33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Human Neuroblastoma Cultures for Biorobotics

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Abstract— **This paper introduces a new biorobotic system using human neuroblastoma cultures and centre of area learning for basic robotic guidance. 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. The main objective of this work will be to control a robot using this biological neuroprocessor and a new simple centre of area learning scheme. The final system could be applied for testing how chemicals affect the behaviour of the robot or to establish the basis for new hybrid optogenetic neuroprostheses based on stimulating optically genetic-modified neurons.**

I. Introduction

MAMMALIAN nervous systems exhibit complex computational functions including sensory functions, motor function and in humans, abstract thought. In particular, pattern recognition exhibited in our olfactory, visual and auditory functions are of particular interest to the abilities to smell, vision are of particular interest to the electronic and computing communities. Meanwhile several approaches attempt to mimic/substitute sensory or neural elements (missing by congenital state or due to pathological processes) in order to enable/restore function by establishing neuro-electronic interfaces.

Our learning experiments were performed in neural cultures containing 120.000 human neuroblastoma SY-5Y, under the assumption that these 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 have shown dynamical configurations, being able to grow and adapt functionally in response to external stimuli over different 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 will be to control a robot using this biological neuroprocessor and a simple centre of area learning scheme. The final system could be applied for testing how chemicals affect the behaviour of the robot or to establish the basis for new hybrid optogenetic neuroprostheses.

Manuscript received January 30, 2010. This work was supported by the Spanish Government through grants TIN2008-06893-C03, TEC2006-14186-C02-02 and SAF2008-03694, Cátedra Bidons Egara, Fundación Séneca 08788/PI/08, CIBER-BBN and by the European Comission through the project "`NEUROPROBES"' IST-027017.

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II. Learning in 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, 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.

A human neuroblastoma SY5Y cell line, that expresses 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 dopaminergic, 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 that are reminders of their cancerous nature, but chemicals can force the cells to dendrify and differentiate in some kind of neuritic growth. The cultured neuroblastoma cells establish synaptic connections. The Figure 1 shows differentiated, neuron-like, and non-differentiated, round cells, neuroblastoma cell bodies growing around the whole electrode population. Differentiated neural cells surround the four electrodes while the rest of the cells are in their growing process. This Figure corresponds to 80.000 human neuroblastoma cells seeded in a no-PEI MEA at 2nd day in vitro (div).

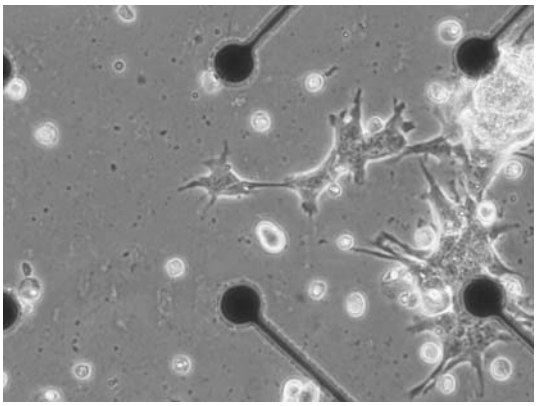

Figure 1: Human neuroblastoma cells over MEA

III. 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 minimizes 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 black and white camera for detecting obstacles.

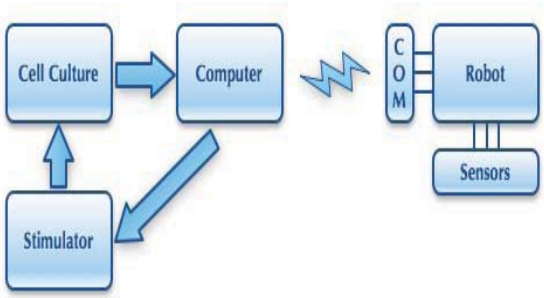

Figure 2: Experimental Setup

The software used is a modified Meabench-based control module. Meabench [3] is a free, open-source, set of programs designed primarily for Linux for multi-electrode data acquisition (DAQ) and online analysis. Meabench directly communicates with DAQ hardware as well as providing real-time visualization. Thanks to its ability to communicate in real-time with stimulator hardware it can be used in closed-loop stimulation experiments. 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 (MultiChannel Systems, Reutlingen, Germany).

