to the organoid reaching the end of its lifespan. Nevertheless, this reduces the resolution of the results for analysis.

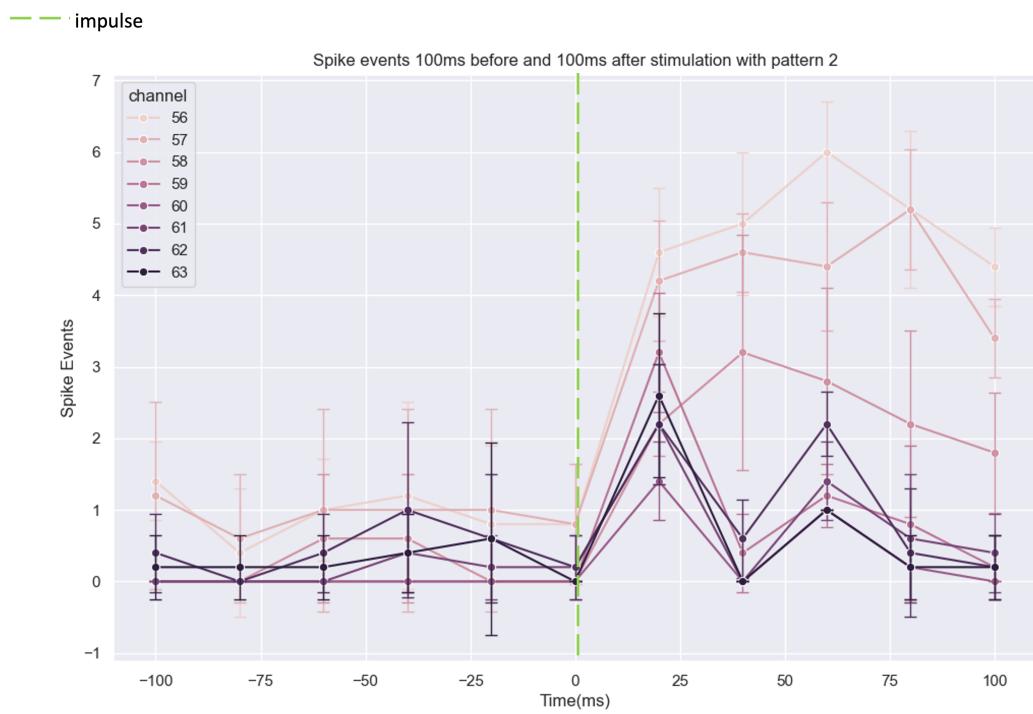


Figure 5.11: Effect of a sequence of stimulation on spiking frequency 100ms before and 100ms post stimulation using the optimal parameters determined ($10\mu\text{A}$ $600\mu\text{s}$, 10 impulses with 0.5 second intervals). Post stimulation of channels 58, 60 and 62 simultaneously (pattern 2).

6 Discussion and Conclusion

6.1 Research Summary

The results hypotheses are discussed in the following section to summarise and discuss main results.

Hypothesis: Parameter Optimisation

Average spiking activity will increase with increasing pulse amplitude. Longer pulse durations will also increase spiking activity until a point where they plateau or decrease the activity. The greater the number of impulses, the greater the number of spike events with a cumulative effect between impulses. Shorter interval durations between multiple impulses should increase spiking activity.

Stimulation parameter optimisation did not show conclusive results and contradicted most of the predicted hypotheses. Lower amplitudes showed more activity at shorter durations, with a significantly high performance exactly at the median parameters of $10\mu\text{A}$ $600\mu\text{s}$. The results of this parameter look almost anomalous, but margin of error is low, show a concise range and high average.

A lack of direct trend or correlations in the parameters may be due to variance in organoid activity on different days or hours of the day. Due to the magnitude of the experiment, some parameters had to be tested on different days, which may have resulted in lack of correlation. Despite contradictory trends and correlations with parameter settings, a clear optimal setting is determined.

