A biological neural network drives a robotic actuator

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Abstract. During the past three years our group experimented the growth of networks of human neural stem cells on a MEA (Microelectrode Array) support. The neurons were stimulated by digital patterns and the output signals were analysed.

In previous experiments, the neurons replied selectively to different patterns and showed similar reactions in front of the presentation of identical or similar patterns.

Analyses performed with a novel Artificial Neural Network called ITSOM showed the possibility to decode the neural responses to different patterns.

In the described experiment, the neurons are connected to a robotic actuator: simulated perceptions stimulate the neurons, that react with organized electric signals. The signals are decoded by the Artificial Neural Network, that drives a minirobot.

1 Introduction

During the past decade several laboratories in the world have carried out experiments on direct interfacing between electronics and biological neurons, in order to support neurophysiological research but also to pioneer future hybrid human-electronic devices, bioelectronic prostheses, bionic robotics and biological computation [1,2,3,4,5,6,7].

During the early nineties a direct interface between nervous cells and silicon has been established. In particular leech neuron have been used, because of their big size [8]. The Fromherz’s group (Max Planck Institute of Biochemistry) first pioneered the silicon/neuron interface and keeps developing sophisticated techniques to optimise this kind of junction [9,10,11].

Many other experiments have been carried out, with different aims: William Ditto and collaborators at the Georgia Tech tried to obtain simple computations from a hybrid leech-electronics creature. As the neurons don’t behave as “on-off” elements, it has been necessary to send them signals and interpret the neural output using the chaos theory [12,13].

In 2000 a team of the Northwestern University of Chicago, University of Illinois and University of Genoa[14] developed a hybrid creature consisting of lamprey neurons connected to a robot. In front of light stimuli, the creature behaves in different ways: follows light, avoids it, moves in circle.

In 2002 Steve Potter (Georgia Tech) created a hybrid creature made by few thousand living neurons from rat cortex placed on a special glass Petri dish instrumented with an array of 60 micro-electrodes, also able to learn from environment [15].
In 2003 the Duke University’s group [16] succeeded in connecting 320 microelectrodes to monkey cells in the brain, allowing to directly translate the electrical signals into computer instructions, able to move a robotic arm. This will be the way to allow disabled people to move paralyzed limbs or electronic prostheses. In 2005 the SISSA group [17] experimented the possibility to use neurons on MEAs (Micro Electrode Arrays) as “neurocomputers” able to filter digital images.

Despite of these astonishing results, the neurophysiological research is far from understanding in detail the learning mechanism of the brain and fails to interpret the cognitive meaning of the signals coming from the neurons.

Our group used MEAs to culture human neural stem cells and an Artificial Neural Network to interpretate the neural signals. This allowed a hybrid neurons/electronics system to learn simulated perceptions and to correctly react to a following presentation of the learnt patterns.

2 Materials and Methods

We cultured human neural stem cells on MEAs, in our case constituted by glass dishes with 96 tungsten microelectrodes (Fig. 1).

Each electrode is connected, by means of a sharp insulated track, to a pad suitable for the external connection.

The distance between electrodes varies between 100 and 200 µm, the diameter of each electrode is around 20 µm.

2.1 Previous experiments

In past experiments our group verified that the cells adhering to the MEAs reply selectively to simulated perceptive stimuli [18,19]. We implemented connection schemata on the MEAs resembling an artificial neural network (ANN) architecture.

In particular, we arranged eight input channels picked from eight electrodes, on which living cells were attached (Fig.2). The cells were cultured on the connection sites of the MEAs and connected each others as in the case of the Hopfield network [20].

The stimulation occurs with a 35mV positive voltage. In order to depolarise the cultured neurons, before every bit, a negative ~35mV pulse is emitted. The pulse length is 10% of the whole bit duration, thus the whole pulse is composed by 10% negative voltage, 90% positive voltage.

As in the Hopfield model, the output channels coincide with the input channels, thus after disconnecting the stimulation circuit and after a short relaxation time (around 10 ms), the output signals were collected from the same electrodes.

![Figure 1: Portion of the MEA support](image_url)
A software simulation highlighted that the minimum configuration suitable to correctly recognize two different input bitmaps (patterns), namely “0”s and “1”s with and without noise, as depicted in Fig. 3, is represented by eight input/output neurons for the Hopfield network.

The patterns were delivered to the hybrid networks as a train of electrical pulses, in form of a digital parallel input, in such a way as to represent every black square of the bitmap as a 35mV pulse.

Our challenge was to verify if, as in the ANN paradigm, the answer given by the network of biological neurons showed an organized and selective reply.

We iterated the experiment with different stimulation lengths, ranging from 1.25 to 25 ms.

The experiments showed that the network reacts with different behaviors depending on the delivered pattern. In particular it reacts to the “0” pattern, constituted by the highest voltage (11111111), emitting the lowest voltages. A control MEA with the only culture liquid, instead, reacts to the “0” pattern with a high voltage, much higher than the neuronal voltages, as correctly expected by a conductive medium.
We used Recurrence quantification analysis (RQA) [21] to evaluate the organization state of the biological networks before and after the training procedure.

