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Information Technologies for Biology Education: Computerized Electrophysiology of Plant Cells

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Abstract. Biology has moved from a bench-based discipline to a bioinformational science in modern times but application of computational and analytical methods of informatics in it is still a problem for many researchers and students of biology. We suggest to integrate cost effective and practical combination of the real and the virtual laboratories into the undergraduate biological science curriculum. This laboratory work illustrates passive and active electrical properties of plant cell membranes while introducing basic principles of electrophysiological recording, data acquisition and analysis. As the object for investigation in this laboratory work large cells of starry stonewort (*Nitellopsis obtusa*) were used. The simple program for experiment control and express visualization of recorded data was developed. Experiment proposed in this paper is easy implemented with a minimum of laboratory equipment, materials and gives an experience of computerized biological experiment.

Keywords: biophysical education, data acquisition system, teaching/learning strategies, computerized teaching laboratory.

Introduction

The application of information technology and informational thinking in the biological sciences leads to the modification of students teaching mode. Biology, like the informatics, is advancing at incredible speed these days. It is very important not only to apply informational thinking and informatics technologies in study of the live world and biotechnologies, but to enrich development of informatics with ideas from informational system of the live world also (Kirvelis and Beitas, 2003). Rapidly increasing power of computers enables detailed simulations of biological systems and inevitably raises the question of whether computers will eventually replace wet labs. Computer-based simulations of experiments provide a cheaper alternative to traditional practical classes. Many experimental problems are concerned with the representation, organization, manipulation, distribution, maintenance, and use of information, particularly in digital form. Virtual laboratories can provide workable alternatives in cases when learning outcomes focus on interpretation and manipulation of data. Because of flexibility in time, location, pace, and process, they are potentially more effective in students learning. In many biology fields virtual teaching laboratories are more ethical also because no real animals are used in them.

Virtual cell laboratories are attempts to use computer simulation for the various cells living process. They are used in scientific studies ("To understand complex biological systems requires the integration of experimental and computational research – in other words a systems biology approach. Computational biology, through pragmatic modeling and theoretical exploration, provides a powerful foundation from which to address critical scientific questions head-on" (Kitano, 2004)) but they can be very successful in a teaching also. Some "virtual cell biology labs" are just complexes of animated images about important cells processes (e.g., NDSU Virtual Cell Learning Environment (2000)) without possibility to interact and experiment. Interactive Lab gives an opportunity to learn about the terms, principles, and techniques of experiments through electronic simulations and modeling of complex phenomena (for example, BioQ Collaborative, 2006). Web-based interactive virtual laboratories incorporate modeling and electronic journal, ability to collaborate with other researchers. ICT technology makes learning more interactive, enjoyable, and customizable. But this is not always the case. None of the models can reflect live processes exactly and completely, also often the critical approach is missing. Biological data are very complex and interlinked. Inquiry based learning, such as laboratory research investigations, develops students' practical, technical and reasoning skills, as well as important scientific habits of mind. The active and rigorous nature of classroom inquiry can significantly enhance students' understanding of science concepts and processes of life. The ability to use laboratory equipment, reagents and possibility to manipulate organisms or experience of real signals processing is an essential learning outcome of a practical class. When inquiry is systematically implemented in a classroom, the student takes increased control of their own learning through the guidance of their teacher. Students must organize data in logical and meaningful ways, make detailed observations, and sometimes transform raw data to reveal patterns, relationships between variables, or to clarify results. But those who have worked with both kinds of laboratories say this question – real or virtual laboratory – misses the point. Each approach offers certain benefits; the challenge is to blend them together successfully, including statistics, physics, computer science, and engineering. Ability to manipulate materials through the use of common tools and laboratory equipment allows students to create simulations and working models in order to test their ideas. Combination of the real and the virtual can provide a cheaper, easier, and less time- and labour-intensive alternative; it produces a better experience then a separate work in real and virtual laboratories.

In this study we suggest cost effective and practical science inquiry approach that can be integrated into the undergraduate biological science curriculum. The experiment and software described in the paper were developed under the EU programme Leonardo da Vinci II within the pilot project "Computerised laboratory in science and technology teaching".