Increased impulses show the expected trend of increasing activity as expected, and a cumulative effect is observed with the first four impulses as predicted. A surprising correlation in interval duration is impulses spaced further apart showed greater activity post impulse, peaking and plateauing at 0.5 seconds. A theory which could explain this is the "temporal binding hypothesis" which claims that information processing between distant neural networks relies on the strength of coherent organisation of activity through synchronous neural oscillations. This relies on 'phase-locking'

of communicative oscillations, and lower frequencies (such as achieved by longer intervals between pulses) have been shown to put fewer constraints on the precision of timing, as phases between low and high excitability are longer. [19] This suggests that longer time intervals that reduce frequency can more easily match the communicative oscillations in the organoid and produce a cumulative effect to peak spiking activity across the organoid.

Hypothesis: Channel-specific spiking distribution

Stimulation of each different channel in the organoid will show a distinct distribution of spatiotemporal spiking activity, with most activity occurring 100ms post impulse.

Stimulation of different channels pertaining to different areas of the organoid have shown that most activity occurred around 100ms post impulse and shown a fairly distinct spiking distribution as expected. Channels adjacent to the target channel/channel being stimulated have shown most activity. This could be due to electrodes being connected downstream or directly in the pathway of nearby channels. Some channels produce a lot less activity, this could be due to the random connection of electrodes to the organoid, as some channels may be more interconnected with a neural network and produce a stronger response.

Hypothesis: Consistency of Response

Immediate response to stimulation may wane over the course over the 20 minute period of constant stimulation, but will still show a significant increase in spiking activity above baseline.

Results show a consistent increase in spiking activity above baseline post impulse throughout 20 minutes of constant stimulation. However, each individual impulse elicited a wide range of spiking activity in the organoid, which suggests the response is unreliable and unpredictable. A pattern of synchronicity was found throughout each set of impulses in one minute, which suggests that spiking activity initially causes a large spike which wanes, and over a period of relaxation regains its ability to cause a large spike. Constant stimulation may not be optimal or feasible for organoid computation, so relaxation periods of 20 seconds or longer would be required for reliable computation.

Hypothesis: Spatiotemporal Sequences of Stimulation

Most spiking activity will be seen 100ms post impulse. Each pattern of stimulation will show a distinct distribution in spiking activity. Stimulation of three channels simultaneously will show increased spiking activity overall when compared to stimulation of only one channel.

Different sequences of stimulation have shown that most activity occurred around 100ms post impulse, and shown a somewhat distinct spiking distribution. It is difficult to determine the significance of this response, as although the total spiking increased significantly, individual channels only elicited up to 7 spike events, so the increase is marginal. Total average spiking was a lot lower than in the previous experiment, with around 20 spike events per pattern, compared with around 150 spike events in the previous response. This could indicate declining of organoid function as the organoid may be reaching the end of its lifespan.

6.2 Discussion

In this section, the overall aims of the project will be discussed.

6.2.1 1. Aim: "To evaluate the reliability and significance of organoid output for computational tasks".

This aim was met somewhat successfully as several of the parameter settings showed a trend and consistency in response, such as increasing impulses show a cumulative effect, and despite contradicting the predicted hypotheses, many parameters some pattern or an optimum was clearly defined. Amplitude and pulse duration did have an element of randomness and unclear pattern, with large margins of error for some values which proved difficult in defining their effect, this may be attributed to noise activity and difference in organoid activity from day to day.

There were some contradictions in trends hypothesised, especially pertaining to stimulation parameters. Deviation from predicted trends could be due to variation in organoid formation and neural connections. Different neural connections could arise from variation in stem cell cultures, resulting in differing activities. Placement of electrodes on the organoid could also have an effect on how what spiking activities may be amplified and recorded. Research on frequencies in

the brain suggests that different waves of activities benefit or amplify neural responses in different ways. For example, low frequency waves show amplification of distal connections. . [19] This is increasingly important when considering matching these parameters can amplify activity during "phase-matching". Tapping into the organoid's synchronicity can also amplify and increase understanding of its spiking output.

6.2.2 2. Aim: "To measure the significance of the organoid's ability to classify tactile data from the NeuroTac."

