LEARNING IN BIOLOGICAL NEUROPROCESSORS USING A CENTER OF AREA METHOD

José M. Ferrández, Victor Lorente
Departamento de Electrónica, Tecnología de Computadores y Proyectos, Univ. Politécnica de Cartagena, Cartagena, Spain

Félix de la Paz, José Manuel Cuadra, José R. Álvarez-Sánchez
Departamento de Inteligencia Artificial, UNED, Madrid, Spain

Eduardo Fernández
Instituto de Bioingeniería, Univ. Miguel Hernández de Elche, CIBER-BBN, Elche, Spain

Keywords: Cultured neural network, Induced plasticity, Multielectrode recordings, Robotic control.

Abstract: Learning in a biological neuroprocessor is analyzed using human neuroblastoma cultures and a center of area method in order to guide a robot to follow the light or the brightest area in a limited scenario. The main setup consists in an inverted microscope where a multielectrode array is attached with the biological cultures. This elements amplifies and send the weak neural signals to a D/A card where analyzing process is achieved, computing the movement of the robot, that is remotely linked to this computer. The robot also sends the a picture of the scenario to the computer in order to stimulate the culture with a center of area scheme. In this paper, it is shown that learning is possible in this culture, and guiding the robot to a desired goal is a feasible task.

1 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 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.

Classical computational paradigms consist in serial and supervised processing computations with high-frequency clocks silicon processors, with moderate power consumption, and fixed circuits structure. In contrast, the brain uses millions of biological processors, with dynamic structure, slow commutations compared with silicon circuits, low power consumption and unsupervised learning. There have been numerous approaches to creating bioinspired parallel processing. However, silicon provides a fundamentally different technological platform to that of neurobiology. Neurons – the core technology component has a huge number of interconnections compared to 3 in traditional transistors. This provides considerably more computational power. Furthermore, this extraordinary connectivity is coupled with natural unsupervised learning based on varying connective efficiency.

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 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 analyzing if populations of neuroblastoma cells are able to process and store information, and if learning can be implemented

M. Ferrández J., Lorente V., de la Paz F., Manuel Cuadra J., R. Álvarez-Sánchez J. and Fernández E..
LEARNING IN BIOLOGICAL NEUROPROCESSORS USING A CENTER OF AREA METHOD.
DOI: 10.5220/0003084003360343
In *Proceedings of the International Conference on Fuzzy Computation and 2nd International Conference on Neural Computation* (ICNC-2010), pages 336-343
ISBN: 978-989-8425-32-4

over this biological structure. The main objective of this work will be to control a robot using this biological neuroprocessor and a simple center of area learning scheme. The final system could be applied for testing how chemicals affect the behavior of the robot or to establish the basis for new hybrid optogenetic neuroprostheses.

2 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 (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 Mg^{++} block. NMDA channels are located at the postsynaptic membrane. When the membrane potential is at rest, the NMDA channels are blocked by the Mg^{++} ions. If the membrane potential is depolarized due to excitation of the postsynaptic neuron, the outward depolarizing field may repel Mg^{++} 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 Mg^{++} 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 Ca^{++} ion which may activate various enzymes for synaptic modification, even nitric 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 Ca^{++} ions into the postsynaptic neuron. The subsequent synaptic modification by Ca^{++} ions is referred to as the maintenance of LTP.

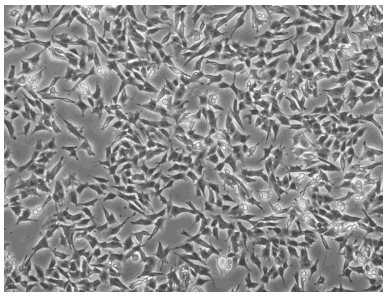

Figure 1: Human neuroblastoma cells, showing different growing stages and neuritic development.

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 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 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.

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, (Rolston et al., 2009; Hales et al., 2010), 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, see Figure 3.

The basic components of the proposed system are

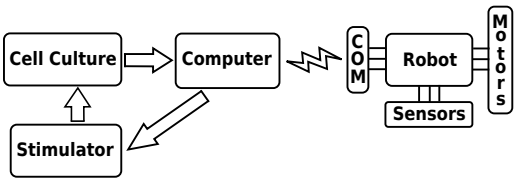

Figure 2: Experimental setup, see text for detailed explanation.

