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An integrated setup for in-vitro optogenetic experiments using AI to localize stimulation

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ABSTRACT

In this proceeding we discuss the recent work involving our developed optogenetic tool, where we use digital light processor (DLP) as a light-stimulation source of neuronal culture and microelectrode array (MEA) system as the sampling unit. In this work we aim at developing an integrated experimental platform which should assist in the study of the structure and the function of neuronal networks. In particular, the setup proposed in this work should serve as an optogenetic tool for *in-vitro* experiments, controlled by a feedback from electrophysiological signals from the network to address specific neuronal circuits. In this manuscript some of the recent results from experiments involving optical stimulation and electrophysiological recording of neuronal cultures are shown. Additionally, we have developed an AI-based model which is trained according the recorded electrophysiological signals and reproduces the functionality and the macro-structure of the culture under test. The description and some preliminary results of this model are also discussed in this proceeding.

Keywords: *in-vitro* neuronal cultures, Electrophysiology, Optogenetics, Signal analysis, Applied Machine-Learning.

1. INTRODUCTION

The study of the brain remains one of the most challenging topics in science. A full understanding of the neuronal dynamics inside a living brain is still far from being achieved. Neuroscience tackles this problem by studying the neuronal functionality as individuals and in networks.[1–3] For this purpose, different disciplines and approaches are used, e.g. electrophysiology, biochemistry, optogenetics and more.

Many neurobiological studies in the last few decades use the intrinsic electrophysiological properties of neurons. One of the most common techniques within this approach is multielectrode array (MEA) technology.[4] It is designed both to stimulate and record electrophysiological dynamics of neurons *in-vivo* and *in-vitro*. MEA is a planar array consisting of between a few tens to a few thousands of electrodes (in the case of HD-MEAs[5]), arranged in a matrix and integrated on a chip. The typical material of the electrodes is TiN (or semiconductors in HD-MEAs). The typical diameter of each electrode could reach tens of micrometers and the spacing between them could reach hundreds of micrometers. The electrodes sense the variation of electrical potential in the vicinity due to flow of ions into or out of the cell body. With a designated electronic system, the electrical signals are probed at high sample rate (up to 100KHz), filtered and amplified and provide a global picture of the network activity with high temporal resolution. Another important feature of MEA systems is their capability to provide electrical stimuli to a specific site (via the electrode), which allows the study of evoked neuronal activity.

As for stimulation of neurons, a novel approach, which became widely used in recent years is *Optogenetics*.[6] This technique uses light to stimulate neurons by introducing light sensitive channel protein, Channel-rhodopsin (ChR2), which serves as a light-gated ion-channel. ChR2 is transfected into a neuronal culture and a single

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neuron can be activated by addressing light with a proper wavelength at the neuron of interest. Fig. 1 presents expression of ChR2 by neurons, emitting green fluorescence. Optogenetics is a very promising technique since it can easily be localized at specific neuronal cell body, other parts of the cells (such as the axon) or at the neuronal synapses. Using integrated photonics optogenetics can be implemented in compact structures.[7]

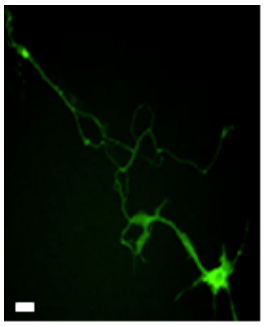

Figure 1. An image of a neuron emitting fluorescence, as a result of an expression of ChR2-GFP, scale bar: 10μm.

We present in this paper some of the recent results obtained from our experiments involving light simulation of ChR2 infected mice neuronal cells, cultured on MEA chips and tested for a response by electrophysiological measurements and data analysis. The work is part of project *"ISLAND"*[8] which aims at assisting in the study of the structure and the functionality of neuronal networks. In particular, the objective of the project is developing an integrated and intelligent platform which reads and writes information from and on neuronal cultures. As a benchmark, the problem of memory formation in small neuronal network is to be examined.

The reading part of the setup is done by sampling the electrophysiological activity of the culture using MEA technology which is highly time-resolved. The writing part is executed by optogenetic techniques using optical instrumentation allowing spatially-patterned light, such as digital light processor (DLP) or integrated photonic circuit (IPC). The core of the project aims to integrate these two parts in a closed-loop, by developing processing and control units. The processing unit should collect the electrical signals from MEA as a feedback of the neuronal activity and map the neurons in the network according to their electrophysiological activity. The control system should then activate the corresponding pattern of stimuli in the optical setup according to the network's map and the assignment. A design of the experimental tool which is considered in this work is depicted in Fig. 2

For the purpose of mapping the neuronal network, we have developed an AI-based computational model which obtains a simplified network (or a graph model), using the data acquired in MEA measurements as training dataset in order to decode the spatio-temporal signal patterns of the neuronal network and to extract its structure. The model is based on Reservoir computer network (RCN) approach,[9] as it uses the fact that around each electrode of MEA, a complex neuronal circuit is embedded. The complexity of these circuits cannot be easily understood from the standard measurement analysis, and hence they are modeled as nonlinear networks with inner random connections. With this model we are able to extract a macroscopic graph representing the structure of the culture under test, where each node of this network represents a neural circuit (population of neurons); and the connections (edges) between them represent the weighted interaction between the populations.