Bioelectrical Phenomena in Cells

Every live cell generates an electrical potential on cell plasma membrane that may result in the flow of electric current. This bioelectricity of cell membranes is related with selective permeability to common ions and has important consequences for many cellular processes. Understanding of bioelectrical mechanisms is important for comprehension of cell functioning.

Electrophysiology has proven to be a powerful tool in understanding of plant physiology and development. This technique has elucidated the many mechanisms underlying the electrical phenomena of cell membrane in neurons and muscle tissue also. Difference of potential across the membranes of all living cells called the resting potential depends on difference in ionic composition on the two sides of the membrane. Membrane potential responds to changes of numerous physiochemical variables as temperature, pH, extracellular and intracellular concentrations of ions and so on (Kitasato, 2003). The membrane potential is measured as a voltage difference across the membrane.

Internodal cells of fresh-water charophytes are known for their ability to generate action potential (Johnson *et al.*, 2002). The giant alga *Nitellopsis obtusa* generates action potentials (AP) in response to mechanical stimulation, injury, or direct electrical stimulation. The bioelectrical response of a charophyte cell is rapid and highly sensitive to chemicals in environment.

Action potentials involve a transient influx of Ca^{2+} to the cytoplasm, effluxes of K^{+} and Cl^{-} (Thiel *et al.*, 1997). The depolarization phase of AP is caused by an influx of Cl^{-}. AP repolarization is comprised of two stages. Repolarization phase is due to the outward K^{+} flow and the activity of the electrogenic pumps at the plasma membrane. It is supposed that the second stage of repolarization during AP is related to the operation of electrogenic H^{+}-pumps in the excitable membrane. K^{+} efflux is controlled by calcium-dependent potassium channels, which close when the $Ca^{2+}{}_{cyt}$ decreases (Thiel *et al.*, 1997).

Object for Investigation of Bioelectrical Phenomena

In laboratory works we propose to use large cells of starry stonewort (*Nitellopsis obtusa*). The algae grow in aquaria without any special care or treatment. It is known that Characean cells are most suitable material for analysis of the electrical characteristics of plant cells (Shimmen *et al.*, 1994). Cells of charophytes are well explored experimental systems. The giant charophyta cell separated from talloma and kept in artificial pond water can he considered to be a single organism that maintains essential physiological characteristics for a long time. The usefulness of Characeaens in the laboratory depends upon the ability of internodal cells to survive after isolation from the plant, and on the large size and regular shape of these cells. These internodes approximate to cylinders in shape and provide seemingly ideal material for many physiological observations and manipulations. Typically the internodal cells used in electrophysiological experiments are about 10 cm length and 1 mm in diameter.

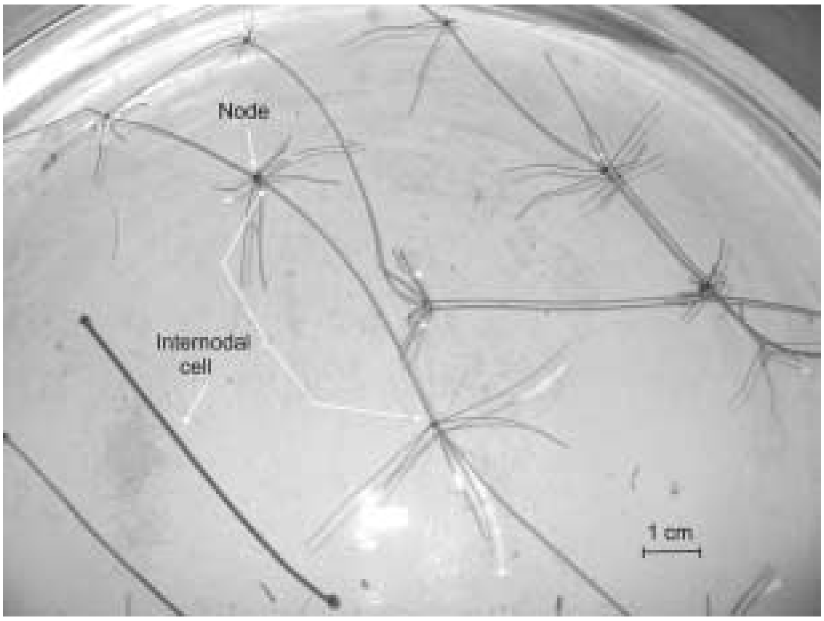

Fig. 1. The internodal cells of *Nitellopsis obtusa*.

