

# Behavior of Living Human Neural Networks on Microelectrode Array Support

R. Pizzi\*, F. Gelain\*\*, A. Fantasia\*, D. Rossetti\* and A. Vescovi\*\*

\*Department of Information Technologies, University of Milano  
Via Bramante 65, 26013 Crema, Italy, [pizzi@dti.unimi.it](mailto:pizzi@dti.unimi.it)

\*\* Stem Cells Research Institute, DIBIT S.Raffaele Milano, Italy

## ABSTRACT

Researchers of the Department of Information Technologies of the University of Milano and of the Stem Cells Research Institute of the DIBIT-S. Raffaele Milano are experimenting the growth of human neural networks of stem cells on a MEA (Microelectrode Array) support.

The Microelectrode arrays (MEAs) are constituted by a glass support where a set of tungsten electrodes is inserted. We connected the microelectrodes following the architecture of classical artificial neural networks, in particular Kohonen and Hopfield networks.

The neurons are stimulated following digital patterns and the output signals are analysed to evaluate the possibility of organized reactions by the natural neurons.

The neurons reply selectively to different patterns and show similar reactions in front of the presentation of identical or similar patterns.

These results allow to design further experiments that improve the neural networks capabilities and to test the possibility of utilizing the organized answers of the neurons in several ways.

**Keywords:** neural networks, stem cells, microelectrode arrays, learning.

## 1 INTRODUCTION

During the past decade several experiments have been carried out on direct interfacing between electronics and biological neurons, in order to support neurophysiological research but also to pioneer future bioelectronic prostheses, bionic robotics and biological computation.

As microelectrodes implanted into brain give rise to rejection and infections, researches are under way, experimenting a direct adhesion between chip and neural tissue. Important results have been achieved at the Max Planck Institute [1], at the Georgia Tech [2], at the Northwestern and Genoa University [3], and at the Caltech[4].

Aim of our work is the development of architectures based on the Artificial Neural Network models on networks of human neural stem cells adhering to microelectrode arrays (MEAs). The MEAs are connected to a PC via an acquisition device that allows to stimulate the neurons with suitable inputs and to acquire the neuron signals in order to evaluate their reactions.

In this way we are investigating on the learning processes of biological neural networks and on the possible technological applications of hybrid biological/electronic networks.

## 2 MATERIALS AND METHODS

The problem of the junction between neuron and electrode is crucial: materials must be biocompatible with the culture environment, and neurons must firmly adhere to the electrodes in order to get maximum conductivity. Our support is constituted by a glass disk with tungsten  $100 \times 100 \mu$  microelectrodes. The distance between the electrodes is around  $20 \mu$ . (Fig. 1). These are connected to the PC via an USB acquisition card (IOTECH Personal Daq/56).

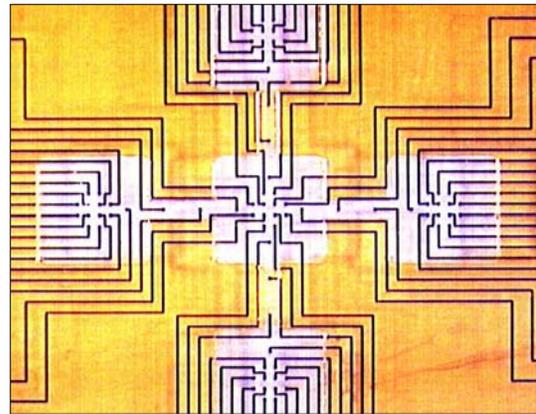


Fig. 1 Portion of the MEA support

Our neurons have been cultured starting from human neural stem cells, multi-potential undifferentiated cells whose main features are the ability of self-renewal and to differentiate into different adult cells[5].

On the MEAs we implemented two kinds of artificial architectures:

- A Kohonen Self-Organizing Map [6], composed by an input layer and an output layer connected in such a way as to classify the input by means of a competition between neurons, and
- A Hopfield network [7], fully interconnected network that stabilizes into equilibrium configurations that correspond to its memories.

We chose these models due to their straightforward architecture and their resemblance to some neurophysiological structures.

Then we realized two hardware/software models in order to discriminate simple patterns. The minimum software configurations able to recognize 8-bit patterns representing “zero” and “one” pure or affected by noise are 1) a Kohonen network with 8 input neurons and 3 output neurons and 2) a Hopfield network with 8 input/output neurons (Fig. 2).

