Image Coding for Robotic Guidance Using Neuroblastoma Cultures

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ABSTRACT:
The main objective of this work is to analyze the computing capabilities of human neuroblastoma cultured cells and to define stimulation patterns able to modulate the neural activity in response to an image for controlling an autonomous robot. Multielectrode Arrays Setups have been designed for direct culturing neural cells over silicon or glass substrates, providing the capability to stimulate and record simultaneously populations of neural cells. If we are able to modify the selective responses of some cells with an external pattern stimuli over different time scales, the neuroblastoma-cultured structure could be trained to process image sequences.

KEYWORDS: Biological processing, image coding, multielectrode array, neural learning, robotic guidance.

1. INTRODUCTION

Using biological nervous systems as conventional computer elements is a fascinating problem that permits the hybridation between Neuroscience and Computer Science. This synergic approach can provide a deeper understanding of natural perception and may be used for the design of new computing devices based on natural computational paradigms. The brain uses millions of biological processors, with dynamic structure; slow commutations compared with silicon circuits, low power consumption and unsupervised learning. This kind of computation is more related to perceptual recognition, due to the natural variance of the perceptive patterns and a priori lack of knowledge about the perceptual domain.

There exist many research approaches based on mimicking this bioinspired parallel processing, not only from the algorithm perspective [1,2], but also from the silicon circuits design. These bioinspired approaches are useful for pattern recognition applications, like computer vision or robotics, however they are implemented over serial and artificial silicon processors with fixed and static structure. A real biological processor with millions of biological neurons and a huge number of interconnections, would provide much more computational power instead of their low transition rates due to high number of computing elements and the extraordinary network capability of adaptation and reconfiguration to unknown environments. This extraordinary capability is related with natural unsupervised learning.

Our learning experiments were performed in neural cultures containing 120.000 human neuroblastoma SY-5Y, under the assumption that this kind of cells are able to respond electrically to external stimuli and modulate their neural firing by changing the stimulation parameters. Such cultured neuroblastoma networks showed dynamical configurations, being able to develop and adapt functionally in response to external stimuli over a broad range of configuration patterns. We are especially interested in analizing if populations of neuroblastoma cells are able to process and store information, and if learning can be implemented over this biological structure. The main objective of this work is coding images acquired by a robot into a stimulation pattern based on a selective stimulation. This stimulation will be applied to the culture in order to change its neural activity and guide the robot movement.

This work describes the process of growing human neuroblastoma cells over MEA (MultiElectrode Array) substrates and tries to change the natural physiologic responses of these cells by external stimulation of the culture provided by the robot sensors. Modifying the global responses of some cells with an external pattern stimuli means adjusting the biological network behavior due to changes in synaptic efficiency or long-term potentiation (LTP). Several groups have followed the same path studying how information is processed and encoded in living cultured neuronal networks by

interfacing them to a robot [3,4]. The cells used in these experiments were extracted from cortical or hippocampal tissue of embryonic rats, but none of them have used human neuroblastoma cells, which can grow and replicate so its plasticity will be higher than in previous studies and we can achieve better results in learning experiments.

We propose a system for determining that the large neuroblastoma Networks developed in cultured MEAs are capable of learning: stablishing numerous and dynamic connections, with modifiability induced by external stimuli, in this case an image acquired by a robot through its camera.

2. HUMAN NEUROBLASTOMA CULTURES

The physiological function of neural cells is modulated by the underlying mechanisms of adaptation and reconfiguration in response to neural activity. Hebbian learning describes a basic mechanism for synaptic plasticity wherein an increase in synaptic efficacy arises from the presynaptic cell's repeated and persistent stimulation of the postsynaptic cell. The theory is commonly evoked to explain some types of associative learning in which simultaneous activation of cells leads to pronounced increases in synaptic strength. The N-methyl-D-aspartate (NMDA) receptor, a subtype of the glutamate receptor, has been implicated as playing a key role in synaptic plasticity in the central nervous system [5], where as dopamine receptors are involved in the regulation of motor and cognitive behaviors. Excitation of both presynaptic and postsynaptic neurons may open the NMDA channels, that it is closely related with Hebbian learning.

Neuroplasticity refers to the ability of the human brain to change as a result of one's experience, that the brain is 'plastic' and 'malleable'. The brain consists of nerve cells (or "neurons") and glial cells which are interconnected, and learning may happen through change in the strength of the connections, by adding or removing connections, and by the formation of new cells. "Plasticity" relates to learning by adding or removing connections, or adding cells. In this paper, neuroblastoma cultures, able of growing and establish new connections, will be the biological platform for inducing platicity in a directed or supervised process.

