A BIOLOGICAL NEURAL NETWORK FOR ROBOTIC CONTROL

Towards a Human Neuroprocessor

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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 external stimuli 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. This paper tries to modulate the natural physiologic responses of human neural cells by tetanic stimulation of the culture. If we are able to modify the selective responses of some cells with a external pattern stimuli over different time scales, the neuroblastoma-cultured structure could be trained to process pre-programmed spatio-temporal patterns. We show that the large neuroblastoma networks developed in cultured MEAs are capable of learning: stablishing numerous and dynamic connections, with modifiability induced by external stimuli.

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 the 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 (Anderson and Rosenfeld, 1998), 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.

Learning is a natural process that needs the creation and modulation of sets of associations between stimuli and responses. For understanding the process of learning, is necessary to define the physiological mechanisms that support the creation and modulation of associations and determine the relation that modulate the configuration between stimuli and responses associations. These mechanisms and relation have been studied by many

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neurophysiological studies at different levels mainly in single cell experimentation.

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, beeing 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 to analyze the computing capabilities of human neuroblastoma cultured cells for controlling a 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 . This paper describes the process of growing human neuroblastoma cells over MEA 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 a external pattern stimuli means adjusting the biological network behaviour due to changes in synaptic efficiency or long-term potentiation (LTP). Therefore, the neuroblastoma-cultured structure could be trained to process pre-programmed spatio-temporal patterns. In what follows, we show that the large neuroblastoma networks developed in cultured MEAs are capable of learning: stablishing numerous and dynamic connections, with modifiability induced by external stimuli.

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 CNS (Bading and Greenberg, 1991), where as dopamine receptors are involved in the regulation of motor and cognitive behaviors. For most synaptic ion channels, activation (opening) requires only the binding of neurotransmitters. However, activation of the NMDA channel requires two events: binding of glutamate (a neurotransmitter) and relief of $Mg2+$ block. NMDA channels are located at the postsynaptic membrane. When the membrane potential is at rest, the NMDA channels are blocked by the $Mg2+$ ions. If the membrane potential is depolarized due to excitation of the postsynaptic neuron, the outward depolarizing field may repel $Mg2+$ out of the channel pore. On the other hand, binding of glutamate may open the gate of NMDA channels (the gating mechanisms of most ion channels are not known). In the normal physiological process, glutamate is released from the presynaptic terminal when the presynaptic neuron is excited. Relief of $Mg2+$ block is due to excitation of the postsynaptic neuron. Therefore, excitation of both presynaptic and postsynaptic neurons may open the NMDA channels, this is closely related with Hebbian learning.

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 long-term potentiation (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 Figure 1. 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.

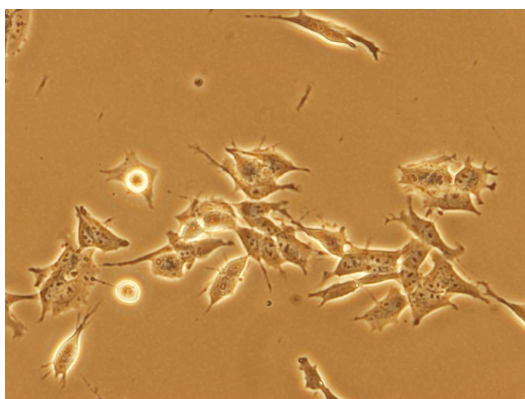

Figure 1: Human neuroblastoma cells.

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.

3 EXPERIMENTAL SETUP

The neuro-physiology setup provides a complete solution for stimulation, heating, recording, and data acquisition from 64 channels. The MEA (microelectrode array) 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 Figure 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 by MCS filter amplifiers 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 microscopes.
- 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 infrared sensors for detecting obstacles.