This project did not successfully meet the goal of using tactile data from the NeuroTac. However, the experiment results do provide clarity on the how input parameters can be optimised to carry out further experiments with the NeuroTac, and the spiking distributions that patterns of stimulation elicit in the organoid, which can be used to determine boundaries for classification of organoid output.

6.3 Limitations

Organoid intelligence is a recent and developing field, which means current research approaches are novel and undetermined. Applying engineering principles to biological matter, especially at the complexity level of organoids, may not always have a straightforward outcome, with factors such as biological regulatory mechanisms, large amounts of noise activity and natural cycles of activity to consider.

Lifespan of the organoid and its stage of maturation can have an effect on spiking activity and it's ability to respond to impulses and produce action potentials. An early stage of maturation of an organoid exhibits the most neuroplasticity and therefore is most likely to respond to stimuli and elicit a stronger spiking response, while a late stage organoid may have exhibited neuronal pruning and be less likely to show a strong response (it may also not have much baseline activity, as functioning is declining closer to end of its lifespan). This is evident throughout this experiment, as initial spiking is much greater in the first experiment compared to the last one.

6.4 Conclusion

This project provides evaluates a brain organoid for computation purposes and provides insight into how different stimulation parameters, stimulation timing and patterns of stimulation can be optimised and used for classification tasks. Key findings include increasing impulses showing a cumulative effect, stimulation impulses showing consistent increase in organoid activity 100ms post impulse, and patterns of spatiotemporal stimulation showing somewhat distinct distributions within areas of the organoid. Existing literature shows primary research on training an organoid for classification tasks using interface technologies, so this project provides an evaluation of how a different organoid could display similar behaviour. This further validates the feasibility of using brain organoids as biocomputers, but also provides some insight into how variation and limitations can affect computational output.

6.5 Future Work

Findings from this project could be implemented to form a translation system to classify tactile data from the NeuroTac. The project determines optimal parameters and trends which could prove useful in computing changes in the NeuroTac into sequences of stimulation. The range of spiking responses determined could be used as a classifier for edge orientation tasks. High activity areas could translate to increased input of stimulation, or a "threshold" of spikes from the NeuroTac can determine when an electrical impulse should be sent to the organoid, in a similar fashion to "integrate-and-fire" neuron models. Outputs can be tested for a significant differences in spiking output, and ranges of spiking events can be used to determine classification. This research could further validate use of organoids for computing classification tasks, and broaden the variation of data that can be computed within the field.

A Appendix

A.1 Stimulation parameter settings:

1. index: int Electrode index [0-127]
2. enable: bool Enabled the stimulation: 0 = false, 1 = true
3. *triggerkey*: int Trigger key: [0-15] digital in connected to the trigger generator [0,15]
4. *triggerdelay*: int Post trigger delay [us]
5. *nbpulse*: int Nb Stim pulse: 0: Single pulse, 1 and more: Pulsetrain
6. *pulsetrainperiod*: int Pulse Train Period [us]
7. *poststimrefperiod*: int Post-Stim Refractory Period [μ s]
8. *stimshape* : StimShape Stimulation Shape: Biphasic, BiphasicWithInterphaseDelay, Triphasic
9. *polarity*: StimPolarity Start polarity of the stimulation: NegativeFirst, PositiveFirst
10. *phaseduration1*: float D1 [μ s]
11. *phaseduration2*: float D2 [μ s]
12. *phaseamplitude1*: float A1 [μ A]
13. *phaseamplitude2*: float A2 [μ A]
14. *enableampsettle*: bool enable amp settle: 0 = false, 1 = true
15. *prestimampsettle*: float Pre stim amp settle [μ s]
16. *poststimampsettle*: float Post stim amp settle [μ s]

17. *enablechargerecovery* : bool enable charge recovery: 0 = false, 1 = true
18. *postchargerecoveryon* : float Post charge recovery on [μs]
19. *postchargerecoveryoff* : float Post charge recovery off [μs]
20. *interphasedelay* : float Interphase delay (for BiphasicWithInterphaseDelay) [μs]