2. EXPERIMENTAL SETUP

The experimental setup in this work consists of a microscopy system, a light driver and a MEA recording system. The light pattern is generated by the DLP device and its supplementary software, and triggered by an external signal generator. The light is then directed via the rear port of the microscope and focused on the sample plane, where the neuronal culture on MEA chip is placed. The MEA chip is inserted inside the MEA system, which records the electrophysiological signals from the culture. The signals are monitored with the MCS experimenter software and further exported to offline analysis. The light triggers are also recorded by the MEA system to deliver the exact timing of the stimuli. The neuronal culture and the expression of ChR2 are imaged with the microscopy system with a super-resolution and spinning-disk confocal microscope.[10] The schematics of the

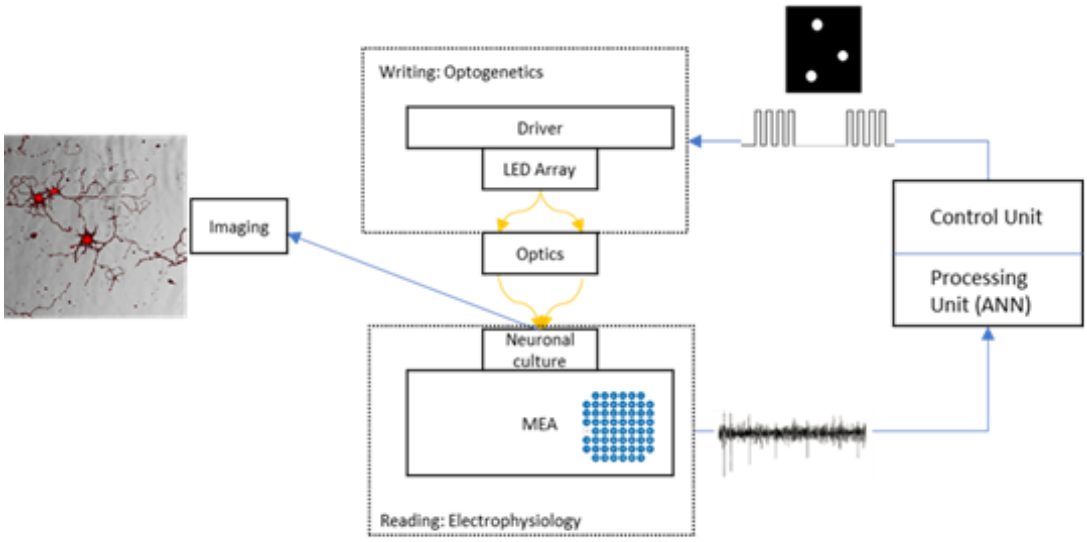

Figure 2. A design describing the general idea of the devised experimental tool

experimental setup is depicted in Fig 3, and a photo of the setup including the MEA, DLP and microscope is shown in Fig. 4