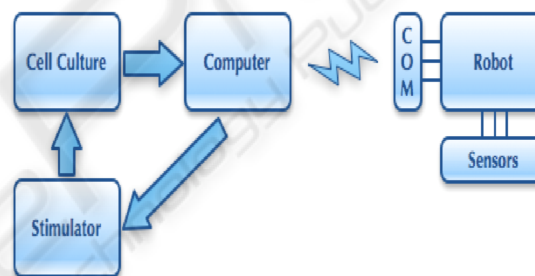

Figure 2: Experimental Setup.

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 mono-layer process, they are washed three time with buffer Phosphate-buffered saline (PBS) for keeping the pH approximately constant. 0,5 per cent 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 centrifugated again during 5 minutes at 1000 rpm.

For seeding the plate cells are stained with trypan blue, (because cells that loose their permeability get colorred 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 Figure 3 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 Figure 3 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).

The electrophysiological properties of the neuroblastoma cultures were analized 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.

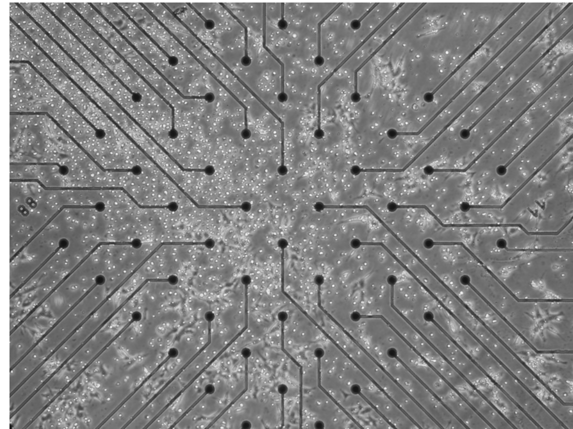

Figure 3: Biological neural network over multielectrode array.

The physiological recordings correspond to neuroblastoma cultures in the range of 1-7 div. They show bursting and spiking activity, with usually negative depolarisations. Figure 4 show 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.

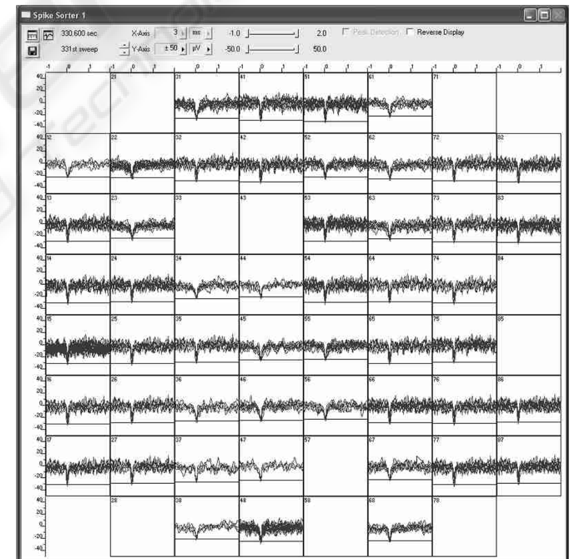

Figure 4: Spontaneus neural activity detected by the multielectrode array.

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 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 (Wagenaar, Pine, and Potter, 2006). The burst usually contains a large number of spikes at many channels, with variable duration, from milliseconds to seconds.

5.1 Tetanic Stimulation

Spontaneous activity was recorded for intervals of 3 minutes before stimulation (PRE-data), and the total number of spikes extracted was counted. The biphasic stimulus consists in a 10 trains of a 100 anodic-first waveform with 1 Volt amplitude delivered to all 60 electrodes in order to propagate a tetanization stimulus to the neuroblastoma culture.

