The development of the experimental setup for measuring the cell membrane electrical potential by Sucrose-Gap Technique

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Abstract. This article describes the development of the experimental setup for measuring the cell membrane electrical potential by Double -Sucrose-Gap Technique. The double-gap isolation method allows the simultaneous measurement of electrical activity and tension output from contracting segments of muscle fibers. This technique has been widely used as a convenient tool for recording of the membrane activities from myelinated or unmyelinated nerves and muscle preparations. This device can be an effective way to provide undergraduate biomedical engineering students with invaluable experiences in neurophysiology. The installation design and its main characteristics are described. The advantages of the described device are the simplicity of the experiment, relatively low cost, the possibility of long-term experiment.

1. Introduction

The study of the various cells in the body is a very important aspect in the investigation of any tissue, organ or organism as a whole, because the actions of cells underlie responses of vast majority of biological systems. However, studies at the cellular level are extremely complex due to the small size and relatively small reaction time of objects. The sucrose gap method allows to measure extracellular action potential parameters fairly easy. Using the intracellular insulating sections of sucrose channels allows to restrict the extracellular shunting and to record the parameters of bioelectric potentials confidently enough.

The main advantages of the method are due to the fact that experimenter does not operate with a single cell, but with a tissue fragment, for example, muscle or nerve fiber [1]. This greatly simplifies experimentation and also reduces the cost of the equipment, because there is no need to use microelectrodes. Today, the devices of this type are not produced in Russia. Devices of this type can be custom-made, however, they are very expensive and designed to work with the nervous tissue, therefore do not allow to obtain data about the relationship between contractile and electrical activity of the investigated muscle tissue. This paper examines the basic principles of the cell membrane potential registration by double sucrose gap method and describes the installation design and its main characteristics.

2. Electrochemical processes in cells

There are a lot of electrochemical processes in living cells, but most clearly they are expressed on the cell membrane surface, which separates the inner cell volume from of the intercellular substance and other cells. All living cells have membrane electrical potential. It is an electric potential difference, existing on inner and outer sides of the membrane and caused by uneven concentrations of various ions in cells and in the extracellular space. Some cells, such as nerve and muscle are able to generate rapidly changing electrochemical impulses, which are used to transmit signals along the membranes of these cells. The membrane potential can be changed under the influence of different origin stimuli (chemical, electrical and others).

The shift in the membrane potential may occur in the negative (hyperpolarization) or positive (depolarization) side. If the cell stays in an unexcited state, the resting potential is formed on the membrane surface. For warm-blooded organisms, it ranges from -55 to -100 mV [1,2]. If resting potential is shifted to the sub-threshold value, then passive electrotonic potential arises. The action potential is a wave of excitement, which moves through the membrane of the living cell in the form of short-term changes in the membrane potential, as a result the outer surface of the membrane becomes negative with the respect to the inner surface of the membrane. An action potential is a physiological basis for a number of electrochemical processes in the cells. The main reason for the initiation of the action potential is achieving a threshold value of the membrane potential bias. The threshold value is the minimum value of the stimulus that can cause a physiological response: muscle contraction, nerve impulse or a secretion from glands. Only upon reaching the threshold value the stimulation causes a self-sustaining process of changing the biopotential value in the cell [Figure 1]. The threshold is determined by multiple conditions. For example, they include the magnitude and duration of the stimulus.

