Neuroelectronic Systems: Binding Neurons to Electric Circuits.

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Abstract

This paper is devoted to description of a new type of information system based on interaction of biological neurons and electronic components - neuroelectronic (NE) system. Main features and components, like neural network sensor, of NE are briefly put into consideration followed with an example of electric interaction between neural network of a snail Lymnaea Stagnalis neurons and electronic MOSFET sensor.

Introduction

Living brain and traditional computer have been always considered as very different information systems. Nevertheless, having completely different information structure biological neural networks and electronic computers operate with trains of electrical signals while coding information on the basic structural level. This a fortunate similarity resulted nowadays in numerous efforts of constructing some kind of hybrid system with direct electric coupling of neurons and electronic components - neuroelectronic (NE) system, a system aimed at elimination of a wide difference gap between living neural networks and artificial computers [1].

In order to build NE system it is necessary to establish direct electrical interaction of alive nervous cells included in a neuron network and artificial component - sensor. It is important to take into account the fact, that interaction should be provided simultaneously in many (hundred, thousand) points of a neural network on a enough long period of time. Traditional electrophysiological methods operating for these purposes with glass microelectrodes cannot be considered acceptable because of complexity of manipulation and size of a construction. Besides, any operation with glass microelectrodes causes the cell death because of membrane rupture.

At the same time, on the basis of micro technologies it is possible to construct an array of flat microtransducers providing strong contact to cell bodies [2]. The recording of neurons electrical activity with the aid of macroelectrode array was carried out for the different cell systems. It was shown that the application of the similar electrode structure allows to detect cooperative and individual electrical activity of neurons in culture of a nervous cells [3]. However, major number of electrodes requires utilization of massive external equipment of an amplification and commutation. Besides, rather large distance between a source of a signal and amplifier arises the big parasitic capacitance and low signal to noise ratio which makes an interpretation of obtained data for a microelectrode system very complex.

On the other hand, modern microelectronic technologies allow creation of an external sensors with the array of active elements (usually field-effect transistors) built-in exactly in the cell placement area [4-5]. Such active sensors have a small distance between a source of a signal and amplifier that open new possibilities for research information processing in neurons.

If made such microtransduces array in the form of a specialized chip with neurons placed in a physiological solution directly on its surface one can name it the neurochip - sensor for neuron networks or neurosensor.

Cell culture for neurosystem.

One of the main element of any NE system are nervous cells. Their behavior in artificial conditions, keeping the property to generate biopotentials, ability of differentiation and saving vital activity during long-lived time (about one month and more) in conditions in vitro determines operation range of the NE system.

Now it is much known about neuron cell. The mechanisms of generation of rest, operation and synaptic potentials, mechanism of spike propagation on a cell and its neurites, morphological structure of a cell and etc. However the following level - interaction of cells and cell-like associations - is researched very poor.

For a creation of NE systems it is important to clarify what is the main functioning unit of the nervous network: one synapse, collection of synapses, site of a membrane, cell or other structures. In this respect culture of neurons creates exclusive possibilities for detection of elementary functioning unities of the nervous system: logical elements, signal converters, memory and integrators.

In culture in pretty wide limits it is possible to change composition of a culture medium, chemical composition of a substrate, add or remove from medium any physiologically active substances with the influence on spike activity and synapses.

The cell of a nervous tissue in conditions of culture in general save the metabolic characteristics of a cell of the whole organism. The values of activity of an oxidizing metabolism, exchange of enzymes, mediators, nucleic acids and protein, ionic carrier often can be comparable to values obtained in situ.

The sensor of electrical activity.

The second main element of NE system is the array of microtransdusers which are capable to record external electrical signals of nervous cells. One main feature of neurons electrical signals is their low power, that is caused by specificity of cells as biological objects. The maximum value of voltage and current amplitude constitute accordingly about 100 mV and 10^{-8} A at a resistance of a cell-like membrane about 10 MOm. It impose a number of the requirements external sensors of cell electrical activity should satisfy.

One of a main requirements, which arises because of high resistivity of a cell membrane, is the necessity to use primary transducers with a high input resistance. Most approaching for this purpose are the field-effect transistors, which have an input resistance about 1 GOm.

For insolation of sensor units from a solution the photoresistive polymer - polyimide is usually used. However thin polyimide film is not stable in a saline solution. This does not allow to carry out long-term experiments without disruption of coating integrity followed with sensor death. Therefore we designed other method based on construction peculiarities of microelectronic component in glass-to-metal frame.

The transistor consists of a silicon chip packed in a steel frame with outputs in glass insulators. The connection of contact sites of a chip (gate, drain, source) with outputs is performed through golden wire with a diameter about 30 microns. For an access to a chip the top of package was cut off, then the wire connecting a contact site of a gate and appropriate output, was detached from output and was drawn up. Three prepared transistors were placed side by side with a distance between gate wires was minimum and then were flooded by polymer. After solidification of polymer, a top was cut forming a flat site with three gold micro contacts. For carrying out of measurements the sensor was built into bottom of the culture camera.