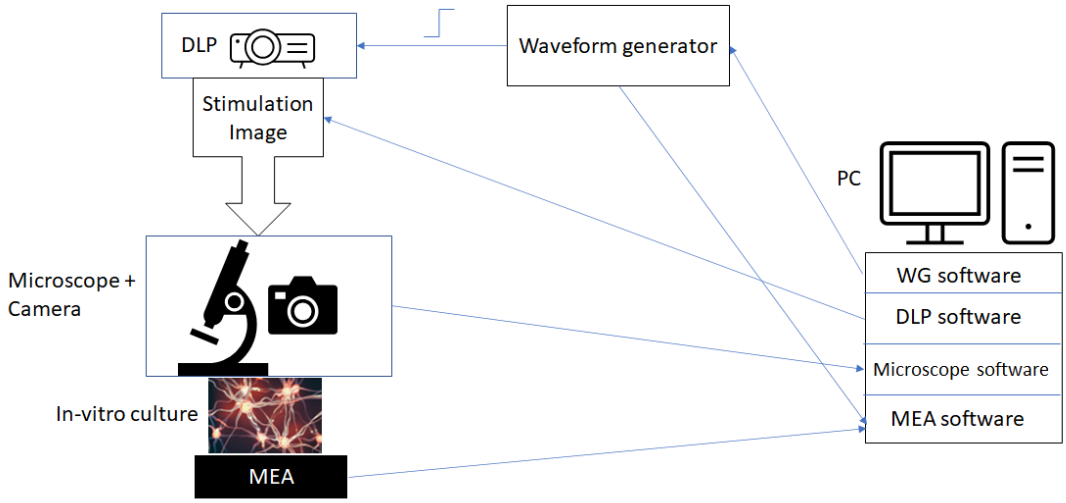

Figure 3. A scheme of the experimental setup used in this work

2.1 Microelectrode Array (MEA)

The electrophysiological signals were recorded using MEA-2100mini system of *Multichannel Systems* GmbH (MCS). The microelectrode array chips used in our experiments were 60MEA-200/30iR-Ti-gr by MCS, which are chips with 60 titanium-nitride electrodes embedded in glass and surrounded by a glass ring. The electrodes are of 30μm diameter, where the horizontal and vertical spacing between each pair of electrodes is of 200μm. The MEA-2100mini system collects the signals through a headstage device. Then the signals undergo amplification and filtering. The system is then connected to a PC through an interface board. The recording is performed using MCS experimenter software, where the signals can be digitally filtered, inceptively-analyzed and tracked in real-time. We sampled the signals at 20KHz. The recorded files are then saved and exported for a secondary offline analysis. The MEA-2100mini system is described in Fig. 5.

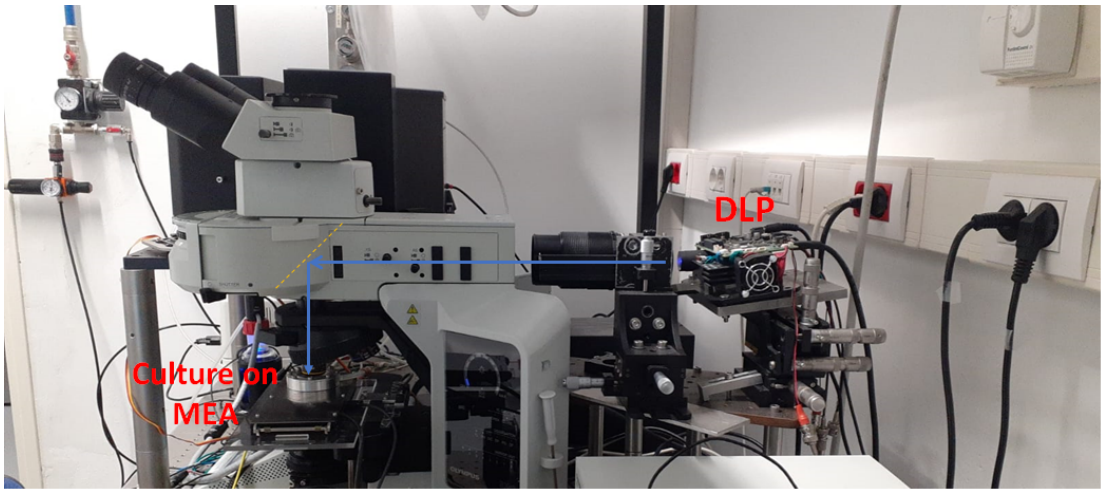

Figure 4. The principal part of the experimental setup used in the optogenetic experiments. Patterned light from the digital light processor (DLP) is directed and focused via microscopy system on top of the neuronal sample located on a microelectrode array (MEA) which measures the response of the culture.