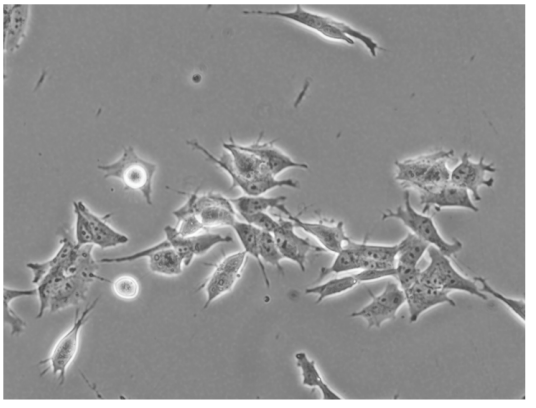
In neuroscience, LTP is a long-lasting enhancement in signal transmission between two neurons that results from stimulating them synchronously. It is one of several phenomena underlying synaptic plasticity, the ability of chemical synapses to change their strength (in chemical synapses transmission is mediated through neurotrasmitters, while in electrical synapses it is achieved by electrical currents). As memories are thought to be encoded by modification of synaptic strength, LTP is widely considered one of the major cellular mechanisms that underlie learning and memory. The term long-term potentiation comes from the fact that this increase in synaptic strength, or potentiation, lasts a very long time compared to other processes that affect synaptic strength. Induction of LTP occurs when the concentration of calcium inside the postsynaptic cell exceeds a critical threshold. In many types of LTP, the flow of calcium into the cell requires the NMDA receptor, which is why these types of LTP are considered to be NMDA receptor-dependent. NMDA receptor-dependent LTP can be induced experimentally by applying a few trains of high-frequency stimuli to the connection between two neurons, this is called tetanization, or tetanic stimulation. So, a tetanic stimulation consists of a high-frequency sequence of individual stimulations of a neuron. It is associated with long-term potentiation, the objective of this work. High-frequency stimulation causes an increase in transmitter release called post-tetanic potentiation [6]. This presynaptic event is caused by calcium influx. Calcium-protein interactions then produce a change in vesicle exocytosis. Some studies [7] use repetitive stimulation for training neural cultures, achieving activity potentiation or depression.

Another important feature of the NMDA channel is that it conducts mainly the Ca2+ ion, which may activate various enzymes for synaptic modification; even nictric oxide has been identified as a relevant element in synaptic regulation. The enhancement of synaptic transmission is called the LTP, which involves two parts: the induction and the maintenance. The induction refers to the process, which opens NMDA channels for the entry of Ca2+ ions into the postsynaptic neuron. The subsequent synaptic modification by Ca2+ ions is referred to as the maintenance of LTP.

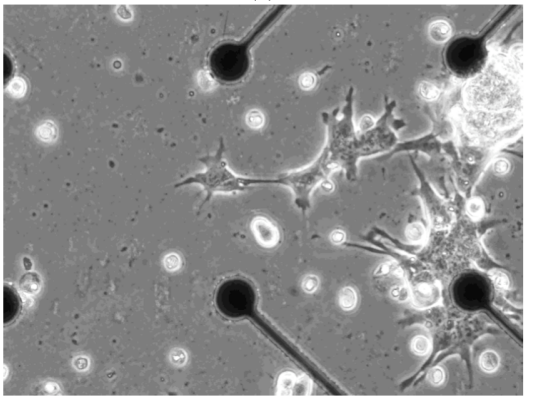
A human neuroblastoma SY5Y cell line, that express clonal specific human dopamine receptors, and also NMDA receptors, will be the biological platform for studying learning in cultured cells.

Neuroblastoma SH-SY5Y cells are known to be dopaminergc, acetylcholinergic, glutamatergic and adenosinergic, so in this line they respond to different neurotransmitters. The cells have very different growth phases, as it can be seen in Fig. 1 b). The cells both propagate via mitosis and differentiate by extending neurites to the surrounding area. The dividing cells can form clusters of cells, which are reminders of their cancerous nature, but chemicals can force the cells to dendrify and differentiate, in some kind of neuritic growth.

As conclusion, neuroblastoma culture cells show electrophysiological responses similar to standard neurons, as potential actions generation sensible to tetrodotoxin (TTX) and acetylcholyn. They have neurotransmitters synthesis process and are able to neuritic growth in culture medium.



(a)



(b)

Fig. 1. (a) Human neuroblastoma cells. (b) Neuroblastoma cells over multielectrode array.

3. EXPERIMENTAL SETUP

The neuro-physiological setup provides a complete solution for stimulation, heating, recording, and data acquisition from 64 channels. The MEA system is intended for extracellular electrophysiological recordings in vitro of different applications that include acute brain, heart, and retina slices; cultured slices; and dissociated neuronal cell cultures.