Algorithm 1 Running stimulation - Parameter Optimisation 1 and 2

Input:, Stimulation parameters

Output:, Experiment start and stop times

```
1: electrode ← 61
2: phaseamplitude1 ← 1 – 10 $\mu$ A
3: phaseamplitude2 ← 1 – 10 $\mu$ A
4: phaseduration1 ← 100 – 600 $\mu$ s
5: phaseduration1 ← 100 – 600 $\mu$ s
6: phasepolarity ← PositiveFirst                                ▷ Initiate Stimulation
7: do Initiate Stimulation
8: instantiate IntanSoftware
9: instantiate Trigger Generator
10: Set stimulation parameters
11: while experiment start do
12:     ← Measure impedance
13:     Disable Variation STD
14:     Send stim parameter
15:     do Print start time
16:     Set trigger key
17:     do Send Trigger
18:     time sleep(1s)                                         ▷ End experiment
19:     Stop experiment
20:     do Print end time
21:     Disable all stimulation
22:     close Trigger Generator
23:     Enable variation threshold
24:     close IntanSoftware
25: end while
```

Algorithm 2 Running stimulation- Parameter Optimisation 3 and 4

Input:, Stimulation parameters

Output:, Experiment start and stop times

```
1: electrode ← 61
2: phaseamplitude1 ← 5 $\mu$ A
3: phaseamplitude2 ← 5 $\mu$ A
4: phaseduration1 ← 300 $\mu$ s
5: phaseduration1 ← 300 $\mu$ s
6: phasepolarity ← PositiveFirst                                ▷ Initiate Stimulation
7: do Initiate Stimulation
8:   Initiate IntanSoftware
9:   Initiate Trigger Generator
10:  Set stimulation parameters
11: while experiment start do
12:   ← Measure impedance
13:   Disable Variation STD
14:   Send stim parameter
15:   do Print start time
16:   Set trigger key
17:   for i in range 1-10 do :
18:     do Send Trigger
19:     Time delay ← 0.01-1s
20:   end for
21:   Time delay (20s)                                              ▷ End experiment
22:   Stop experiment
23:   do Print end time
24:   Disable all stimulation
25:   Close Trigger Generator
26:   Enable variation threshold
27:   close IntanSoftware
28: end while
```

Algorithm 3 Retrieving data

Input:, Impulse trigger time

Output:, Spike events

```
1: do Initiate Database
2: Input: start time
3: Input: end time
4: Print trigger times from data base ← start time - end time
5: for i in range 1-10 do :
6:     s1 = trigger time
7:     Get spike event count ← s1 + 0.3 seconds
8:     do Print event count
9:     Filter for channels 56-63
10:    for n in range 56-63 do :
11:        do Record spike count
12:    end for
13: end for
```

References

- [1] L. Smirnova, B. S. Caffo, D. H. Gracias, *et al.*, Organoid intelligence (oi): The new frontier in biocomputing and intelligence-in-a-dish, *Frontiers in Science* [online], vol. 1 2023, 2023, ISSN: 2813-6330. DOI: 10.3389/fscı.2023.1017235. available from: <https://www.frontiersin.org/journals/science/articles/10.3389/fscı.2023.1017235>.
- [2] H. C. Z. A. C. T. Z. W. H. L. J. T. M. G. K. M. F. Guo., Brain organoid computing for artificial intelligence, *bioRxiv* 2023, 2023.
- [3] C. M. Kim SH, Application of human brain organoids-opportunities and challenges in modeling human brain development and neurodevelopmental diseases, *bInt J Mol Sci* [online] 2023, 2023. DOI: 10.3390/ijms241512528. available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10420018/#:~:text=Brain%20organoids%20are%20three%2Ddimensional,found%20in%20the%20human%20brain..>
- [4] Z. S. Medvedev SP Shevchenko AI, Induced pluripotent stem cells: Problems and advantages when applying them in regenerative medicine, *Acta Naturae* [online] 2010, 2010. available from: [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3347549/#:~:text=Induced%20pluripotent%20stem%20cells%20\(ipSCs,animal%20and%20human%20differentiated%20cells..](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3347549/#:~:text=Induced%20pluripotent%20stem%20cells%20(ipSCs,animal%20and%20human%20differentiated%20cells..)
- [5] E. Kurtyś, "*neuroplasticity-in-vitro*", Accessed on 21/02/24, Dec 18, 2023. available from: <https://finalspark.com/neuroplasticity-in-vitro/>.
- [6] FinalSpark, "*a closer look at our neurospheres*", Accessed on 21/02/24, May 24, 2022. available from: <https://finalspark.com/a-closer-look-at-our-neurospheres/>.
- [7] B. S. B. L. O. S. S. L. and R. A., *Mass generation, neuron labeling, and 3d imaging of minibrains*, 2021. DOI: 10.3389/fbioe.2020.582650, 202.