RQA is a non-linear statistics procedure suitable for physiological time series. The resulting plots show how the vectors of the dynamical system represented by the time series are near or distant each other. More specifically, RQA represents the (Euclidean) distances between all pairs of vectors and codes them as colors. 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 leads to interesting results.

First of all we observed that signals coming from similar bitmaps gave rise to similar recurrent plots. Moreover, the plots in Fig. 5 show the self-organization of a single output channel before and after stimulation.

Fig. 5a shows one output channel of the network before stimulation. Colours are cold and unstructured, showing lack of self-organization. Fig. 5b is the plot of the same output channel after the training phase. In this case the uniform bands further widen, showing that the signal remains self-organized in time.

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

Moreover, the RQA analysis shows that the network behaves differently depending on the input signal and on the different channels.

The biological network is also able to answer selectively to different patterns. The signal behavior changes depending on the network channels, and similar patterns give rise to similar answers.

2.2 The new experiment

On the basis of these results we designed a new experimental set-up with the aim to develop a system able to decode the signals coming from a networks of neurons stimulated by digital patterns.

The whole hybrid system is shown in Fig. 6.

The first phase of the experiment consists of stimulating the neurons with a set of simulated perceptions in form of four digital patterns.

Figure 5: a- RQA plot of one output channel before stimulation
     b – RQA plot of the same output channel after training
An electronic stimulation circuit converts digital 8-bit signals (patterns), generated by the software resident on the PC, from electrical signals generated by the National Instruments PCI-6036E DAQ with a logical level 0-5V, into electrical pulses with voltage and current suitable to stimulate neurons. The stimulation occurs with a 100 mV positive voltage followed by a brief -100 mV depolarization pulse. The stimulation frequency is 433 Hz, the sampling rate is 10 kHz.

As the electrophysiology of neural stem cells is not well known at the moment, these stimulation parameters have been chosen after a series of tests devoted to optimize the neural response, reaching the most physiological reactions.

Each pattern is constituted by a matrix of 8 x 8 bits. Every bit lasts 300 ms. The cells have been stimulated 2.4 sec for each pattern. Each stimulation has been followed by a 1 sec pause and has been repeated 10 times for each pattern, in order to allow the neurons to learn.

The four digital patterns are depicted below (Fig. 7).

Rows represent the 8 simultaneously activated channels, columns represent time. Each square represents one bit: if it contains a dot, this means that a stimulation has been activated, otherwise there is no stimulation. At the end of the tenth stimulation, the reactions of the cells, collected after disconnecting the stimulation circuit and after a 10 ms relaxation time, have been recorded and processed by the Artificial Neural Network.

Once the training phase was finished, a testing phase was carried out. During this phase we sent to the neurons several stimulations corresponding to one of the four patterns, in a random order.
The reactions of the neurons have been sent to the Artificial Neural Network, that classified the answers on the basis of the neural reactions recorded after the training phase.

2.3 Decoding the neural signals

The Artificial Neural Networks supplies, in form of an integer number, the direction that will drive the robot. This number is converted into a TTL signal that is directed to the parallel port of the PC. A suitable circuit converts it into an infrared signal that drives the robot.

It must be stressed that the ANN works just on the signals emitted by the biological neurons 10 ms after the end of the stimulation, thus the ITSOM's task is not to classify the digital patterns, but to decode the biological reactions to different patterns.

The model of ANN, a novel architecture called ITSOM (inductive tracing self organizing map), was selected considering that a self-organizing architecture was necessary, as we had not a set of examples to train it. The ITSOM was tested in the past with electrophysiological [22], correctly showing their organized structures.

The extremely low processing time makes this model very effective in case of real-time applications. The dynamical properties of artificial neural networks and of the SOM in particular are well known [23,24,25,26]. In particular it can be shown that, even if the winning weights may vary at any presentation epoch, their temporal sequence tends to repeat itself. Such a sequence constitutes chaotic attractors that univocally characterize the input element that has determined them (Fig. 8).

Figure 8 – The ITSOM Network identifies a series of winning neurons in time

In this way, due to the countless variety of possible combinations among winning neurons, the configurations allow to finely determine the correct value.

It should be stressed that the ITSOM's crucial feature is that the network does not need to be brought to convergence, as the cyclic configurations stabilize their structure within a small number of epochs.

After interrupting the network processing phase, an algorithm is needed that codifies the obtained chaotic configurations into a small set of outputs.

To this purpose the cumulative scores related to each input have been normalized following the distribution of the standardized variable \( z \) given by:

\[
z = \frac{x - \mu}{\sigma}
\]
where $\mu$ is the average of the scores on all the competitive layer weights and $\sigma$ is the root mean squared deviation. Once fixed a threshold $0 < \tau \leq 1$, we have put

$$
\begin{align*}
z &= 1 \quad \text{for} \quad z > \tau, \\
z &= 0 \quad \text{for} \quad z \leq \tau.
\end{align*}
$$

In this way every winning configuration is represented by a binary number with as many 1’s and 0’s as many the competitive layer weights. Then the task of comparing these binary numbers is straightforward.