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 37 ^{o}C in 5% CO2 and humidify atmosphere.

IV. Tetanic Stimulation

In Neurobiology, a tetanic stimulation consists of a high-frequency sequence of individual stimulations of a neuron. It is associated with long-term potentiation, which is the objective of this work. High-frequency stimulation causes an

increase in transmitter release called post-tetanic potentiation [1]. This presynaptic event is caused by calcium influx. Calcium-protein interactions then produce a change in vesicle exocytosis. Some studies [2] use repetitive stimulation for training neural cultures, achieving activity potentiation.

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

V. Robotic Control

For controlling the direction of the robot we propose to compute the winner neurons (that is the ones that increase more its firing characteristics most) resulting from neural activity recorded in the human neuroblastoma culture stimulated using a centre of area method. In Mechanics, the branch of Physics which studies the movement of bodies according to causes that produce it, there exists a magnitude called the centre of mass. For a discrete system, the position of the centre of mass is the weighted mean of particle coordinates in the system, the weights being the relative contributions of each particle to the system's total mass. This centre of mass concept can be applied to robot environment modeling, by replacing the mass with the accessible area detected. Note that this Centre of Area concept is a construction that emerges from the visual perception of the robot. Figure 3 shows the image captured by the robot camera while walking through a cave, divided in 64 labeled visual areas. This segmented image is being digitized in three gray levels, black, white and gray, to provide three different stimulations to the neural culture, no stimulation, high tetanization, and medium stimulation respectively.

The resulting stimulation configuration is shown in Figure 4. Gray boxes correspond to no-stimulation, red boxes correspond to medium tetanization while blue 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. From this example, it is expected that electrodes that cover the centre of area of the gray and white image, that is the electrodes at bottom center of the image, electrodes 48 and 58, will increase their activity more relative to the remaining electrodes. This winner neuron coordinates will be provided to the robot to guide his movement. In the new robot position, the camera will send a new image, and the information will be passed to the computer to induce a selective tetanization of the biological neural network for changing the resulting direction vector.

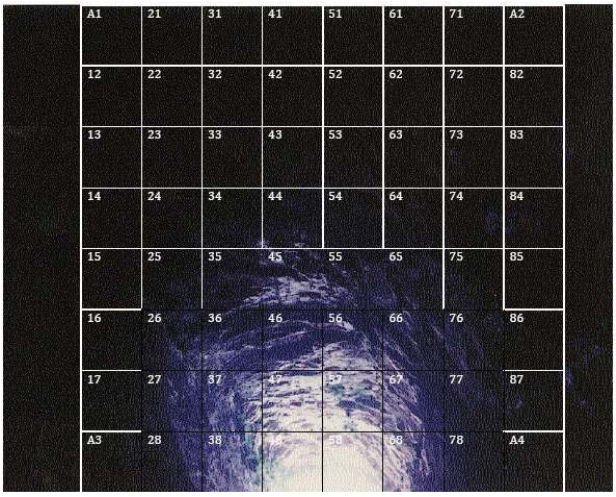

Figure 3: Image acquired by a robot over MEA

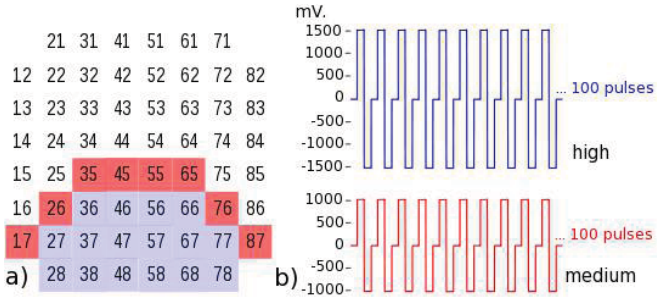

Figure 4: Selective electrode tetanization

VI. Results

The electrophysiological properties of the neuroblastoma cultures were analized 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 [4][5], 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 signal to noise ratio (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 depolarisations. Figure 5 shows the spiking activity of the neural population with an automatic detection level for each electrode. This is very convenient when multiple channels exist for extracting spikes.