- [8] Y. X. M. X. P. Z. W. G. L. R. Q. M. Z. J. W. F, Physiological electric field: A potential construction regulator of human brain organoids, *Int J Mol Sci* [online] 2022, 2022. DOI: 10 . 3390 / ijms23073877. available from: <https://pubmed.ncbi.nlm.nih.gov/35409232/>.
- [9] R. Pramoditha, "the concept of artificial neurons (perceptrons) in neural networks", Accessed on 13/04/24, Dec 26, 2021. available from: <https://towardsdatascience.com/the-concept-of-artificial-neurons-perceptrons-in-neural-networks-fab22249cbfc>.
- [10] B. Schrauwen, D. Verstraeten, and J. Campenhout, An overview of reservoir computing: Theory, applications and implementations, *Proceedings of the 15th European Symposium on Artificial Neural Networks* Jan. 2007, pp. 471–482, Jan. 2007.
- [11] J. E. Smirnova L. Caffo B., Reservoir computing with brain organoids, *Nat Electron* [online] 2023, 2023. DOI: 10 . 1038 / s41928 - 023 - 01096 - 7. available from: <https://www.nature.com/articles/s41928-023-01096-7>.
- [12] A. K. Maan, D. A. Jayadevi, and A. P. James, A survey of memristive threshold logic circuits, *CoRR* [online], vol. abs/1604.07121 2016, 2016. arXiv: 1604 . 07121. available from: <http://arxiv.org/abs/1604.07121>.
- [13] Wikipedia, "neuromorphic engineering", Accessed on 10/03/24, 2024. available from: https://en.wikipedia.org/wiki/Neuromorphic_engineering.
- [14] E. Farquhar, C. Gordon, and P. Hasler, *A field programmable neural array*, 2006. DOI: 10 . 1109/ISCAS.2006.1693534.
- [15] C.-S. Poon and K. Zhou, Neuromorphic silicon neurons and large-scale neural networks: Challenges and opportunities, *Frontiers in Neuroscience* [online], vol. 5 2011, 2011, ISSN: 1662-453X. DOI: 10 . 3389/fnins.2011.00108. available from: <https://www.frontiersin.org/journals/neuroscience/articles/10.3389/fnins.2011.00108>.
- [16] B. Ward-Cherrier, N. Pestell, and N. F. Lepora, *Neurotac: A neuromorphic optical tactile sensor applied to texture recognition*, 2020. arXiv: 2003 . 00467 [cs.R0].

- [17] FinalSpark, "biological neuroplatform up and running", Accessed on 21/02/24, Feb 10, 2023. available from: <https://finalspark.com/biological-neuroplatform-up-and-running-2/>.
- [18] B. F. Jordan FD. Kutter M. Comby J-M and K. E, Open and remotely accessible neuro-platform for research in wetware computing, *Front. Artif. Intell* [online] 2024, 2024. DOI: 10.3389/frai.2024.1376042.
- [19] A. F. C. Beste C. Münchau, Towards a systematization of brain oscillatory activity in actions, *Commun Biol* [online], vol. 6, no. 137 2023, 2023. DOI: <https://doi.org/10.1038/s42003-023-04531-9>.