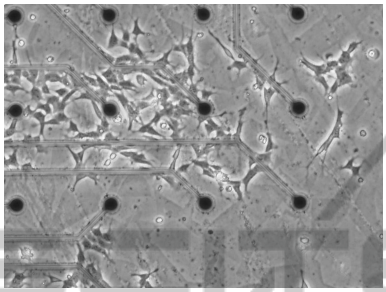

Figure 3: Neuroblastoma cells over multielectrode array, the picture shows a rectangle with sides about 1 mm. long.

shown in Figure 2. These components are:

1. 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, see figure , and can be cultivated for up to several months. Almost all excitable or spontaneously active cells and tissues can be used.
2. 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.
3. 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.
4. The robot sends information about the environment to the computer using a bluetooth link. The sensor consists in infrared sensors for detecting obstacles.

It has been developed a system that provides a complete robotic control platform over remote neural cultures. The system includes 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). Using this software in conjunction with MEABENCH is specially intended for close-loop experiments.

The software developed consists of the following programs:

1. Cult2Robot: The main program. It has been developed as a MEABENCH module, so it can read spikes information from MEABENCH spike detector and compute a direction vector based on MEA neural activity. The direction vector is calculated based on the number of spikes per electrode in t seconds and it can be weighted by the height and width of the spikes. This direction vector can be sent to a robot to control its movement.
2. Stg_control: This program controls a general-purpose two channel stimulus generator.
3. BT_server: Non-blocking Bluetooth server that uses RFCOMM protocol to receive characters from a specific MAC, process the information and do some action, this protocol is used for remote controlling the robot.
4. Remote environment: the computer that is communicating directly with the robot include TCP-IP facilities for reading/sending information to the bio-hybrid platform, wherever it is located.

Bluetooth client and server will be used with a humanoid robot (Robonova, Hitec Robotics) and a two-wheeled robot (e-puck, www.epuck.org) to send and receive information about obstacles.

The system comprises:

1. A bio-hybrid robot control: It includes the development of a whole bio-hybrid hardware/software platform with for robotic guidance.
2. A remote communication system with the neural culture: It has been developed a remote environment for communicating the robot with any bio-hybrid control platform through TCP-IP links.
3. Creation of a bio-hybrid robotic control: Design the neuromorphic processes, e.g. obstacle avoidance tasks, implemented over the bio-hybrid system.

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 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 μm. 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 CO_2 and 95 per cent O_2 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. 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 1 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).

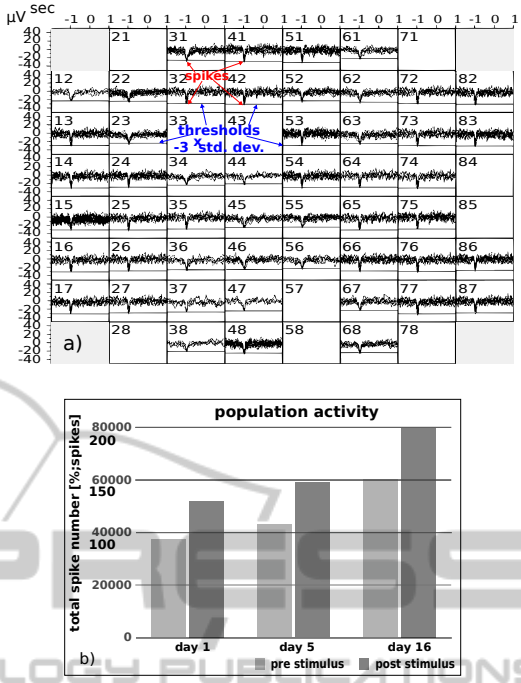

Figure 4: a) Spontaneous neural activity detected by the multielectrode array. b) Induced neural activity by tetanization stimuli.

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. Figure 4 a) show the spiking activity of the neural population with an automatic detection level for each electrode. This is very convenient if one has multiple channels for extracting spikes.

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 et al., 2006; Rolston et al., 2007; Madhavan et al., 2007; Esposti et al., 2009). 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 et al., 2003). This presynaptic event is caused by calcium influx. Calcium-protein interactions then produce a change in vesicle exocytosis. Some studies (Jimbo et al., 1998) use repetitive stimulation for training neural cultures, achieving activity potentiation or depression.