Table 1: z-scores obtained from presentations of the same pattern

| Pattern R, first presentation | 0 1 0 0 1 1 0 0 1 1 1 1 0 0 1 0 |
| Pattern R, second presentation | 0 1 0 0 1 1 0 0 1 1 1 1 0 0 1 0 |
| Pattern R, third presentation | 0 1 0 0 1 1 0 0 1 1 1 1 0 0 1 0 |
| Pattern R, fourth presentation | 0 1 0 0 1 1 0 0 1 1 1 1 0 0 1 0 |
| ........................................ | ............................................ |

In Table 1 we represented the codes generated by the ITSOM elaborating a set of signals emitted by the cells after stimulating them many times with the same digital pattern. The table shows that the ITSOM generated always the same code for all the presentations of the same pattern.

In this example, after the first presentation, during competition the networks “won” 0 times in node 1, 6 times in node 2, 1 in node 3, 0 in node 4, 4 in node 5 and so on up to 20 epochs. After the z-score procedure we obtained the code as in the first row of Table 1. With the second presentation, the network “won” 0 times in node 1, just 4 times in node 1, 0 in node 2, 0 in node 3, 4 in node 4 and so on, but the z-score provided the same code.

Despite the lack of a detailed neurophysiological interpretation of the signal tracing, the ITSOM network has allowed to distinguish the different information contents of the signals.

We could show that similar patterns give rise to output signals containing similar chaotic attractors corresponding to the same ITSOM code, whereas different patterns lead to attractors corresponding to different codes.

3 Results and Discussion

In the described experiment we tested the hybrid system with 25 random patterns.

In Tables 2 and 3 the results of our experiment are displayed.

Observing the tables we can draw the following considerations.

The classification percentage of the “F” and “B” patterns reach high values (80% and 83.33%). Once eliminated the non classified signals, the B pattern is recognized with a 100% percentage. All the values are very far from the random value (25%).

In order to estimate the quality of this classification we elaborated two-classes confusion matrixes as reported in Table 4.

For each confusion matrix we can define four important parameters: False Positive (FP), False Negative (FN), True Positive (TP), True Negative (TN).
Table 2: Robot Performances

<table>
<thead>
<tr>
<th>Input</th>
<th>Directions</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pattern F</td>
<td>Pattern B</td>
</tr>
<tr>
<td>Correct classification</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Wrong classification</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>No classification</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total of the yielded patterns</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>% Classified</td>
<td>100%</td>
<td>83.33%</td>
</tr>
<tr>
<td>% Correctly classified</td>
<td>80%</td>
<td>83.33%</td>
</tr>
</tbody>
</table>

Table 3: Classification Percentage

If we define Sensitivity and Specificity of a test by means of the following formulas:

\[
Sensitivity = \frac{TP}{TP + FN} \times 100
\]

\[
Specificity = \frac{TN}{TN + FP} \times 100
\]

we obtain for the four patterns (Table 5):

Table 4: Confusion Matrixes of the model

<table>
<thead>
<tr>
<th>Confusion matrix of a pattern « P »</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>Non-P</td>
</tr>
<tr>
<td>P</td>
<td>TP</td>
<td>FN</td>
</tr>
<tr>
<td>Non-P</td>
<td>FP</td>
<td>TN</td>
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</tbody>
</table>

Confusion matrix pattern F

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Non-F</th>
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<tbody>
<tr>
<td>F</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Non-F</td>
<td>1</td>
<td>17</td>
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</table>

Confusion matrix pattern B

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Non-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Non-B</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

Confusion matrix pattern L

<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>Non-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Non-L</td>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

Confusion matrix pattern R

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>Non-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Non-R</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Pattern F</td>
<td>Pattern B</td>
<td>Pattern L</td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>45.45%</td>
</tr>
<tr>
<td>Specificity</td>
<td>94.44%</td>
<td>100%</td>
</tr>
</tbody>
</table>

These results allow us to consider the effectiveness of our hybrid classifier quite satisfactory.

4 Conclusions

In this work we describe a hybrid biological/artificial system composed by a human neural network and an Artificial Neural Network. The output of the biological neurons constitute the input of the ANN, that classifies the electrical signals coming from the neurons, self-organizing on the basis of the temporal series of the neural electrical signals.

The neurons show the ability of memorizing and learning digital patterns, so that the ANN can decode their reactions to the learnt patterns and drive a robotic actuator.

The hybrid system, tested with 25 random patterns has obtained a correct classification of the four patterns with percentages respectively of 80%, 83.33%, 42.86%, 42.86%.

The evaluation of the proposed model presents an accuracy of 80.11% and a precision of 90.50%.

Better performances can be reached in the future by a better tuning of the Artificial Neural Network. A new algorithm is under study, that substitutes the z-score procedure. During an off-line experiment the new procedure has been able to reach better performances in the classification of the proposed patterns.

We also plan to experiment more complex patterns, possibly yielded by means of sensors mounted on the robot.

Aim of this kind of research is on one side to improve the knowledge of the neurophysiological learning and memory functionalities; on the other side to evaluate the feasibility of biological computation, or of non-invasive neurological prostheses, able to improve or substitute damaged nervous functionalities.

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