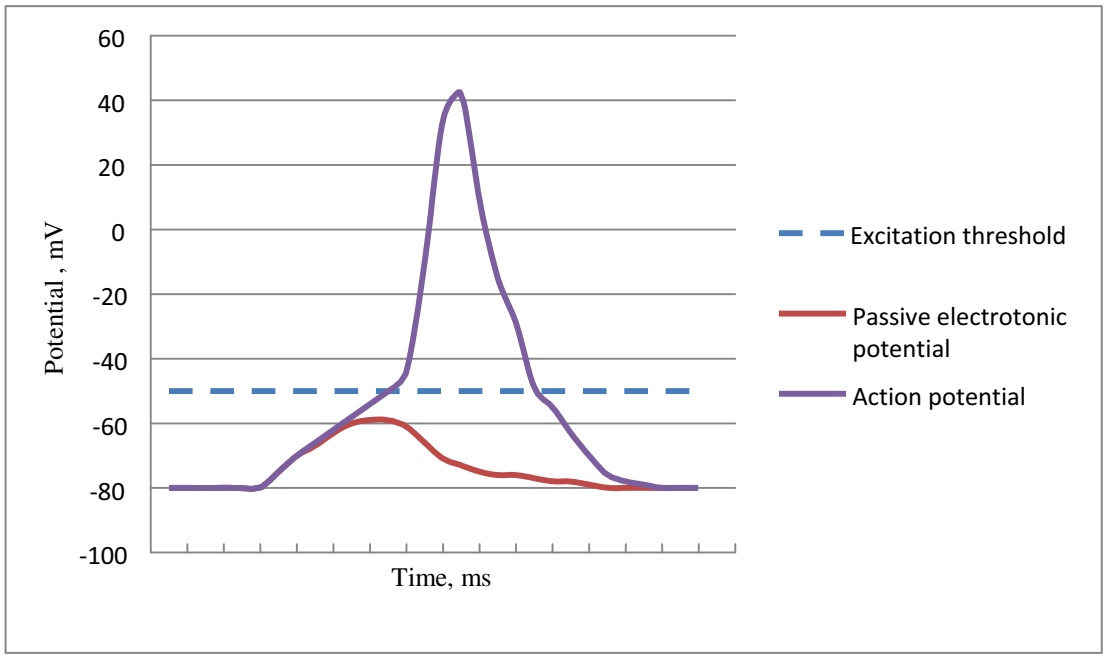

Figure 1. Changing of the potential depending on the strength of stimulation

The sucrose gap method is an extracellular way for the registration of the action potential parameters. When placing electrodes on the surface of nerve or muscle fiber, which is located in saline, the diversion will be ineffective because of the shunting effect of the solution. To eliminate the electrical bypass, fiber section, which is located between electrodes, perfused with a solution containing no ions [Figure 2]. Under one of the electrodes the saline is replaced with an isotonic solution of potassium chloride which eliminates transmembrane potential in this region which is equivalent to the electrode connection to the intracellular content of the fiber. To allow abduction of the

transmembrane potential and its management, the fiber is divided into 5 sections. The middle portion is tested and perfused with saline. Through the adjacent portions on both sides sucrose solution is passed and the edge portions on both sides are perfused with an isotonic potassium chloride solution. A pair of measuring electrodes is located on both sides of one of the sucrose sections and a pair of exciting ones on both sides of the other [3]. In multicellular specimen there is an effective electrical connection between neighboring cells by which smooth muscle tissue has distinct conductive properties. The spatial constant is 1-3mm. [4]. Thus, the sucrose gap method can be used for measuring the membrane potential of smooth muscle or nerve fiber. This method has several advantages. One of them is the possibility of continuous measurement of the membrane electrical parameters changes caused by different influences in particular physiologically active substances, temperature, electric current. In addition, the sucrose gap makes it relatively easy to implement simultaneous recording of electrical and contractile activity of smooth muscle.

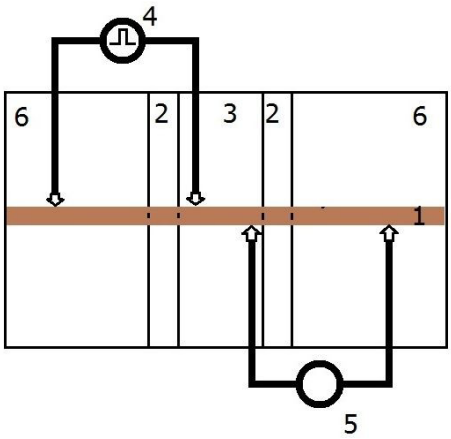

Figure 2. The scheme of the double sucrose gap device. 1 – a muscle fiber; 2 - sucrose gaps, insulating intercellular areas; 3 - the working chamber filled with Krebs solution; 4 - measuring electrodes; 5 - stimulating electrodes; 6 - working chamber filled with isotonic KCl solution.

3. Development of the device

All electronic components of the device are placed in the single housing. On the top of the housing there is a working area, where a sample of nerve or smooth muscle tissue is situated. At the bottom of the working chamber located the control and recording electrodes, which are made of platinum wire. To maintain the purity of the solutions in the chamber sucrose solutions, Krebs' solution, and KCl solution supplied to the working sections through the special channels throughout the experiment. To maintain the desired temperature the heating element and temperature sensor are placed in the working area [Figure 3]. Abduction of the solutions implemented by the internal channels. Used solutions gather in the special containers, which are removable to facilitate maintenance. The main elements of the device are made by three-dimensional printing technology.