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 4 b) 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 behavior. 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

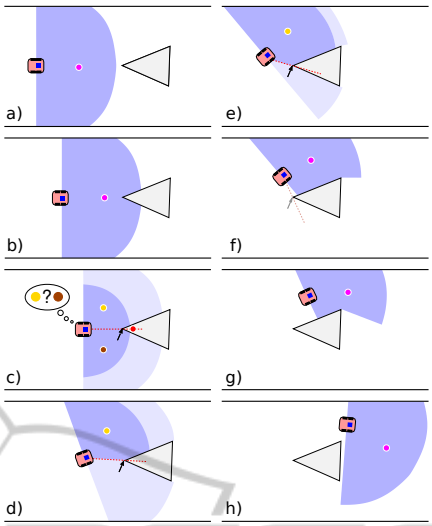

Figure 5: Obstacles avoidance using area center. These pictures form a sequence showing how a robot overcomes an obstacle using the center of area method. The current advance sector is the dark blue area, discarded sectors are in light blue. a) Area center (magenta/dark circle) is accessible: follow it (using full advance sector). b) Area center is still accessible: follow it (using full advance sector). c) Area center became inaccessible (red/dark circle): shrink and split the advance sector, choose one side and turn to its area center (yellow/lighter or brown/darker circles), remember (local coordinates) the split point (marked with arrow). d) Left side has been chosen: as robot turns the sector is expanded to the right to cover the split point and increase restricted advance sector radius as function of sector amplitude increment, follow sector area center (yellow/light circle). e) Robot is going past the split point: continue expanding the sector, continue following sector area center (using restricted advance sector). f) Split point has been gone past, the advance sector has been expanded to its initial radius and amplitude: forget split point, continue following sector area center (magenta/dark), the full advance sector has been recovered. g) Area center is accessible: follow it (using full advance sector). h) Robot has overcome the obstacle, area center is accessible: follow it.

change, getting a potentiation effect on the spontaneous firing, modulating in this way the culture neural activity.

5.2 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) resulting from neural activity recorded in the human neuroblastoma culture stimulated using a center of area method (Álvarez Sánchez et al., 2010; Álvarez Sánchez et al., 2009).

This method is a new brand of navigation meth-

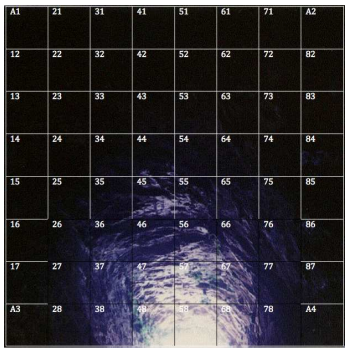

Figure 6: Image acquired by a robot over MEA.

ods, at this time a reactive version has been developed. The method computes the center of area of a frontal sector, the advance sector, of the robot free perceived area and use its position for robot driving. The robot approximately follows the center of area path, see Figure 5. The idea is that the center of area is normally a safe place to go, but when an obstacle lies near robot path, the center of area could became inaccessible inside the obstacle, so there is no way to follow it. In this case the advance sector is split in two shorter sectors using a point of the obstacle as a reference. If the centers of area of both sectors are accessible one of them is chosen, randomly or by some external preference. If only one center is accessible it is selected. If both centers are inaccessible, no way, then the method determines the center of area of the robot shrunk rear area and robot turns to it as an escape maneuver. Successive splits may be done, if needed, so robot can drive through complex configuration of obstacles in a safe way, even passing through narrow places and following smooth paths.

A modification of the method makes it capable of goal reaching avoiding obstacles, still being, at least at these preliminary stages of its development, a reactive method.

Note that this center of area concept is a construction that emerges from the visual or ranging perception of the robot. In Figure 6, it can be seen a simulation of a robot walking through a cave. This image will be digitized 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.