Internodal cells of Characeae are intensively used for studies in electrophysiology (Beilby *et al.*, 1997; Kitasato, 2003), ion transport (Demidchik *et al.*, 2001; Shimmen, 2001) cytoplasmic streaming (Kikuyama *et al.*, 1996), and ion channel activity, (Berecki *et al.*, 2001; Tazawa, 2003), as well as to study ecophysiological characteristics (Sakalauskas and Daktariunas, 1999).

Equipment for Implementation of Electrophysiological Experiment

The laboratory work described in this paper is a part of teaching material for the undergraduate students of biophysics and illustrates passive and active electrical properties of cell membranes while introducing basic principles of electrophysiological recording, data acquisition and analysis. The cost of equipment can be a critical barrier for introducing electrophysiology exercises in biology teaching programs. Proposed experiment is easy implemented with a minimum of laboratory equipment and materials. When a microscope with video camera is available, the observations of experiment can be demonstrated for all students of the class or for remote students in distant learning.

The equipment for electrophysiological experiments includes multifunction data acquisition system (DAQ), which is a common device for all experiments introduced into the course of biophysics, Plexiglas chamber with Ag/$AgCl_2$ electrodes for cells stimulation and biopotential registration.

The multifunction data acquisition device used by us is "e-Biol" system (Daktariunas, 2004), which implements features (programmable amplifier, differential inputs, user defined multiplexing of DAQ inputs and other) which are essential for realisation of experiments with biological objects. Membrane potentials of up to 8 cells of green alga *Nitellopsis obtusa* were simultaneously recorded using a plexiglass chamber. In experiments with microelectrodes an additional multichannel biopotential amplifier was used. The measured cells biopotentials were coded as 12 bits words (or 16 bits – depending on modification of DAQ "e-Biol") in each channel and saved to file. The cells were stimulated electrically, chemically and mechanically. The number and quality of outputs (two 12 bits analog outputs and 24 bits ones) of the "e-Biol" DAQ allow implementation of any stimulation algorithm. Simplified block scheme of data acquisition and control device "e-Biol" is shown in Fig. 2.

Any other data acquisition device can be used in described laboratory works, but few modules are essential for such experiments – amplifier with low input current especially if microelectrodes are used, analog digital converter and timer. Sampling frequency of bio-potential is low, so the data acquisition device can be supplied with interface to any computer port for data input/output, such as COM, LPT or USB. In more complex laboratory works data acquisition device must include multiplexer for multichannel registration, digital-analog converter and module of digital information output for electrical stimulation of the cell.

Next and very important part of equipment is software. Standard full set of software for computerized experiments encompass procedures of control of experiment parameters, data acquisition, data visualization, and data analysis as a finished product. This kind of software usually complicates modification of experiment by user, because of source code unavailability and very limited range of methods and algorithms for data analysis.

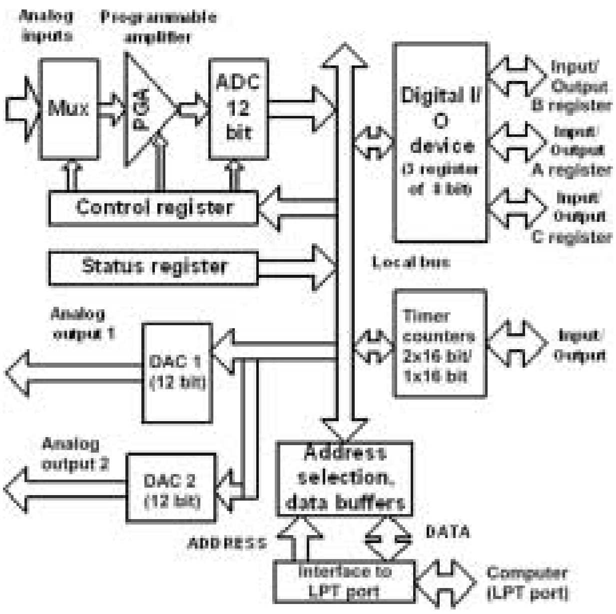

Fig. 2. Simplified block scheme of data acquisition and control device "e-Biol".