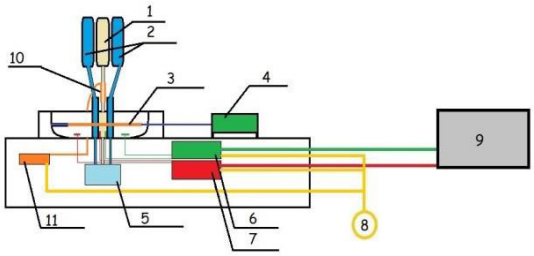

Figure 3. Schematic diagram of the device. 1- Krebs solution; 2 - sucrose; 3 - the study tissue; 4 - tension sensor; 5 - tank for waste collection; 6 – oscilloscope; 7 - pulse generator; 8 - power supply; 9 - output data to a personal computer; 10 - The heating element and temperature sensor; 11 - Temperature control board

During the development of the device, two variants of working sections were considered. In the first variant [Figure 4] each sucrose section is arranged as the single element in the form of a parallelepiped with the openings on opposite sides. On the lateral sides of the sections a latex thin film is attached. Between the sucrose sections the oblique strip with thickness of 1.5 mm is installed. In the formed gap comes the Krebs solution. The advantage of this design is the simplisity of its production. The disadvantage is the complexity in the preparation of the experiment, the low reliability of the sealing system, the need for long-term preparations for the experiment. The second option [Figure 5] suggests the production of individual walls. Each wall is hollow inside and is connected to a pneumatic system. The baffle has two round-shaped holes of 8 mm diameter on opposite sides in which two clamping rings are installed [Figure 6]. The rings fix the sealing rubber membrane of cylindrical shape. The air channels within the walls allow to regulate the pressure inside the membrane modifying the final dimensions of the transverse channel inside the baffle. The main advantages of this technical solution are the simplicity of setting up and using of the installation and its enhanced reliability. The disadvantage is the difficulty in its production. The small size of the clamping rings requires the high accuracy in the production, as well as the careful selection of the materials.

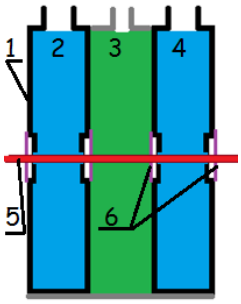

Figure 4. The section embodiment type 1.
1 - Sucrose section module; 2.- Sucrose solution; 3 - Krebs solution; 5 - Sample; 6 - Rubber seals

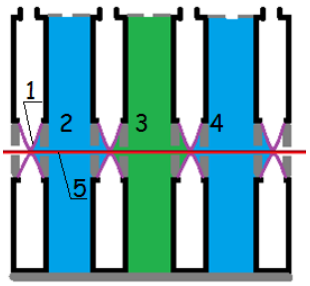

Figure 5. - The section embodiment type 2.
1 - The rubber sealing ring; 2 - sucrose solution; 3 - Krebs solution; 5 – Sample.

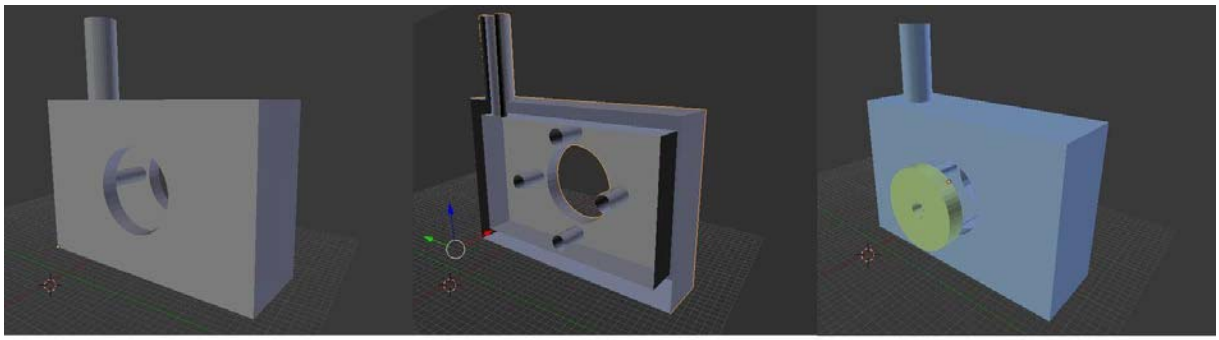

Figure 6. The section embodiment type 2. 1 - general view; 2 - view in section; 3- partition together with a sealing ring

4. Specifications

The design of the device provides the ease of use and high reliability. Reliable installation is due to the fact that all electronic components are placed in a single robust housing and protected against dust, moisture and strokes. Electronic components provide high accuracy and reliability of the data acquisition.

Table 1. Installation parameters.

Parameter	Value
The accuracy of the electric potential measurement	10^{-6} V
Time resolution	10^{-4} sec
Diameter of the study sample	0,1 – 4 mm
Temperature stability	0,1^{o} C

5. Conclusions

The sucrose gap method has a number of advantages. This method makes it relatively easy to implement simultaneous recording of electrical and contractile activity of smooth muscle. The obtained data allow us to investigate the relationship between changes in the cell membrane parameters and temperature settings, physical effects, effects of damaging factors of various origins, including side effects of medications. The advantages of the described device are the simplicity of the experiment, relatively low cost, the possibility of long-term experiment. Similar devices will be produced for higher education and research institutions for the purpose of students training in medical and biological fields, as well as research.

6. Acknowledgements

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