The resulting stimulation configuration is shown in Figure 7 a). White 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

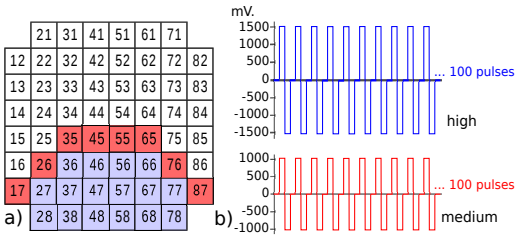

Figure 7: Selective electrode tetanization corresponding to data acquired from image shown in Figure 6. a) MEA representation showing three groups of electrodes, white ones are not stimulated, light blue ones are stimulated with high tetanization and red (darker) ones are stimulated with medium tetanization. b) High and medium tetanization pulses train, anodic first waveform.

pulses, Figure 7 b). From this example, it is expected that electrodes that cover the center 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.

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 37^{o}C in 5% CO_2 and humidify atmosphere.

The electrophysiological properties of the neuroblastoma cultures were analyzed by recording the spontaneous activity of the network. In vitro neuroblastoma networks showed spontaneously firing. This firing rates changed during the culture development with marked day differences and the global rate was 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) have 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 showed bursting and spiking activity, with usually negative depolarizations. It was used also an automatic detection level for each electrode. This is

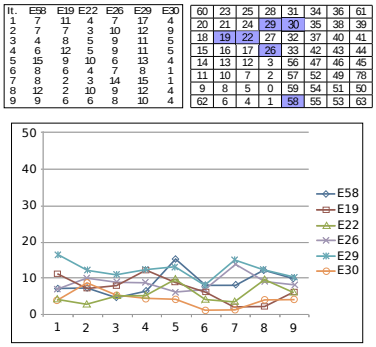

Figure 8: Spontaneous neural activity detected previous to tetanization. Spikes number (*y*-axis) from six electrodes were recorded 9 times (*x*-axis). Left table shows data in numeric format, right table shows a MEA representation with selected electrodes marked out. Spikes number is always below 20.

very convenient if one has multiple channels for extracting spikes.

The standard deviation of each data trace was used to estimate its spike threshold and computing the spikes per channel. A time interval of 500 ms was 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 8 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 Figure 7 was applied, the spiking characteristics of the neuroblastoma culture changed. The computed spikes per channel is shown in Figure 9 during the tetanization process. It can be seen that the most significant increment registered is at electrode 58, that matches the center 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 Figure 10 it can be seen a clearly potentiation effect in electrode 58 about 4 times, 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 changes last for seconds, in this case a biped robot will be the perfect candidate due to its limited

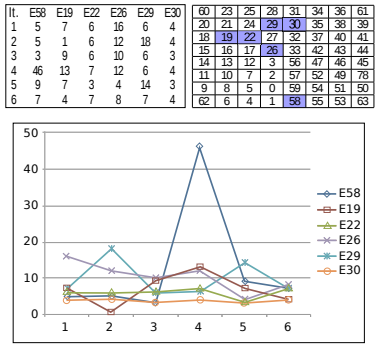

Figure 9: Spontaneous neural activity detected during tetanization. It shows a considerable increase in activation at electrode 58. See figure 8 caption for more explanations.

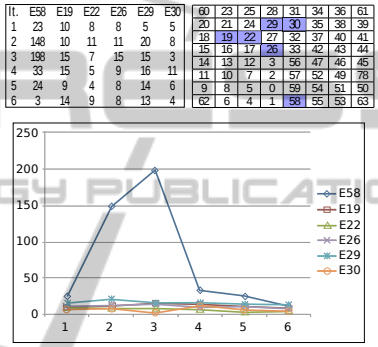

Figure 10: Spontaneous neural activity detected after selective tetanization. Note the different *y*-axis scale in this figure respect to figures 8 and 9. Activation at electrode 58 is bigger than before.

movement. The robotic control will be refined in future works, while in this paper the plasticity of the center of area stimulation is presented.

6 CONCLUSIONS

Learning in cultured neuroblastoma networks by a stimulation process requires identifying the correct stimuli to provide to the neurons in culture. 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, (Bakkum et al., 2007; Bakkum et al., 2008b; Bakkum et al., 2008a; Chao et al., 2008), 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 stimu-

lation. Our results indicate the existence of a clear facilitation mechanism in response to the tetanization stimuli at different stages of cell development. By selective tetanizing some parts of the culture, the network changes its firing characteristics in seconds, modifying in this way its electrical behavior, and it has been shown that if the brightest area of the scenario, induces more stimulation in its corresponding part of the culture, then the increase in the firing properties of the neurons that represent the area where light is detected is observed. Future work consists in determining the optimal stimulation to apply for inducing permanent firing changes in the culture. These aspects will then constitute the basis for analyzing the behavior change by adding chemicals to the culture, and for designing new optogenetic hybrid learning schemes. A more detailed robotic control will be also studied analyzing the culture time responses.