The basic components of the proposed system are shown in Fig. 2. These components are:

- A microelectrode array is an arrangement of 60 electrodes that allows the simultaneous targeting of several sites for extracellular stimulation and recording. Cell lines or tissue slices are placed directly on the MEA and can be cultivated for up to several months. Almost all excitable or spontaneously active cells and tissues can be used.
- Raw data from the MEA electrodes are amplified filter amplifiers from MCS (MultiChannel Systems, Reutlingen, Germany). with custom bandwidth and gain, which are built very small and compact using SMD (Surface Mounted Devices) technology. The small-sized amplifier combines the interface to the MEA probe with the signal filtering and the amplification of the signal. The compact design reduces line pick up and keeps the noise level down. The amplifiers are mounted over an inverted microscope.
- The analog input signals are then acquired and digitized by the MC-Card that is preinstalled on the data acquisition computer, that supplies the power for the amplifiers, and the pattern stimuli to the stimulators.
- The robot sends information about the environment to the computer using a bluetooth link. The sensor consists in a camera placed on the robot.

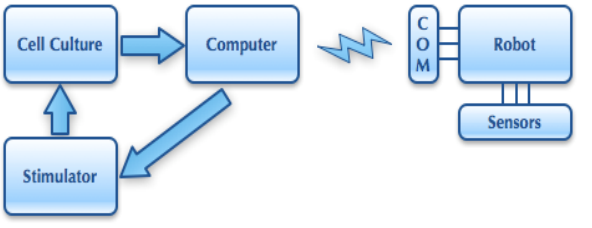


Fig. 2. Experimental Setup.

We have developed a system that provides a complete robotic control platform over neuroblastoma cultures. The system includes five free, open-source, console-based programs written in C/C++ for real-time robotic applications with embodied cultures. All of this software has been developed for the Linux Operating System and MCS hardware. Using this software in conjunction with MEABENCH is specially intended for close-loop experiments.

The software developed consists of the following programs:

- *Cult2Robot:* The main program. It has been developed as a MEABENCH module, so it can access to all of the data streams that MEABENCH provides. Cult2Robot read spikes information from MEABENCH spike detector and compute a direction vector based on MEA neural activity.
- *Stg_control:* This program controls a general-purpose stimulus generator for current and voltage-driven electrical stimulation, STG 1000 series (MultiChannel Systems, Reutlingen, Germany). The STG may be connected to a PC via RS232 port or via USB port. Stg_control make possible program and download custom functions to the STG, start/stop the stimulation, reset the device or send any command described in the STG100x RS232 Communication Manual.
- *Electrode_select:* This module allows configuring the MEA1060BC amplifier

following the MEA protocol from MCS [6]. With the Electrode_select program, it is easy to change the electrode selection during the experiment, e.g. to use stimulating electrodes for recording and vice versa. It also has almost all the features that its Windows counterpart 'MEA_select', such as blanking electrodes, changing MEA, using wait for optimizing the electrode's behaviour or reset the device.

- *BT_server:* Receiving environmental information from the robot is a primary objective. BT_server is a non-blocking Bluetooth server that uses RFCOMM protocol to receive characters from a specific MAC, process the information and do some action, e.g. it can be used to call another program when p. eg. an obstacle is detected (An 'O' has been received).
- *BT_client:* Sending information to the robot is also important to establish a bidirectional link and transmit the outputs to the robot. BT_client sends ASCII characters via Bluetooth using RFCOMM protocol.

4. METHODS

Human neuroblastoma cultures were produced using the commercial line SH/SY5Y . Neural cells were then plated on Micro-Electrode Arrays - MEAs (MultiChannel Systems, Reutlingen, Germany). Initially the nitrogen frozen cells, was immersed in a 37-degree bath, and centrifuged at 1000 rpm during 5 minutes. When cells have grown in a uniform monolayer process, they are washed three times with buffer Phosphate-buffered saline (PBS) for keeping the pH approximately constant. 0,5 percent trypsin was added to the solution in order to re-suspend cells adherent to the cell culture dish wall during the process of harvesting cells. The cells were kept in the incubator for 5 minutes and passed through a 40-microm-cell strainer (Falcon, Bedford, MA) to remove large debris. Finally the cells are transferred to a specific medium in order to inactivate trypsin, and centrifuged again during 5 minutes at 1000 rpm.

For seeding the plate cells are stained with trypan blue, (because cells that loose their permeability get colored with this solution) and counted with a Neubauer chamber. Finally, 80.000 or 120.000 total neuroblastoma cells have been placed over the MEA substrate.