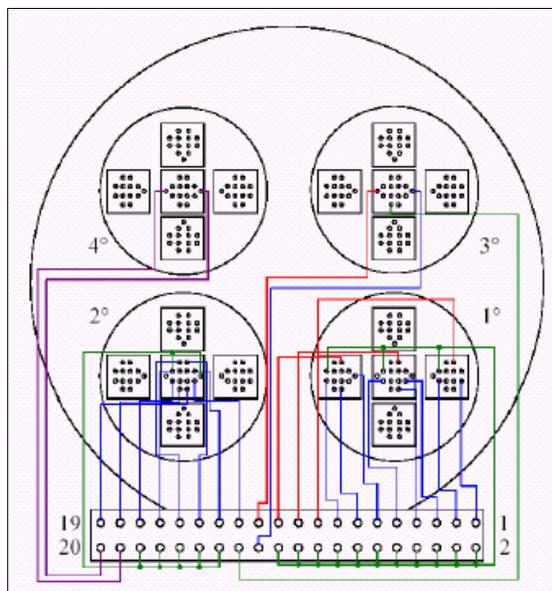


Fig. 2 Circuits of the hybrid neural networks on the MEA

These networks have been implemented on the MEAs, culturing the stem cells on the connection sites. The software allows to design the input patterns, that are converted into suitable electrical stimuli (similar to the natural action potentials) at 40 Hz by a custom hardware device. The signals are also sampled at 40 Hz. This frequency has been chosen because of its probable involvement in the most advanced brain tasks [8][9].

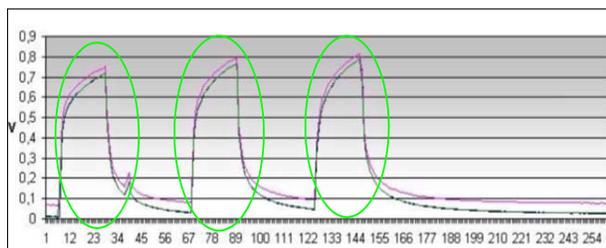


Fig. 3 Culture liquid output during stimulation with “zero” patterns

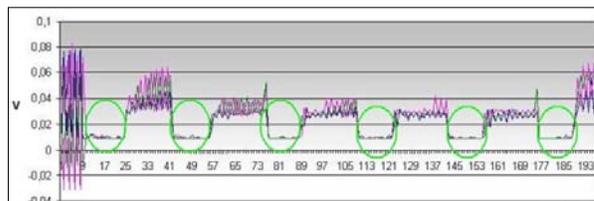


Fig.4 Network output during stimulation with “zero” patterns

In order to check if the signals received by the acquisition device were actually coming from neurons, we measured the reactions of the only culture liquid, without cells (Fig. 3), comparing them with those coming from the cells (Fig. 4).

You can see that the network reacts to the pattern “zero”, constituted by the highest voltage (“11111111”), emitting the lowest voltages. The culture liquid, instead, answers to the “zero” pattern with a high voltage, as expected by a conductive medium.

In Fig. 5 you can see the reaction of the Kohonen network after stimulation with “zero” patterns, pure and affected by noise (green circles), and with “one” patterns (red circles), pure and affected by noise. Similar effects have been shown by the Hopfield network.

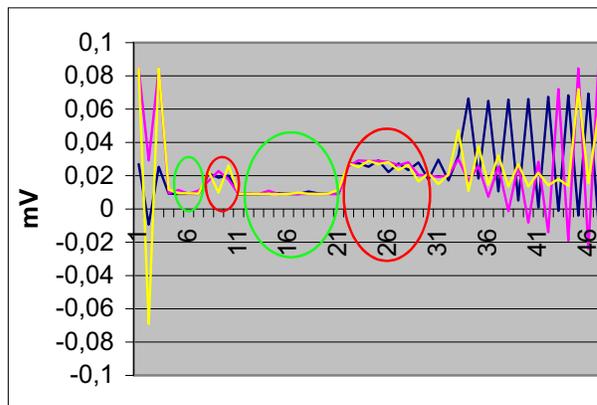


Fig. 5 Network output during training

At the end of the experiment we measured the network output in order to evaluate if the neurons had “stored” information in some way. Differently from the only culture liquid, that shows the same behaviour before and after experiment, the network output retains different voltages.

After the experiment, the output signals have been analysed using the Recurrent Quantification Analysis [10].

This non-linear analysis tool processes the time series in a multi-dimensional space. In this phase space the time series represents the dynamical system to be analysed.

The Recurrent Plots show how the vectors in the reconstructed space are near or distant each other. The Euclidean distances between all the vector pairs are calculated and translated into colour bands. Hot colours (yellow, red, orange) are associated to short distances between vectors, cold colours (blue, black) show long distances. Signals repeating fixed distances between vectors are organized, signals without repeating distances are not. In this way we obtain uniform colour distribution for random signals, but the more deterministic and self-similar is the signal, the more structured is the plot.

The analysis of our data using the RQA method lead to interesting results. Signals coming from similar bitmaps gave rise to similar Recurrent Plots. Moreover, in the following figures you can see the self-organization of a single output channel before stimulation, during the training, during the testing phase and after stimulation.

Fig. 6a shows one output channel of the Kohonen network before stimulation. Colours are cold and unstructured, showing lack of self-organization.