In neurobiology, 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 (Antonov, Antonova, Kandel, 2003). This presynaptic event is caused by calcium influx. Calcium-protein interactions then produce a change in vesicle exocytosis. Some studies (Jimbo, Robinson, and Kawana, 1998) use repetitive stimulation for training neural cultures, achieving activity potentiatiation or depresion

Once the tetanization stimulus was applied to the whole population 5 minutes after the stimulation a 3 minutes interval was recorded (POST-data). Only neuronal signals which had at least a 2:1 signal:noise ration were valued as "spikes". Again, the total number of spikes extracted was counted. This process was made for cultures at 1 day in vitro (div), 5 div and 16 div. Figure 5 represents the counted spikes with bar charts for the different recordings. The conclusion from this Figure is:

1) While the neuroblastoma culture is growing new connections are created, and the number of spikes increases as the culture expands over the MEA.
2) After a tetanic stimulation the cells continue with their increased spiking rate, providing a persistent change in the culture behaviour. When this change in the network response lasts, these changes can be called learning.

In all the experimentation performed, tetanic stimulation was applied as training method, and the electrophysiological properties of the neuroblastoma culture change, getting a potentiation effect on the spontaneous firing, modulating in this way the culture neural activity.

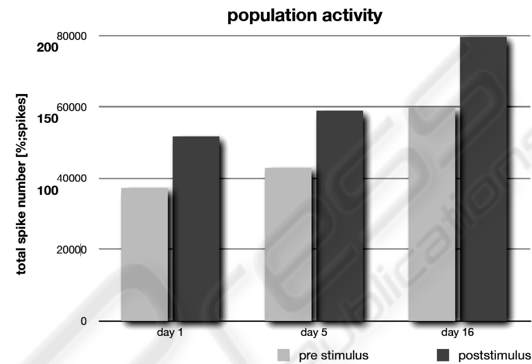

Figure 5: Induced neural activity by tetanization stimuli.

5.2 Robotic Control

For controling the direction of the robot we propose to compute the vector resulting from neural activity recorded in the human neuroblastoma culture. This vector will be provided to the robot in order to guide his movement. The sensors will detect the obstacles, 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. In Figure 6, the selected electrodes for the tetanization are shown in order to selective induce a persistent change in the biological neural network behaviour.

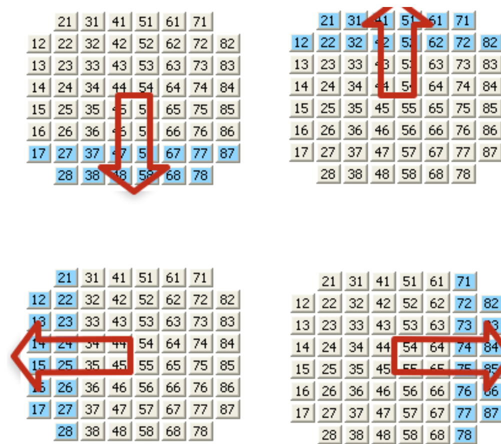

Figure 6: Selective electrode tetanisation.

When the robot detect an obstacle in his left path, an stimilation signal will be sent to the system for tetanizing the right tissue. By tetaniztion 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 will be achieved, and the direction vector will point to the right in this particular case.

We expect to apply some basic Braitenberg principles to the system in order to study the biological neural network behaviour induced by a tetanization learning scheme.

6 DISCUSSION

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.

Tetanization consists in high-frequency stimulation to the culture, in order to cause an increase in transmitter release called post-tetanic potentiation. The results illustrate the existence of qualitatively different responses to stimulation. Our results indicate the existence of a clear facilitation mechanism in response to the tetanization stimuli at different stages of cell development. Since this kind of stimulation has been used in attempts to induce plasticity in neuroblastoma, refining some crucial aspects of the stimulation is still indispensable.

It is very important to adjust the frequency of the train pulses of the stimulation for suppressing bursting in the culture. While in vivo networks suppress bursting naturally with the tissue development and sensory inputs, ex-vivo cultures need to reduce this synchronized activity by adjusting the stimulation parameters. Also, for superimposing a desired behaviour on the biological networks it is necessary to stimulate locally some part of the culture in order to facilitate some parts of the networks, or achieve some kind of electrical stimulation that depress the local activity of a restricted location. With this local potentiation-inhibition scheme the culture global behaviour could be controlled.

Future work consists in determine the optimal stimulation to apply for inducing permanent firing changes in the culture, 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.

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