Maintaining cells in culture is essential for studying their physiological properties. Cell culturing is dependent on the growth surfaces and cells must adhere to the electrode substrate in order to establish the best connection with the electrodes material. For most cultures coated tissue culture plates are prerequisite for seeding. The most commonly used coatings are positively charged polymers. In this work, the insulation layer (silicon nitride) of some of the plates was pre-treated with polyethyleneimine (PEI), showing no advantages compared with no covered plates.

The neuroblastoma cultures are maintained in a 37 degree humidified incubator with 5 per cent CO2 and 95 per cent O2 with serum-free Neurobasal medium. Under the aforementioned conditions we were able to record stable electrophysiological signals over different days in vitro (Div). The medium was replaced one-half of the medium every 5 days.

5. RESULTS

The cultured neuroblastoma cells establish synaptic connections. In Fig. 3 a) it can be seen differentiated and non-differentiated neuroblastoma cell bodies growing around the whole electrode population. The dendritic arborescence is more evident in the magnification Fig. 3 b) where differentiated neural cells surround the four electrodes while the rest of the cells are in their growing process. This figure corresponds to 80.000 neuroblastoma cells seeded in a no-PEI MEA at 2nd day in vitro (div).

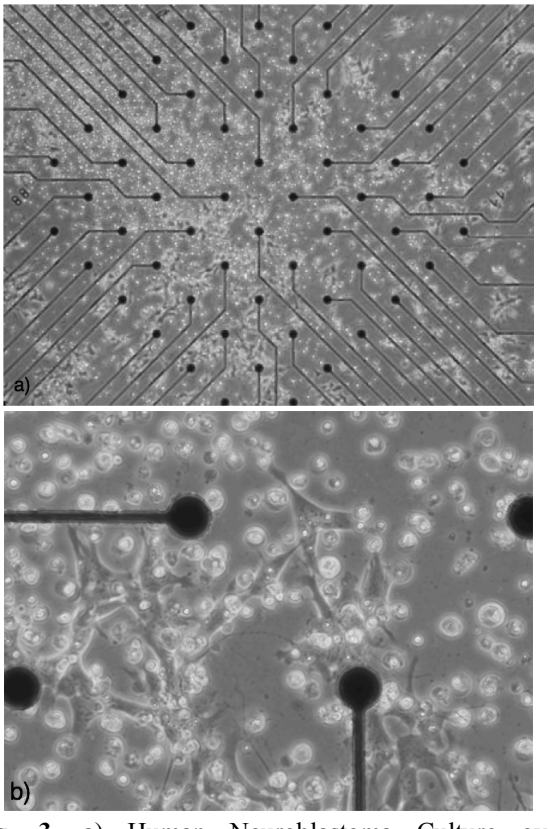


Fig. 3. a) Human Neuroblastoma Culture over Multielectrode Array (80.000 cells). b) Magnification of the culture shown in a).

The electrophysiological properties of the neuroblastoma cultures were analyzed by recording the

spontaneous activity of the network. Time course of experiments was over 15 days; recordings were done using two MCS-Meas with two neuroblastoma cell cultures (but only in one the cells survived till day 15). In vitro neuroblastoma networks show spontaneously firing. This firing rates change during the culture development with marked day differences and the global rate is closely related to the age of the network.

The physiological recordings correspond to neuroblastoma cultures in the range of 1-7 div. They show bursting and spiking activity, with usually negative depolarizations. Fig. 4 shows the spiking activity of the neural population with an automatic detection level for each electrode. This is very convenient if you have multiple channels for extracting spikes.

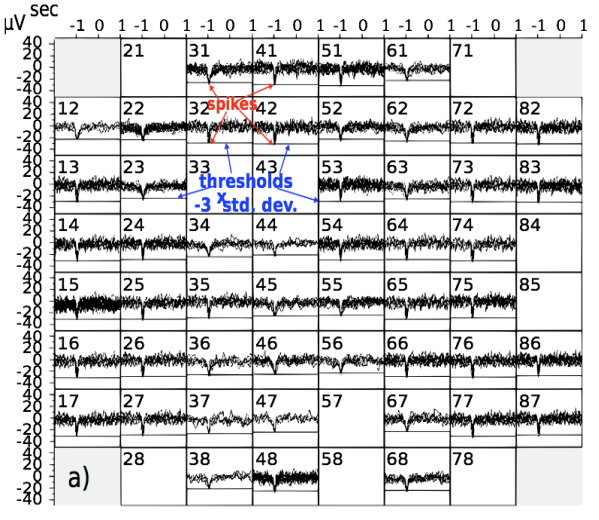


Fig. 4. a) Spontaneous neural activity detected by the multielectrode array.