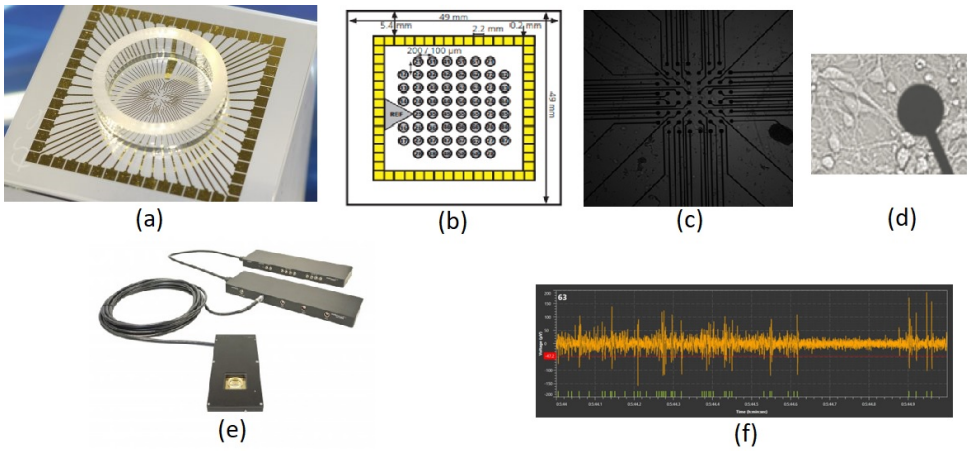

Figure 5. MEA system. (a) MEA glass chip, containing the electrode array. (b) Electrode array scheme. (c) A microscope image of the array. (d) A close-up of an electrode surrounded by cells. (e) The electronic system (MCS MEA-2100mini): including headstage, amplifier and interface board. (f) Signal acquisition and spike detection performed on MCS experimenter software: close-up on one of the channels

2.2 Digital Light Processor (DLP) and the optical setup

The system is a DLP E4500, which includes 3 LEDs, optics, a WXGA DMD (Wide Extended Graphics Array Digital Micro-mirror Device) and a driver board. The light engine can produce approximately 150 lumen at 15W LED power consumption. The blue LED (488nm) which is used in this work has a power of 600mW. The light from the LEDs impinges on the DMD which has 1039680 mirrors arranged in 912 columns by 1140 rows with a diamond array configuration. Each of these mirrors has two main possible inclinations that reflect the light in a different direction. This system allows to get patterned illumination with pre-loaded and custom patterns that can be chosen through the DLP E4500 software. Moreover, these patters can be pulsed in time, with both an internal or external trigger, with a nominal precision down to μs. The system supports 1-, 2-, 3-, 4-, 5-, 6-, 7-, and 8-bit images with a 912 columns $\times$ 1140 rows resolution. These images are pixel accurate, meaning that each pixel corresponds to a micro-mirror on the DMD. The light coming from the DLP system is collimated and aligned to the optical path of the microscope from the rear port of the system. As can be seen in FIG. The light from the DLP is collected by a macro TAMRON 90mm AF2.5 objective and the light pattern is imaged on the sample plane, where the MEA chip is located, a by 10$\times$ objective, while passing through a dichroic mirror

(Chroma T505lpxr-UF1) which acts like a high-pass filter, reflecting all the wavelengths smaller than 505nm.

The ChR2-infected culture could be in parallel imaged using a microscopy system, where the signal of the green fluorescent protein (GFP) expressed by the ChR2 infected cells, is transmitted through a dichroic filter and detected by a CMOS camera. The culture image allows to direct the desired light pattern from the DLP directly to the region of interest in the culture. Fig. 6 shows an image of ChR2 expressing culture on top of MEA.

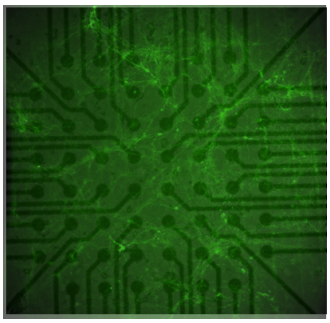

Figure 6. An image of ChR2 expressing culture on top of MEA. Vertical/horizontal spacing between 2 electrodes: 200μm

More details on the microscopy setup and the DLP properties can be found on previously reported experimental design, found in Ref. 10.

2.3 Cell Cultures

To seed neurons on MEA chips we used primary neuronal cells isolated from the E17/E18 embryonic cortex of C57BL/6 mice. Briefly, after 17/18 days of gestation, we decapitated embryos and dissected their cortex in a standard (solution) glucose-enriched dissection buffer beneath a laminar flow cabinet. Then we removed the dissection buffer, added 5ml 0.5% trypsin-EDTA and left it for 20 minutes in the incubator. Next to stope trypsin we added 5ml DMEM medium containing 10% FBS and gently pipetted the solution containing a slurry of mixtures of various cells to obtain a uniform solution. We employed a cell strainer with a 70μm pore size to isolate neurons, and then we used a conventional counting chamber to count the number of cells present in the fluid to separate as much as we need. After then we centrifuge the separated cells with 1800 rpm for 5 min to discard the superficial solution (DMEM with 10% FBS) and then added seeding medium as much (Neurobasal, 10% FBS, and P/S) so the cell concentration becomes equal to 1700 cells/μl. We seeded 80μl of cell solution in a droplet for each chip. The droplet was applied in the center of the chip, which was priorly coated by Poly-D-Lysine (PDL) and laminin. After 2 hours and 30 minutes we removed the surface medium and add a feeding medium (Neurobasal, 1% B27, 1% Glutamax, and 1% P/S).To improve our cultures situation in the MEA chips we enrich our coating with Laminin which helps the cells by nourishing and supports growing attached neurons. With this additional support we could have cell for 42 days alive and electrically active.