In the other hand, a vast number of powerful software for data visualization, processing and analysis (e.g., OriginLab, MatLab) that can be used for implementation of any method or algorithm are proposed to the market. Some tasks can be realized with commonly used spreadsheet software. We propose to implement simple programs for experiment equipment control with minimal data visualization, analysis and saving abilities. More advanced data processing is passed to specialized software for data analysis. We developed a simple program for data acquisition that works in conjunction to data analysis freeware program Data Master 2003 (Data Master 2003, 2004).

This program controls hardware of data acquisition device, acquires data and writes data to computer memory and displays the data in real time. The program also is used to define parameters of experiment – sampling interval, number of samples, gain coefficient of programmable amplifier, numbers of channels to be used and etc. At the end of experiment the program transfers data from computer memory to spreadsheet and graphics of Data Master. Screenshot of the program is shown in Fig. 3. Program was written in Borland Delphi.

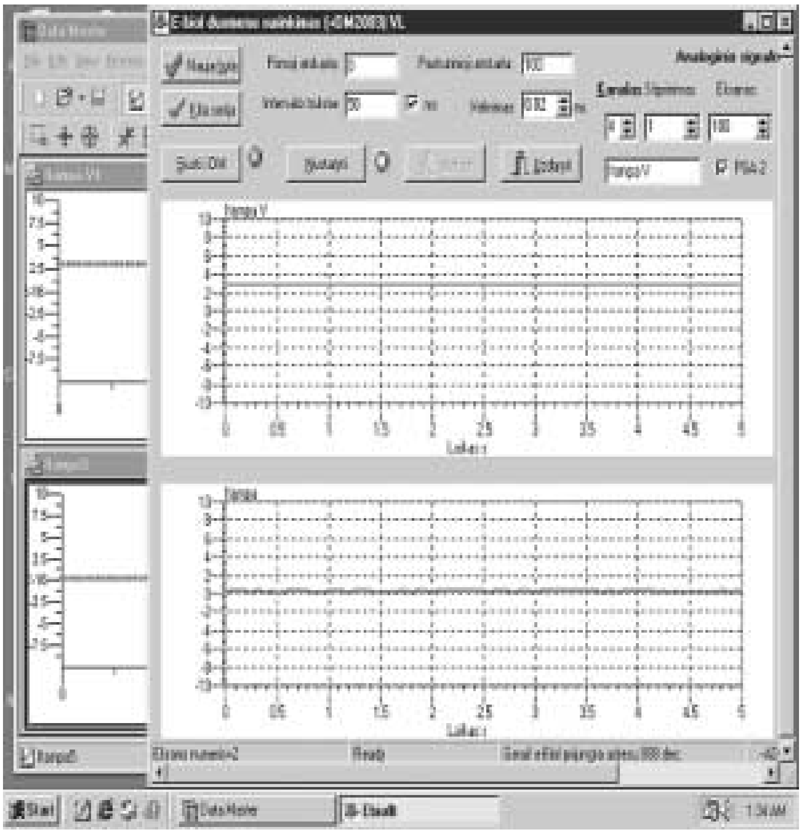

Fig. 3. Screenshot of the Data Master program.