The standard deviation of each data trace is used to estimate its spike threshold. A time interval of 500 ms is used to calculate the standard deviation. By fixing the factor, by which the standard deviation is multiplied, the sign of the factor determines whether the spike detection level is positive or negative, only values above this will be extracted as spiking activity. A value between -1 and -4 of the standard deviation is appropriate for most applications the threshold was fixed at standard deviation equal to -3 with respect to the electrode activity in order to identify spikes embedded in the noisy signals. During the neuroblastoma development, a wide range of population bursting or synchronized activity has been observed, according to some studies in neural cultures preparations [9]. The burst usually contains a large number of spikes at many channels, with variable duration, from milliseconds to seconds.

In our first approximation we used tetanic stimulation for programming the culture [6,7,10] getting temporal changes in the culture behavior.

5.1. Robotic Control

In previous papers [10,11] we proposed to compute the vector resulting from neural activity recorded in the human neuroblastoma culture and this vector was provided to the robot in order to guide its movement. The sensors detected the obstacles, and the information was passed to the computer in order to induce a selective tetanization of the biological neural network for changing the resulting direction vector.

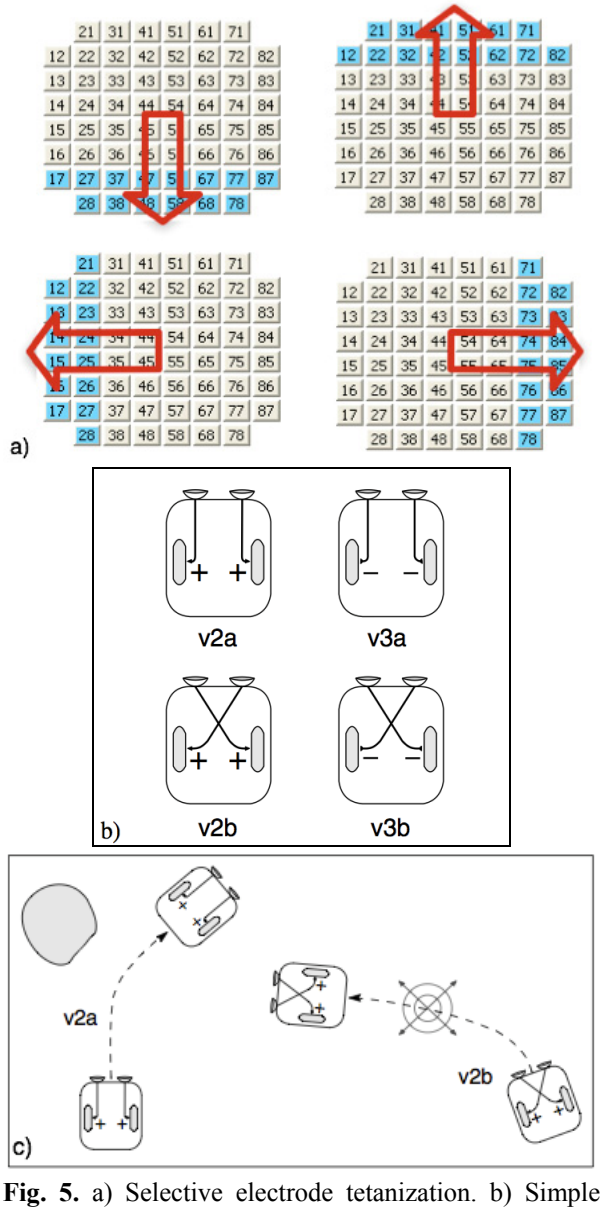


Fig. 5. a) Selective electrode tetanization. b) Simple Braitenberg's vehicles showing sensory motor connections. c) Braitenberg's vehicles behaviors.

In Fig. 4a, the selected electrodes for the tetanization are shown in order to selectively induce a persistent change in the biological neural network

behavior. This robotic control system aimed to implement Braitenberg's vehicles [12] with the following scheme: an increase in the neural activity of lateral part of the culture will imply an increase of the speed in the corresponding robot motor, (Braitenberg's vehicle v2a, see Fig. 5b) and 5c)).

If the scheme is transposed in a cross-modal connection, which is every part of the culture is connected to the opposite lateral motor, the robot behavior changes, orienting its direction to the stimulus source, Braitenberg's vehicle v2b. So, when the robot detected an obstacle in his left path, a stimulation signal was sent to the system for tetanizing the right tissue. By tetanizing the electrodes of the right part of the array, an increase in the firing rate of the neural cells that lie in the part of the culture was achieved, and the direction vector pointed to the right in this particular case.