3. EXPERIMENTAL PROCEDURE

Our experiments included spontaneous activity recordings between 10 and 15 minutes. These recordings were performed to evaluate the performance of each culture in terms of spike and bursts rates. Later, we also used this data for the modeling part (Section 4). In addition we tested each culture for response to optical and electrical stimuli.

3.1 Primary data analysis

Following MEA recordings, the raw data was exported from MCS software and analyzed using a custom code written in MATLAB. First the raw signals recorded on MEA were digitally filtered with a band-pass Butterworth filter with cutoff frequencies 0.3 and 3 KHz. Spikes were detected using a threshold detection method,[11] setting the threshold to 6 times (negative) of the noise value for each of the channels, where the noise is defined as the

median value of the signal standard deviation sampled in 1s windows along the recording. Noisy channels were eliminated from recording or analysis according to fixed criteria.

With the detected spike trains we implemented burst detection and network burst detection algorithms, similar to which can be found in literature.[12,13] For the analysis of light-evoked activity (as well as electrically-evoked), we analyzed the response of the network with post-stimulus time histogram (PSTH).[14]

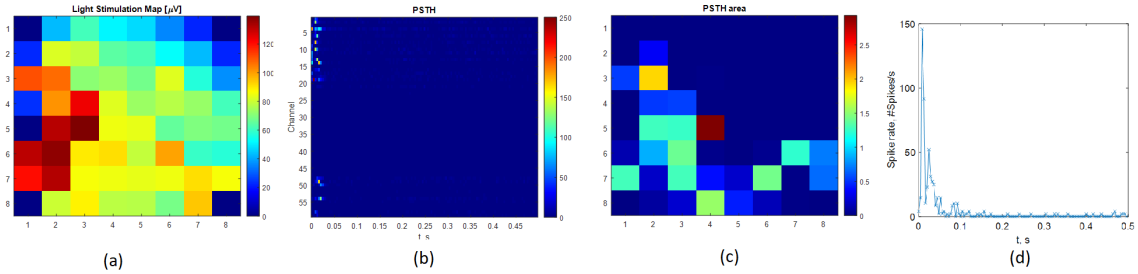

Figure 7. An example of MEA measurements analyzed data in a light stimulation experiment. (a) Map of photo-electric response of the electrodes following illumination, which (to some extent) describes the illuminated area on top of MEA. (b) Post-stimulus time-histogram (PSTH)- indicates the neuronal response to light stimultaion for each channel of the MEA. t=0 is the stimulus time (c) Map of the PSTH area at each electrode- describes the total neuronal response of the network. (d) PSTH of one of the channels

3.2 Optical stimulation and MEA recording

In these experiments we performed MEA recordings as a response to optical stimulation, using the DLP as the light source. The stimulation temporal pattern was as following: the light pulse length was 20ms and the period of stimulation sequence was 2s (0.5Hz frequency); in total we applied 300 pulses in each recording (10 minutes of total recording time). We tested the response of the network, both for wide-field illumination (i.e., under the field-of-view of the optical system), and illumination of a smaller spot (of a few up to a few tens of microns in diameter) around a small population of neurons. Fig. 8 shows experimental results of the neuronal response to light stimulation both for spot and wide-field illumination. These experiments demonstrate the responsiveness of the culture to light following ChR2 infection and also the network connectivity, when stimulating locally in one part and recording a response in other parts of the network.

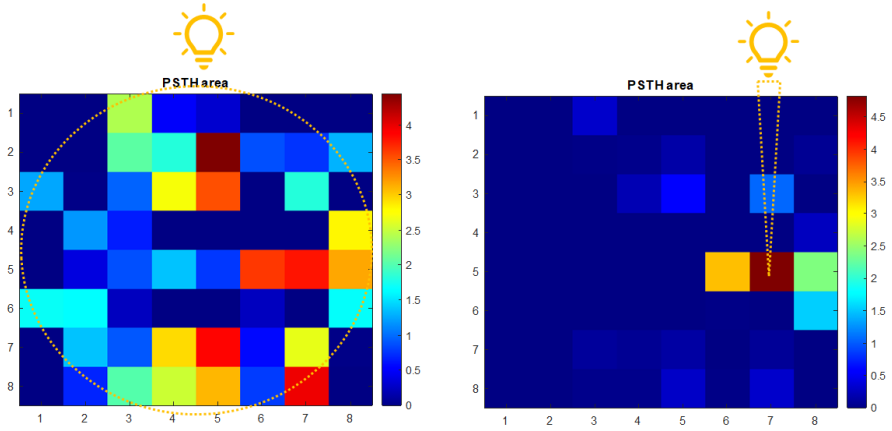

Figure 8. Measurement of culture's response to light stimuli. Each pixel represents the response of a MEA electrode. The measure of the response is the area under the PSTH curve. Left: Response to wide-field illumination (approximately all over the electrode array). Right: Response to spot illumination around electrode #75 (the light spot size was around 50μm in diameter)