Implementation of Experiment

The freshwater alga *Nitellopsis obtusa* were cultured in tap water at room temperature. Prior to an experiment, internodal cells (second or third internode below the tip of growing alga) were separated from neighboring cells. The internodes were kept in the dark at least overnight in artificial pond water (control solution for electrophysiological testing) containing 0.1 mM KCl, 1.0 mM NaCl, 0.5 mM $CaCl_2$, and 1.0 mM TRIS buffer, pH 7.5 (adjusted with NaOH). The extracellular registration of membrane parameters (resting and action potential) are carried out according to the K'-anaesthesia method of [14]. Up to several internodes are placed in a plexiglass chamber having distinct yet identical sections (a-pool), as seen in Fig. 4. One end of the cell is placed in the larger a-pool. The node and small segment (about 1 mm) of each cell is positioned in the common central c-pool, filled with 100 mM KCl in control solution. Electrical isolation between the c- and a-pools is achieved with 5% boric Vaseline junctions (v), as well as by an air gap (ag) between them (Fig. 4). The cell parts in the a-pools are continuously bathed with control or test solutions.

Bioelectrical signals are obtained from one general reference electrode placed in a reference pool (r), similar to an a-pool, and from appropriate measuring electrodes (me) in (a) pools (Fig. 4). Through out the study, electrodes made of chlorinated silver wire with both high stability and low electrode potential are used. The electrical contact between (r) and (c) pools is enabled through the P-shaped glass tube bridge (b) plugged with 3% (w/v) agar solidified in a 3M KCl solution. The placing of reference electrode (re) in (r) pool instead of in the (c) pool was necessary to avoid the appearance of chlorine ions as a potential component that may interfere in later measuring of the RP value. Such interference could occur due to alteration of the Cl^- concentration difference between the constant c-pool and changing test solutions. Action potentials are elicited by injecting depolarizing current between two pools using Ag/AgCl wires.

Electrical signals from electrodes are led directly (or through the high input impedance preamplifier module when microelectrodes are used) to the input of the differ-

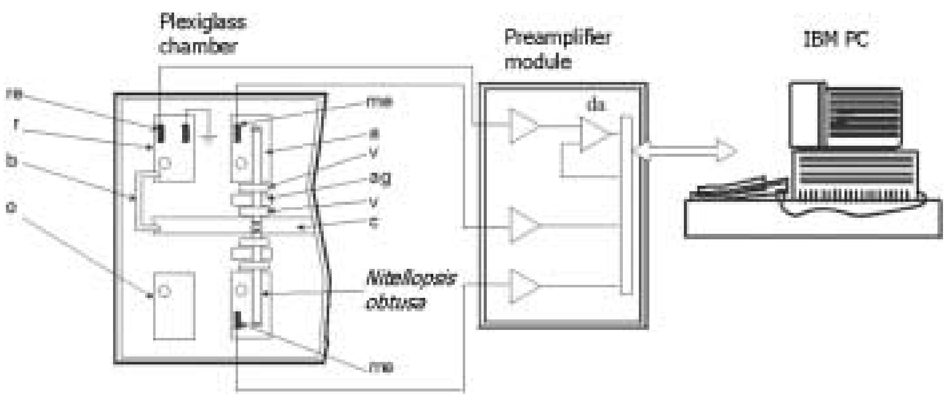

Fig. 4. Schematic diagram of the experimental set-up. See text for details.

ential amplifier (ad, Fig. 4) of multifunction data acquisition system. Sampling frequency for registration of resting potential (RP) was selected as 1 Hz.

Students Work

The laboratory works on algal cells electrophysiology can be organized according inquiry based approach in this way: the general applications where electrophysiological cell methods can be used are related with two topics:

- Electrical potentials in plants and algae are similar to potentials in animal neural cells. How plants and algae can be used as models of animal neurons? In what way the use of algal cells can be more convenient then use of animal neurons? On what cellular mechanisms the similarity of plant/algal and animal cells is based?
- Electrical potentials of algae depend on chemical composition of water in what they dwell. How this electrophysiological sensitivity of cells can be used in evaluation of quality of environment?