Coding images for robotic guidance makes it difficult to use the previous scheme. Because of that, we propose to apply a simple centre of area method, presented in earlier papers [13,14]. In this method we compute the winner neurons (that is the ones that increase more its firing characteristics) resulting from neural activity recorded in the human neuroblastoma culture stimulated using a centre of area method [15, 16].

This centre of area concept is a construction that emerges from the visual or ranging perception of the robot. In Fig. 6, it can be seen a simulation of what robot would see walking through a cave. This image will be digitalized in three grey levels, black, white and grey, in order to provide three different stimulations to the neural culture, no stimulation, high tetanization, and medium stimulation respectively.

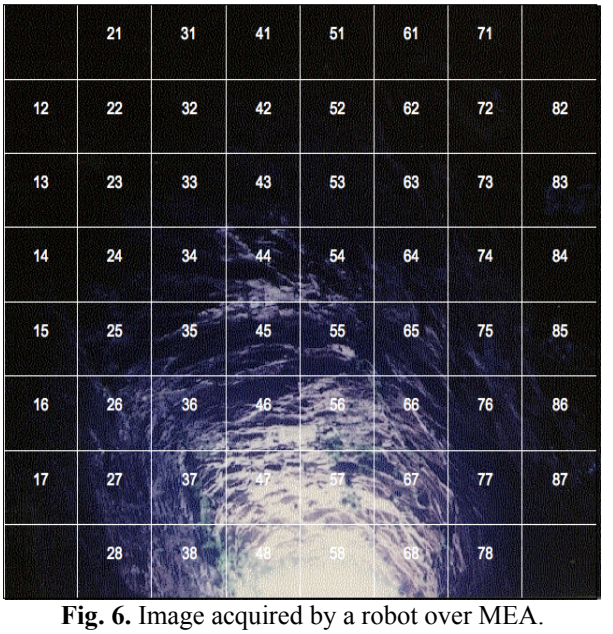


Fig. 6. Image acquired by a robot over MEA.

The resulting stimulation configuration is shown in Fig. 7a. Red boxes correspond to no-stimulation; blue boxes correspond to medium tetanization, while white electrodes will deliver high tetanization according with the acquired cave image. Medium tetanization will consist in five trains of a hundred anodic first pulses with 1 V amplitude, while high tetanization will provide 1,5 V anodic first pulses, Fig. 7b. From this example, it is expected that electrodes that cover the centre of area of the grey and white image, that is the electrodes centered at the bottom of the image, electrodes 48 and 58, will increase more their activity. This winner neuron coordinates will be provided to the robot in order to guide its movement. In the new robot position the camera will send a new image, and the information will be passed to the computer in order to induce a selective tetanization of the biological neural network for changing the resulting direction vector.

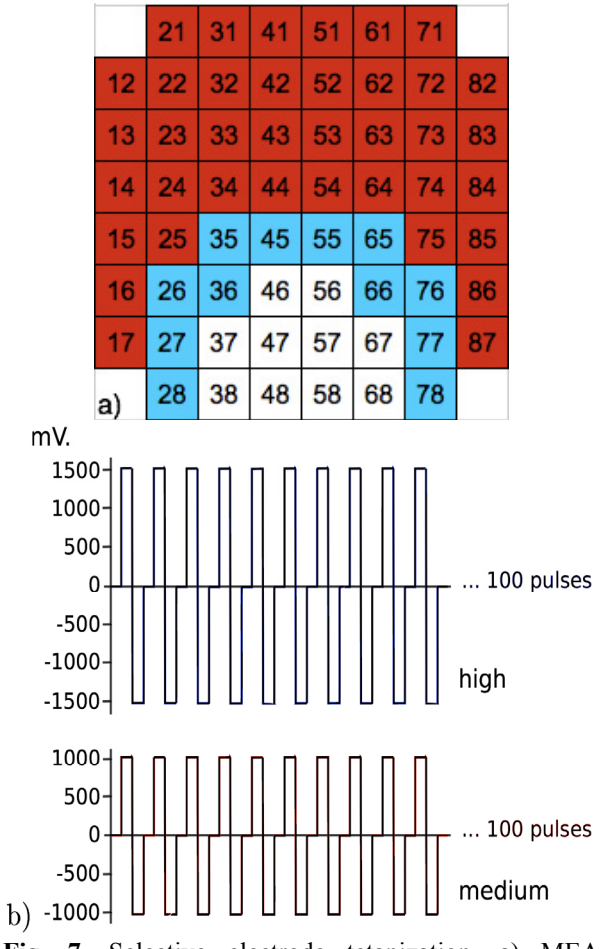


Fig. 7. Selective electrode tetanization. a) MEA representation showing three groups of electrodes, red ones are not stimulated, light blue ones are stimulated with medium tetanization and white ones are stimulated with high tetanization. b) High and medium tetanization pulses train, anodic first waveform.