We have also performed a set of experiments where we tested the competence of our system to induce long-term potentiation (LTP) in the culture using light, followed by a demonstration of forming engrams.[15] The LTP

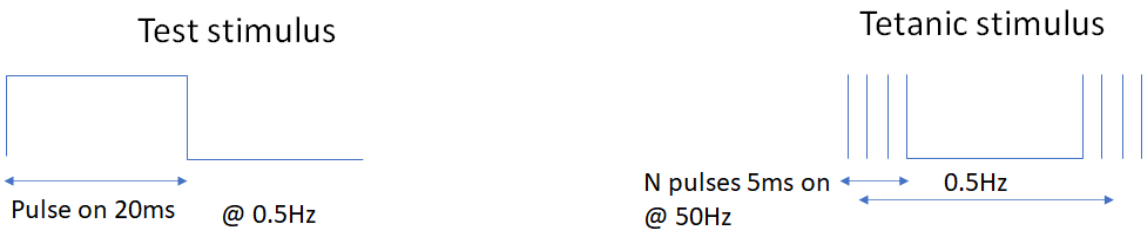

Figure 9. Temporal patterns of light stimuli used in experiments: Test stimulus- used to measure the response of the culture; Tetanic stimulus (high frequency)- used to induce potentiation of synaptic connections in the neuronal cultures.

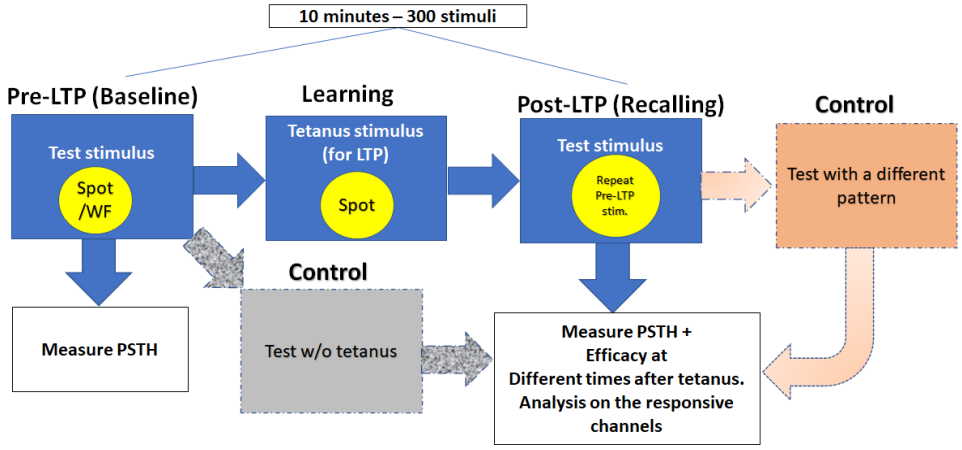

Figure 10. Experimental protocol of LTP test

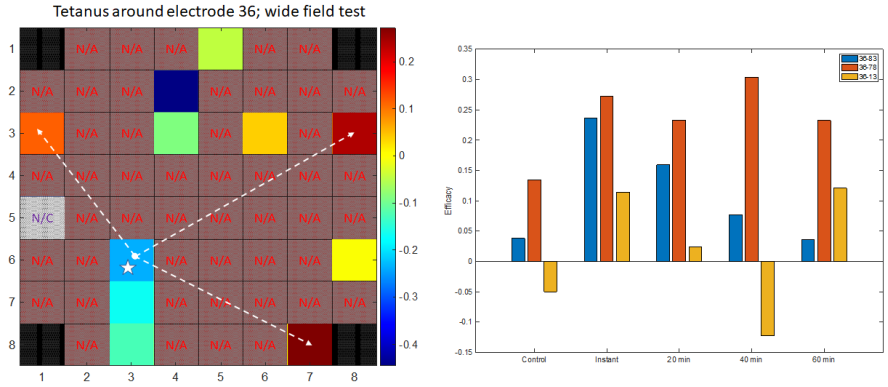

Figure 11. An example of LTP experiment result: The tetanic stimulus was applied around electrode # 36 (marked with a star), and the test stimulus was applied in a wide-field illumination. A change in the response is measured between the pre-and post-tetanic phases at the different electrodes. Left: the change in the response for each of the electrodes (N/A- not analyzed- due to low response; N/C- not connected). Right: The change of the response (efficacy) in specific connections at different times after the tetanic stimulation.

is induced by stimulating part of the network with high-frequency stimulation (known as tetanic stimulation)- Fig 9. In particular, we tested how the the response of the network changes following tetanic stimulation at a specific location (spotted tetanic stimulus). The diagram of the experimental procedure is shown if Fig. 10. Some results of LTP experiments are shown if Fig. 11 . This part of the work is still in progress, as we study the optimal configuration for LTP and engram demonstration.