Students are asked to search literature databases about possible use of algal cell electrophysiology and to analyze it. On the basis of information they collect and presented descriptions of laboratory equipment for these experiments they are asked to find directions how measurements of electrical potentials can be used in research of mentioned problems. Students are expected to present ideas how electrophysiological methods can be used as a tool for research of:

- differences and similarities of electrophysiological phenomena of neural and algal cells,
- influence of biologically active substances on activities of membrane ion channels,
- influence of water pollutants on rest and action potentials.

For example, it is expected that students will find that differences in ion channels composition of animal and algal cells determines differences in potentials generation that can be demonstrated in mathematical models (that are realized as a computer software, for example, “Neuron”) and in wet experiments.

The full solutions of mentioned problems can be realized in practical works that are organized as unstructurized large practicum where time limits aren’t critical. In practical courses that are divided to small cycles the solving of big problems must be divided to solving of small ones also. In this case after students comprehend and explicate the wide context of electrophysiological investigations with algal cells the continuum of possible experiments can be divided into few parts and, depending on time available, each part can be executed as a separate laboratory work.

First part. Resting potential as a reaction to changes of main ion concentration.

In this stage of the laboratory work students can observe and investigate:

1) very negative resting potentials (up to -250 mV) of the cell;
2) dependence of resting potential from different pH or concentration of potassium and sodium ions in surrounding solution.

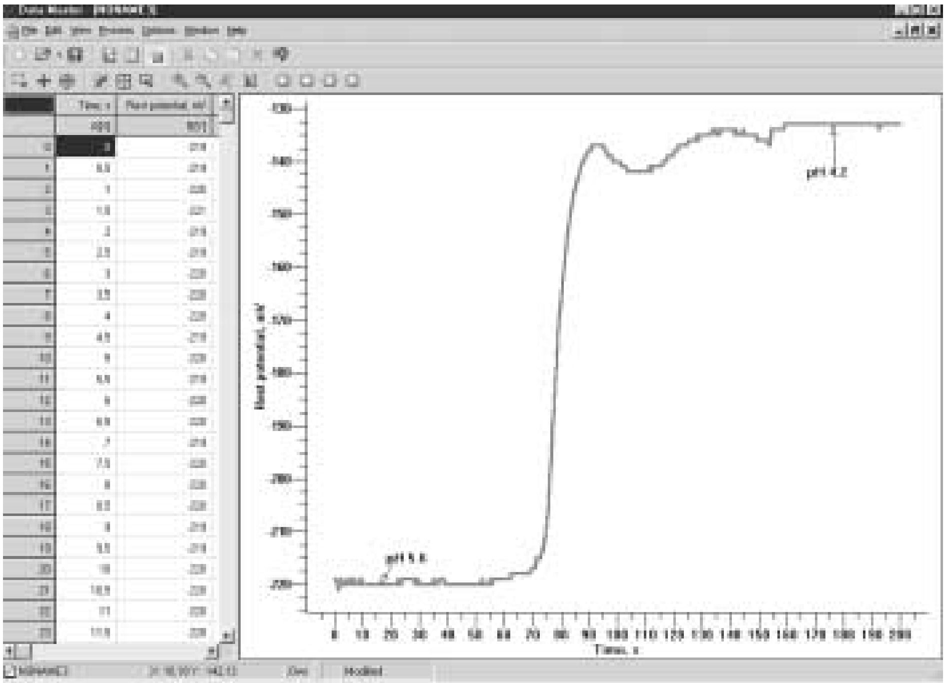

Fig. 5. Resting potentials of *Nitellopsis obtusa* membrane. Resting potential changes depend on pH of used solutions.

Second part. Action potential and characteristics of action potential.

During this experiment students can observe and investigate:

1) action potential as a result of electrical or mechanical stimulation;
2) magnitudes with depolarizing peaks over 0 mV;
3) AP durations of seconds;
4) refractory periods up to several minutes.

Third part. Processing (noise removing) and analysis of experimental data, application of theory and statistics knowledge for practical purposes:

1) Nernst potentials calculation for the ions distributed across the *Nitellopsis* cell membrane to hypothesize the ions responsible for the resting potential and for the depolarizing phase of the AP. These calculations suggest that K^+ is responsible for the resting potential and that Ca^{2+} influx and Ca^{2+}-activated Cl^- efflux are responsible for depolarizing phases of the AP.
2) Simple statistical data processing. For this calculation the experimental data collected in laboratory works by all students of the group can be used.
3) Comparison of *Nitelopsis obtusa* cells and other cells (for example neurons) reactions to applied stimulus.