The computer codes the image into a selective tetanization using software developed for us called im2MEA. This software allows loading an image and getting a three gray level image and its corresponding stimulation (see Fig. 8). It allows to specify the gray levels thresholds for selectivity and provides the commands needed to apply such stimulation with a MultiChannel Systems Stimulator STG100x. Im2MEA is still under development and our goal is to achieve with it an autonomous scheme for coding images for robot guidance.

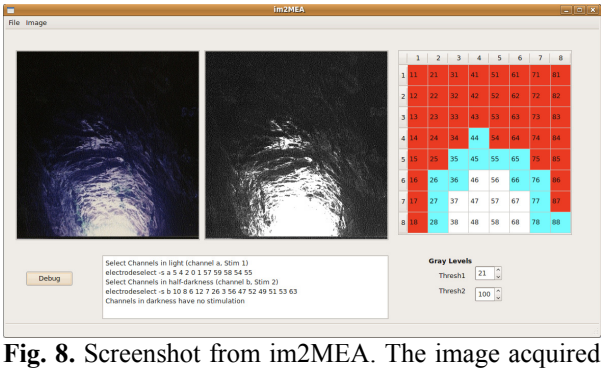


Fig. 8. Screenshot from im2MEA. The image acquired by a robot (left) is transformed into a three gray levels image (centre) and finally transformed into stimulation (right).

It.	E58	E19	E22	E26	E29	E30
1	7	11	4	7	17	4
2	7	7	3	10	12	9
3	4	8	5	9	11	5
4	6	12	5	9	11	5
5	15	9	10	6	13	4
6	8	6	4	7	8	1
7	8	2	3	14	15	1
8	12	2	10	9	12	4
9	9	6	6	8	10	4

60	23	25	28	31	34	36	61
20	21	24	29	30	35	38	39
18	19	22	27	32	37	40	41
15	16	17	26	33	42	43	44
14	13	12	3	56	47	46	45
11	10	7	2	57	52	49	78
9	8	5	0	59	54	51	50
62	6	4	1	58	55	53	63

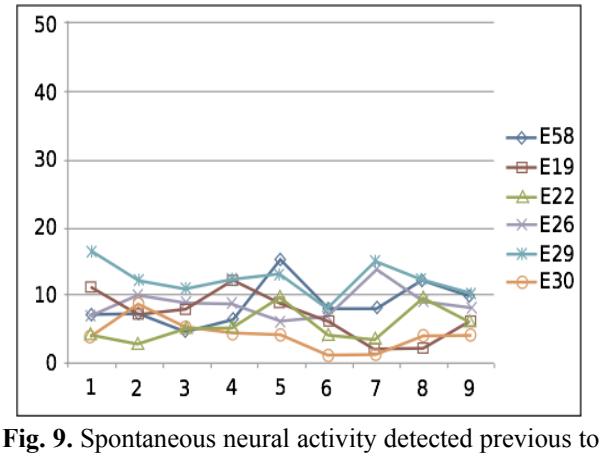
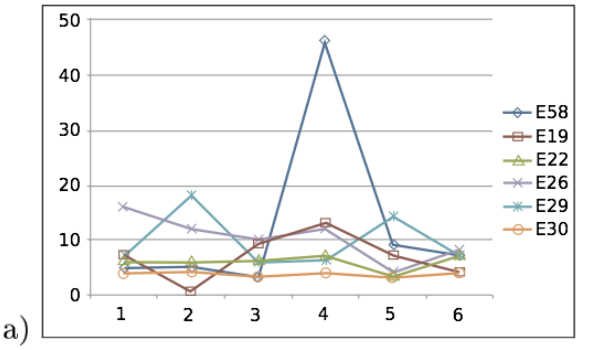


Fig. 9. Spontaneous neural activity detected previous to tetanization. Spikes number (y-axis) from six electrodes were recorded 9 times (x-axis). Left table shows data in numeric format, right table shows a MEA representation with selected electrodes marked out. Spikes number is always below 20.