ACKNOWLEDGEMENTS

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.

REFERENCES

Álvarez Sánchez, J. R., de la Paz López, F., Cuadra Troncoso, J. M., and de Santos Sierra, D. (2010). Reactive Navigation in Real Environments Using Partial Center of Area Method. *Robotics and Autonomous Systems*. In press, http://dx.doi.org/10.1016/j.robot.2010.05.009.

Álvarez Sánchez, J. R., de la Paz López, F., Cuadra Troncoso, J. M., and Rosado Sánchez, J. I. (2009). Partial Center of Area Method Used for Reactive Autonomous Robot Navigation. In Mira, J., Ferrández, J. M., Álvarez, J. R., de la Paz, F., and Toledo, F. J., editors, *Bioinspired Applications in Artificial and Natural Computation*, volume 5602 of *LNCS*, pages 408–418. Springer Verlag.

Antonov, I., Antonova, I., and Kandel, E. (2003). Activity-dependent presynaptic facilitation and hebbian LTP are both required and interact during classical conditioning in aplysia. *Neuron*, 37(1):135–147.

Bading, H. and Greenberg, M. (1991). Stimulation of protein tyrosine phosphorylation by NMDA receptor activation. *Science*, 253(5022):912–914.

Bakkum, D. J., Chao, Z. C., and Potter, S. M. (2008a). Long-term activity-dependent plasticity of action potential propagation delay and amplitude in cortical networks. *PLoS One*, 3(5):e2088. Online Open-Access paper.

Bakkum, D. J., Chao, Z. C., and Potter, S. M. (2008b). Spatio-temporal electrical stimuli shape behavior of an embodied cortical network in a goal-directed learning task. *Journal of Neural Engineering*, 5:310–323.

Bakkum, D. J., Gamblen, P. M., Ben-Ary, G., Chao, Z. C., and Potter, S. M. (2007). MEART: the semi-living artist. *Frontiers in NeuroRobotics*, 1(5):1–10. Online Open-Access paper.

Chao, Z. C., Bakkum, D. J., and Potter, S. M. (2008). Shaping embodied neural networks for adaptive goal-directed behavior. *PLoS Computational Biology*, 4(3):e1000042. Online Open-Access paper, supplement, and movie.

Esposti, F., Signorini, M. G., Potter, S. M., and Cerutti, S. (2009). Statistical long-term correlations in dissociated cortical neuron recordings. *IEEE Transactions on Neural Systems & Rehabilitation Engineering*, 17(4):364–9.

Hales, C. M., Rolston, J. D., and Potter, S. M. (2010). How to culture, record and stimulate neuronal networks on micro-electrode arrays (MEAs). *JoVE*, 39. doi: 10.3791/2056. Online video tutorial: http://www.jove.com/index/Details.stp?ID=2056.

Jimbo, Y., Robinson, H., and Kawana, A. (1998). Strengthening of synchronized activity by tetanic stimulation in cortical cultures: application of planar electrode arrays. *IEEE transactions on Biomedical Engineering*, 45(11):1297–1304.

Madhavan, R., Chao, Z. C., and Potter, S. M. (2007). Plasticity of recurring spatiotemporal activity patterns in cortical networks. *Physical Biology*, pages 181–193.

Rolston, J. D., Gross, R. E., and Potter, S. M. (2009). A low-cost multielectrode system for data acquisition and real-time processing with rapid recovery from stimulation artifacts. *Frontiers in Neuroengineering*, 2(12):1–17. Online Open-Access paper.

Rolston, J. D., Wagenaar, D. A., and Potter, S. M. (2007). Precisely timed spatiotemporal patterns of neural activity in dissociated cortical cultures. *Neuroscience*, 148:294–303.

Wagenaar, D. A., Pine, J., and Potter, S. M. (2006). An extremely rich repertoire of bursting patterns during the development of cortical cultures. *BMC Neuro-science*, 7:11.