4. MODELING

For studying the structure and functionality of the neuronal network under test, we have developed a computational model, based on Reservoir computer network (RCN) approach.[9] In this model we consider the fact that each electrode samples the electrophysiological signals resulted from the activity of the neuron ensemble (may consist of up to few tens of neurons) found in its vicinity (see Fig. 5(d)). We hence defined the domain of the MEA measurement as the *macroscopic domain*, which is described by the network in question, where the real neuronal structure which is sampled by each node is considered as the *microscopic domain*. The data unit which is contained in each of these nodes is a sample of the electrophysiological signals expressed in the instantaneous spike-rate measured in a specified time window, which is defined as the integration time of each micro-circuit.

The architecture of the proposed model in a form of artificial neural network (ANN) is shown in Fig. 12. As can be seen in Fig. 12, the ANN consists of three layers:

- Input layer: transforms the data from the macroscopic domain into a larger size domain, where each of the nodes transforms into a reservoir larger network, each representing the micro-circuit found around each electrode.
- Reservoir layer: this layer integrates the input signals with the reservoir state, which depends on the history of the signals (carrying memory). In addition the reservoir layer applies a nonlinear function on its current state and yields the next reservoir state.
- Output layer: transforms the reservoir state at each step back into the macroscopic domain while coupling between all the micro-reservoirs. The transformation in this layer is linear and the weights of the couplings are trainable.

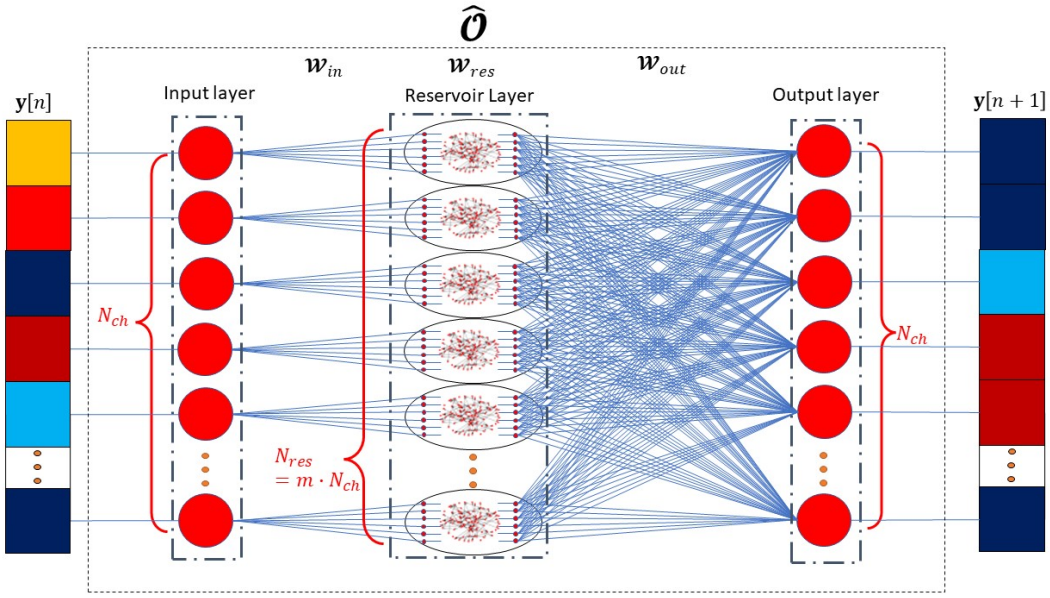

Figure 12. The archetecture of the ANN used in the modeling part

The recurrent dynamics of this scheme is described by:

$$y[n+1] = \mathcal{W}_{out}\mathbf{x}[n] \tag{1}$$

where,

$$\mathbf{x}[n] = \mathbf{f}_{NL}(\mathcal{W}_{in}\mathbf{y}[n] + \alpha\mathcal{W}_{res}\mathbf{x}[n-1]) \tag{2}$$

where $\mathbf{y}[n]$ is the network state at time step n; $\mathbf{x}[n]$ is the reservoir state at time step n; $\mathcal{W}_{in}$,$\mathcal{W}_{res}$ and $\mathcal{W}_{out}$ are matrices of weights of the input, reservoir and output layers, respectively; α is the memory parameter and $\mathbf{f}_{NL}$ is a nonlinear function describing the reservoir non-linearity.