In presented laboratory work simulated data of neurons responses are used because experiments with real neurons are much trickier then experiments with algal cells. Bioeth-

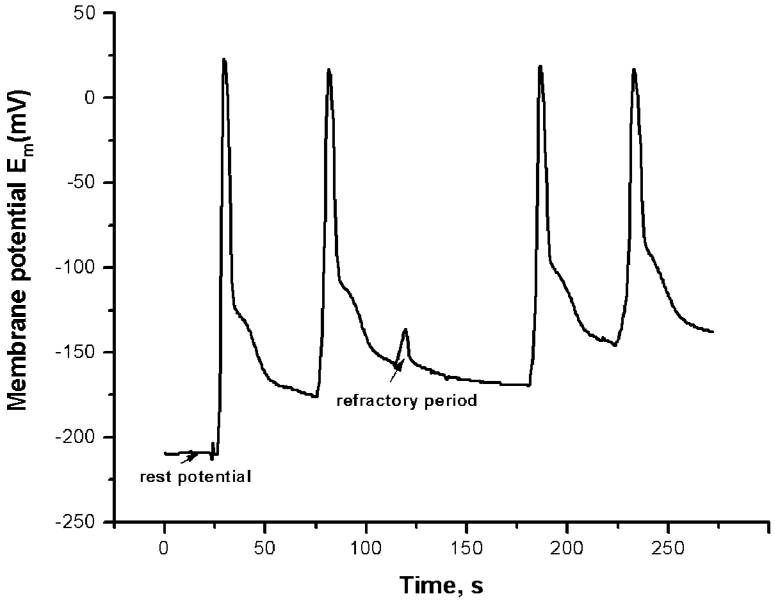

Fig. 6. Resting and action potentials of *Nitellopsis obtusa* membrane. Changes of magnitude of action potential depend on interstimular interval.

ical requirements limit the use of animal objects in similar experiments also. Programs *Neuron* (Huguenard and McCormick, 1994) or *NeuroDynamix* (Friesen and Friesen, 1994) are used for simulation of neural cell membrane potentials.

The real experiments as ones presented here give experience of real experiments with real biological objects. Virtual experimental part (simulated experiments with mathematical model of neuron biopotentials) gives experience on processes in more tricky biological objects. This doubled experiment can serve as an experimental/simulational core of more enriched learning complex. In case when potential of World Wide Web is used experiment can work as nucleus for seminar part, where students search for additional information, articles and reviews in scientific journals, databases and news strips and discuss issues related to wide range of problems from research in plant ant animal electrophysiology to environmental expertise, prostheses of sensory organs and brain-computer interfaces.

Summing Up

Presented experiment system is a base on what students create an authentic understanding of scientific skills, processes, and habits of mind, interacting with materials, measurements, variables and informational technologies application. Information search in multiple sources (for example, articles in data bases of scientific magazines) and analysis help to connect and to fuse students' observations and the observations of others in students'