It.	E58	E19	E22	E26	E29	E30
1	5	7	6	16	6	4
2	5	1	6	12	18	4
3	3	9	6	10	6	3
4	46	13	7	12	6	4
5	9	7	3	4	14	3
6	7	4	7	8	7	4

60	23	25	28	31	34	36	61
20	21	24	29	30	35	38	39
18	19	22	27	32	37	40	41
15	16	17	26	33	42	43	44
14	13	12	3	56	47	46	45
11	10	7	2	57	52	49	78
9	8	5	0	59	54	51	50
62	6	4	1	58	55	53	63



It.	E58	E19	E22	E26	E29	E30
1	23	10	8	8	5	5
2	148	10	11	11	20	8
3	198	15	7	15	15	3
4	33	15	5	9	16	11
5	24	9	4	8	14	6
6	3	14	9	8	13	4

60	23	25	28	31	34	36	61
20	21	24	29	30	35	38	39
18	19	22	27	32	37	40	41
15	16	17	26	33	42	43	44
14	13	12	3	56	47	46	45
11	10	7	2	57	52	49	78
9	8	5	0	59	54	51	50
62	6	4	1	58	55	53	63

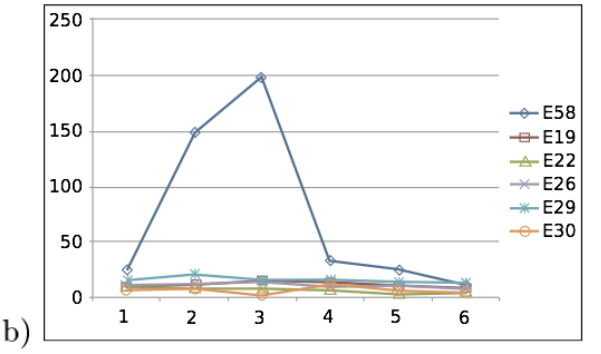


Fig 10. Spontaneous neural activity detected during a) and after b) selective tetanization. In b) was used the same selective stimulation as in a). Note the difference between y-axes scales in a) and b), it shows a considerable increase in activation at electrode 58. See Fig. 9 foot for more explanations.

Again, human neuroblastoma cultures were produced using the commercial line SH/SY5Y. Cell culture of SH SY5Y was grown in DMEM (Gibco) completed with 10% of fetal bovine serum at 37oC in 5% CO2 and humidify atmosphere. The electrophysiological properties of the neuroblastoma cultures were analyzed by recording the spontaneous activity of the network. In vitro neuroblastoma networks show spontaneously firing. This firing rates change during the culture development with marked day differences and the global rate is closely related to the age of the network. Recordings of Neuroblastoma SH-SY5Y has the disadvantage of having a very low signal to noise ratio. As we have shown in previous

papers, the electrophysiological properties of the culture change with the age of the culture, getting a potentiation effect in the spontaneous firing. A young neuroblastoma culture (1-5 DIV) has a low spontaneous firing activity, with a signal to noise ratio barely higher than 1:1. A mature neuroblastoma culture (1-15 DIV) has a higher spontaneous firing activity and its SNR may be higher than 2:1, but still is lower than SNR of other cells. The physiological recordings correspond to neuroblastoma cultures in the range of 1-7 div. They show bursting and spiking activity, with usually negative depolarizations. It was used also an automatic detection level for each electrode. This is very convenient if you have multiple channels for extracting spikes.

Spontaneous activity was recorded for intervals of 5 seconds before stimulation, and the total number of spikes extracted was counted for each channel. Fig. 9 shows the total number of spikes per channel, and a graphic visualization of this data for six different electrodes located at different positions at the neural culture.

When the tetanization configuration shown in Fig. 7 was applied, the spiking characteristics of the neuroblastoma culture changed. The computed spikes per channel is shown in Fig. 10a) during the tetanization process. It can be seen that the most significant increment registered is at electrode 58, that matches the centre of area of the provided image, guiding in this way the robot to the light. When the image of the cave was presented once again, that is the same selective stimulation was provided, the registered activity was again modified. In Fig. 10b) it can be seen a clearly potentiation effect in electrode 58 about 4 times, while the rest of the electrodes didn't show any significant increase.

6. DISCUSSION AND CONCLUSION

Learning in cultured neuroblastoma networks by a stimulation process, without the involvement of a natural adaptation process to the environment requires identifying the correct stimuli to provide to the neurons maintained ex vivo. These neuroblastoma networks form a large culture covering the whole electrode array and generating a rich dendritic configuration. The connectivity can be modulated by external stimulation as has been described in many studies, but also the activity of the network can be modulated with the appropriate stimulation scheme. We use a stimulation configuration based on the imaged captured by a robot camera. It is very important to adjust the frequency of the train pulses of the stimulation for guiding the robot to the light

Future work consists of determining the optimal stimulation to apply for inducing permanent firing changes in the culture using the image captures, and the strategies for connecting the robot sensors to the stimulation patterns. These aspects will then constitute the basis for inducing stable goal-directed plasticity, and hence for designing new biological neuroprocessors applied to robotics.

7. ACKNOWLEDGMENT

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