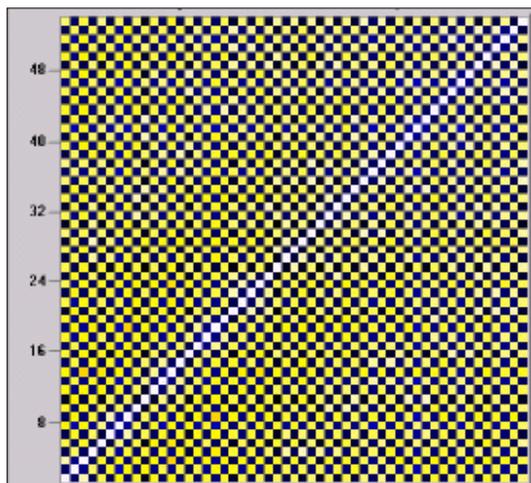


Fig. 6a RQA plot of the Kohonen network before training

The training phase shows a change in the structure. The Recurrent Plot of the channel during the testing phase shows wide uniform hot colour bands corresponding to a high organization. Fig. 6b is the plot of the output channel after the end of stimulations. In this case the uniform bands further widen, showing that the signal remains self-organized in time.

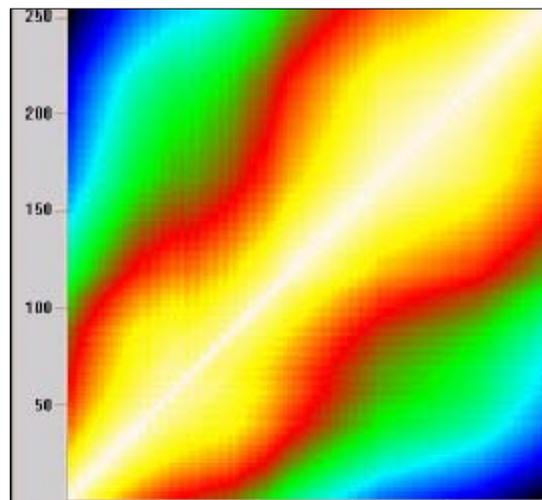


Fig. 6b RQA plot of the Kohonen network after stimulation

This analysis shows that introduction of organized stimuli modifies the network structure and increases the information content even after the end of stimulation, suggesting a form of learning and memorization.

We applied the same procedure to the output signals coming from the Hopfield network.

Fig. 7a shows one channel after stimulation with the pattern “zero”: we see wide organized bands with peculiar features, different from the other channels. Fig. 7b shows the same channels after stimulation with pattern “one”.

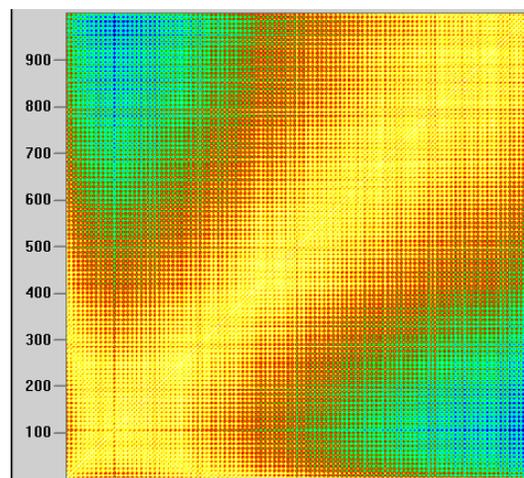


Fig. 7a RQA plot of one channel of the Hopfield network after stimulation with “zero” pattern

This analysis shows that the network behaves differently depending on the input signal and on the different channels.

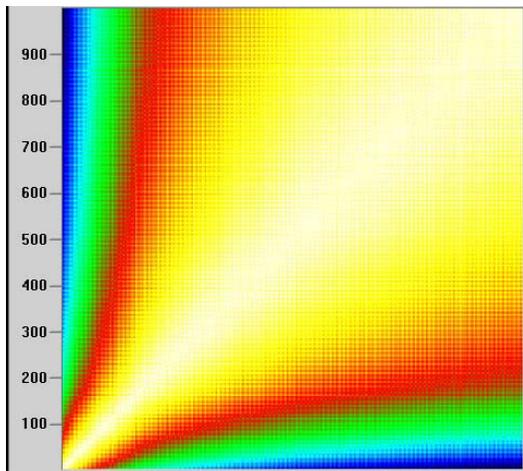


Fig. 7b RQA plot of one channel of the Hopfield network after stimulation with "one" pattern

### 3 CONCLUSIONS

After a qualitative analysis of the output signals of the networks we can reasonably affirm that the networks show an organized behavior after the stimulation with patterns, and they are able to answer selectively to different patterns. The signal behavior changes depending on the network channels, and similar patterns give rise to similar answers.

Thus we can say the networks have shown a form of selective coding, highlighting a strong self-organization as a reply to stimulation.

Other experiments with more complex patterns and new output analyses are under way. In particular, we are using non-linear methods (including Artificial Neural Networks) to discriminate the output responses in form of dynamical attractors. Preliminary analyses give the hope that discriminant interpretation of the information content kept by the networks is actually possible.

In this case we would be able to use these outputs in several ways, ranging from robotics to biological computation to neuroelectronic prostheses.

In the future we will improve both the cell growing on the MEA supports and the measure methods and tools. We will also increase the number of connections in such a way as to build more complex networks and to test their learning abilities on more complex patterns.

### ACKNOWLEDGEMENTS

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