In this scheme, the training is applied only on the output weights of $\mathcal{W}_{out}$ matrix, and since the the relation in Eq. 1 is linear, it is sufficient to train it with linear regression. The training data is collected from the pre-processing of MEA measurements (Section 3.1), where it is arranged as time-binned spike trains over events of network bursts (spontaneous activity) or post stimulus time windows (evoked activity). The diagram describing the data collection, training and validation of the model is shown in Fig. 13. After the model is trained (and after some mathematical manipulations) it is possible to describe the given culture in terms of macroscopic connectivity between the nodes of the network. And also it is possible to test this network as a response to various stimuli. In Fig.14 we present some of the results obtained in this part of the study. More detailed

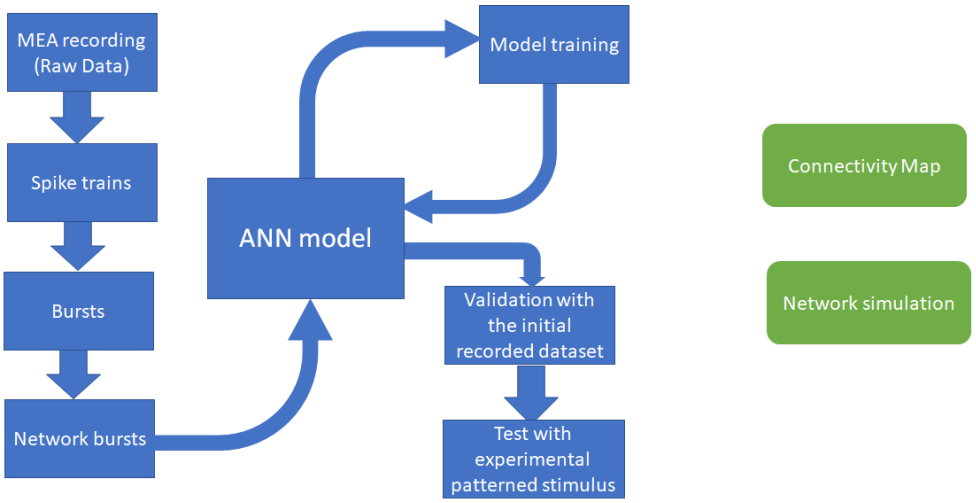

Figure 13. A diagram describing the modeling procedure

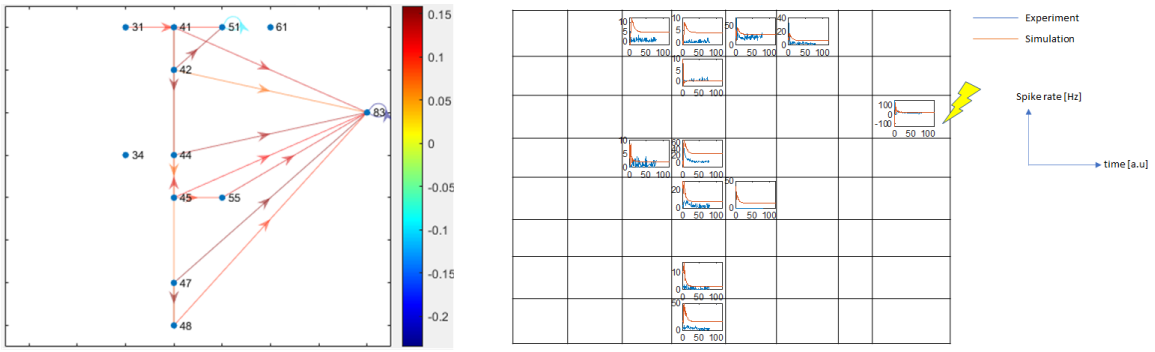

Figure 14. Results obtained from the ANN model. Left: Connectivity map describing the weighted connections between different nodes, where each node represents an electrode in MEA measurements, which in fact describes the neuronal circuit around that electrode (The Map shows connections of weights above a specific threshold). Right: Simulation of the network response to stimulation at electrode #83 and comparison with the experimental response

description of this work will be presented in future publications.

5. SUMMARY

In this proceeding we discussed the recent work progress, where we performed optogenetic stimulation of ChR2-infected neuronal culture using DLP and electrophysiological reading using MEA technology. Additionally we have developed a computational AI-based model which creates a macroscopic mapping of the neuronal network and is able to simulate the given culture under test. This tool should eventually be evolved into a feedback module between the electrophysiological signals and the optical stimulation.

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