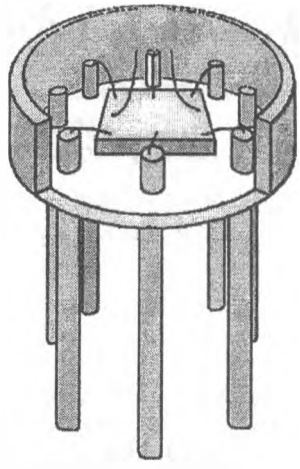

Fig. 1. A construction of the microelectrode sensor on the basis of the field-effect transisor.

Described method allows to create insulating coatings within the width of 2 mm., that provides reliable protection of a chip against salts of a solution without usage of photopolymers. The golden wire as electrodes has chemical resistance and biological inertness necessary for carrying out of long-term experiments.

Registration of electrical signals of neurons.

Detected external signals of neuron usually have a high level of inphase interferences. Therefore, to reject interference, system of an amplification should connect transducers in differential manner.

In an input cascade of the amplifier the differential circuit of field effect transistors was used. One transistor achieves signal from the neuron another from a reference electrode. The transistors were switched on in the saturation mode, therefore the drain-sources currents of transistors were modulated by the voltage on the gates. The drain voltage was fed to a differential amplifier, which magnified a signal,

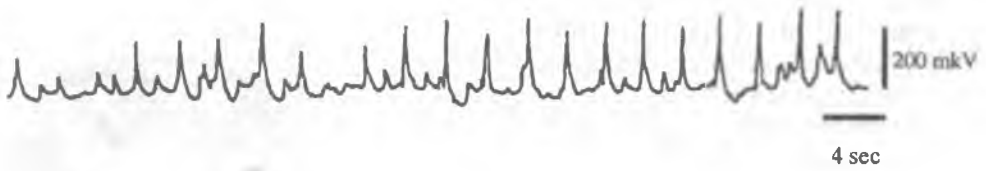

Fig. 2. Action potentials of a neuron on a background of synaptic potentials registered with application of sensors based on MOSFET.

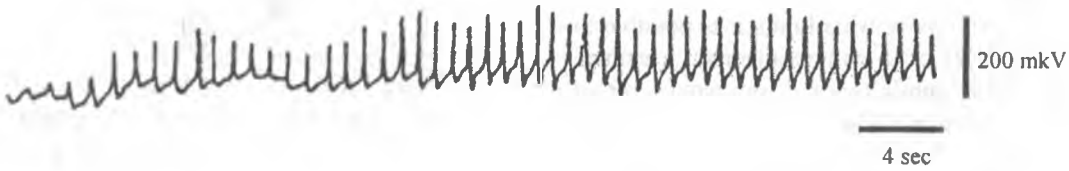

Fig. 3. A spike train of a neuron registered with application of sensors based on MOSFET.

rejecting a zero signal current and inphase interferences on the inputs.

The given technique was successfully applied for long-term monitoring of electrophysiological activity of neurons of a snail Lymnaea stagnalis.

For culturing of a nervous tissue of a snail Lymnaea stagnalis the following media composition was used: 30 % L-15 medium, glucose - 400 mg/ml, antibiotics (penicillin - 100un/ml, streptomycin - 50un/ml), 70 % of a saline solution of the following composition: NaCl 3 g/L; MgCl 0,14 g/L; KCl 0,127 g/L; CaCl 0,455 g/L. The isolated ganglions of snail nervous system were cultured within several weeks. During this time the electrophysiological activity of a neurons was recorded. The detection of activity was carried out on the basis of above described sensor, which was embedded in the bottom of neurons culture chamber.

An electrical activity of snail nervous tissue recorded with the help external sensor represented in a fig. 2-3.

References

1. Bove M., Grattarola M., Tedesco M. and Verreschi G. (1994). Characterization of growth and electrical-activity of nerve-cells cultured on microelectronic substrates - towards hybrid neuro-electronic devices. - Journal Of Materials Science Materials In Medicine V.5, P: 684-687.

2. Bove M., Grattarola M., Martinoia S., Verreschi G. (1995). Interfacing cultured neurons to planar substrate microelectrodes - characterization of the neuron-to-microelectrode junction. - Bioelectrochemistry and Bioenergetics V.38, P: 255-265.

3. Breckenridge L. J., Wilson R. J. A., Connolly P., Curtis A. S. G., Dow J. A. T., Blackshaw S. E., Wilkinson C. D. W. (1995). Advantages of using microfabricated extracellular electrodes for in-vitro neuronal recording. - J. Neurosci. Res. V.42, P: 266-276.

4. Hammerle H., Egert U., Mohr A., Nisch W. (1994). Extracellular recording in neuronal networks with substrate integrated microelectrode arrays. - Biosensors & Bioelectronics V.9, P: 691-696.

5. Janossy V., Toth A., Bodocs L., Imrik P., Madarasz E., Gyevai A. (1990). Multielectrode culture chamber: a device for long-term recording of bioelectric activities in vitro. - Acta Biol Hung V.41, P: 309-320.