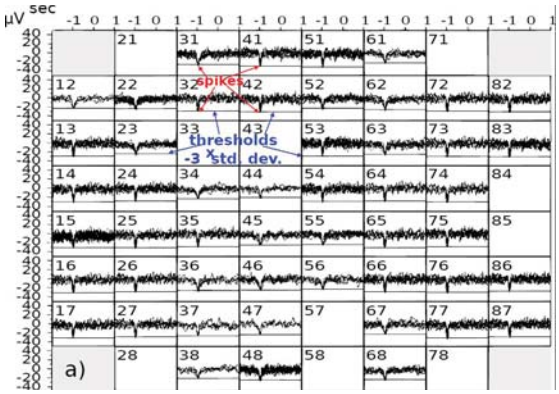

Figure 5: Spontaneous neural activity detected by the multielectrode array

The standard deviation of each data trace is used to estimate its spike threshold and computing the spikes per channel. A time interval of 500 ms is used to calculate the standard deviation. The threshold was fixed at standard deviation equal to -4 with respect to the electrode activity in order to identify spikes embedded in the noisy signals. Spontaneous activity was recorded for intervals of 5 seconds before stimulation, and the total number of spikes extracted was counted for each channel. Figure 6 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. Spontaneous neural activity detected previous to tetanization. Spikes from six electrodes were recorded 9 times. Table A shows data in numeric format, table B shows a MEA representation with selected electrodes marked out. Spikes number is always below 20. Figure 6C shows the temporal evolution of the selected electrodes marked, represented numerically in Table A, with no significant activity on any electrode.

A

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

B

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	50
9	8	5	0	59	54	51	50
62	6	4	1	58	55	53	63

C

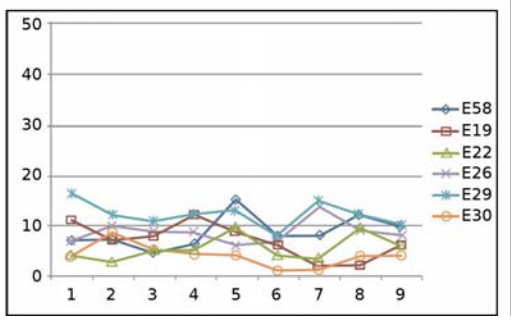

Figure 6: Spontaneous neural activity detected previous to tetanization

When the tetanization configuration shown in Figure 4 was applied, the spiking characteristics of the neuroblastoma culture changed. The computed spikes per channel is shown in Figure 7 during the tetanization process. It can be seen that the electrode that registers the most significant increment is electrode 58, that matches the centre-of-area of the provided image, guiding in this way the robot to the light. A clear potentiation effect of above 4 times in electrode 58 can be seen, while the rest of the electrodes did not show any significant increase. In this way, selecting the orientation of the robot as the neuron or group of neurons that increase in a more quantitative aspect its firing characteristics, it is possible to guide the robot to the light or the brightest area of the discretized scene. This change lasts for seconds, in this case a biped robot will be the perfect candidate due to its limited movement. The robotic control will be refined in future works, while in this paper the plasticity of the center of area stimulation is presented.

A

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

B

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

C

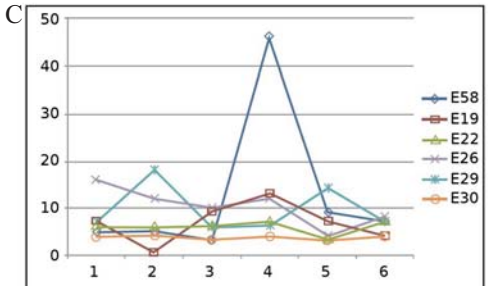

Figure 7: Spontaneous neural activity detected during tetanization

VII. Results

Human neuroblastoma cultures have electrophysiological behaviour similar to neural cultures and their activity can be modeled using tetanization schemes. 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. Future work consists of determining the optimal stimulation to apply for inducing permanent firing changes in the culture. These aspects will then constitute the basis for analizing the behaviour change by adding chemicals to the culture and by designing new optogenetic hybrid neuroprostheses which permit stimulating only with light the neural cultures.

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