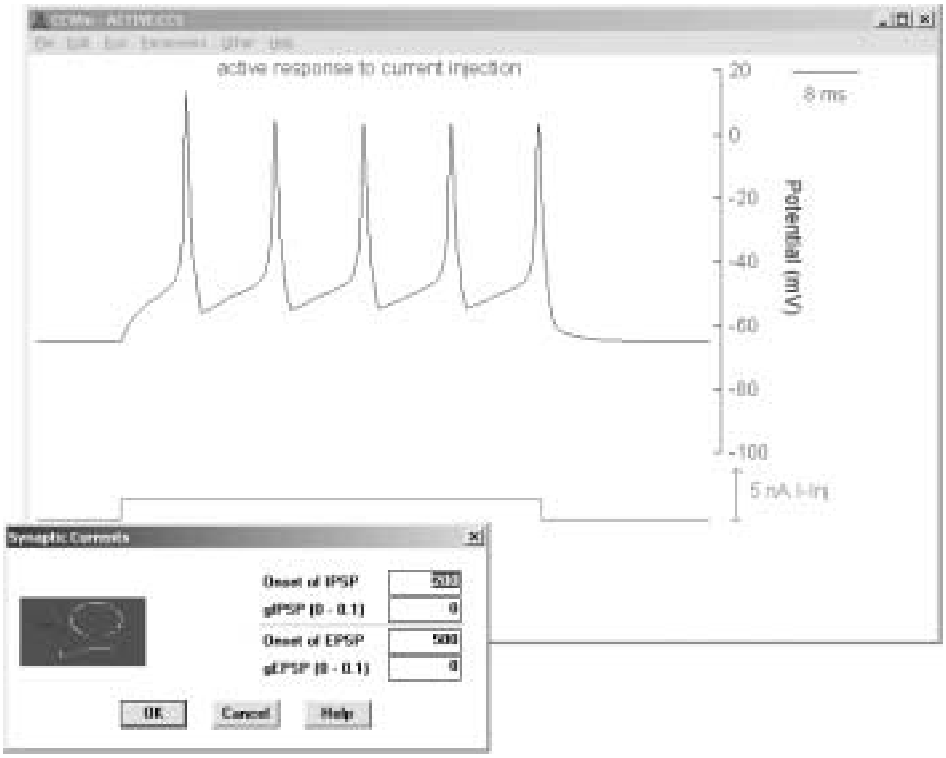

Fig. 7. Simulated action potentials of neuron. Simulated by program *Neuron*.

minds. Study of cell biology by virtual means is helpful, it helps to imagine invisible process in the cell, but wet experiment gives and requires more knowledge integrated from different fields of science even though biological object investigated in wet experiment is a simple one. The students must understand physiology of object, chemistry of used materials, methods of signal registration and data analysis. Lack of knowledge from any field of science necessary for real experiment gives artifacts or loss of experimental data (Sorgo *et al.*, 2002). Investigative science is the way in which science should be taught but the difficulties of managing classroom inquiry, need for expensive hardware, and software, modern bioethical requirements stop wide implementation of purely experimental learning. So combination of wet (real) experiment and virtual experiment that enables virtual experiments with tricky, expensive and bioethically restricted objects, enhanced by discussion and internet-extracted information is the necessary alloy for production of knowledge necessary for specialists in nature sciences and technologies. Presented computerized laboratory work is used in teaching laboratory for biology students but it can be used in education of agriculture, sylviculture, medicine, computer sciences specialists also.

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Informacinių technologijų panaudojimas biologijos mokyme: kompiuterizuoti elektrofiziologiniai augalinių ląstelių matavimai

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Šiuolaikinė biologija gali būti priskiriama informacinių mokslų kategorijai, tačiau informacinio mąstymo bei analitinių metodų taikymas nemažai daliai biologijos studentų bei tyrėjų vis dar išlieka problema. Šiame straipsnyje siūlome, kaip realų ir virtualų elektrofiziologijos laboratorinius darbus sujungti į vieną darbą, kuris galėtų būti įtrauktas ne tik į biologijos, bet ir į medicinos, žemės ūkio, bioinformatikos studijų programas. Laboratoriniame darbe, naudojant menturdumblio Nitellopsis obtusa ląsteles, tiriamos pasyvios ir aktyvios augalinių ląstelių membranų elektrinės charakteristikos, susipažįstama su elektrofiziologinių signalų registravimo ir analizės principais. Eksperimento kontrolei ir surinktų duomenų registravimui bei tiesioginiam pavaizdavimui buvo sukurta paprasta kompiuterinė programa. Siūlomam eksperimentui atlikti užtenka minimalios laboratorinės įrangos ir medžiagų. Derinant jį su virtualiais eksperimentais, įgyjamos ne tik elektrofiziologijos, bet ir informatikos, signalų teorijos, duomenų analizės, chemijos žinios bei gebėjimai.