

## КОНЦЕПЦІЯ БІОСЕНСОРА ТА ВВІД ДАНИХ ДО БІОМЕДИЧНИХ ІНФОРМАЦІЙНИХ СИСТЕМ

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У статті представлено детальну характеристику технічних пристроїв — нейроподібних біосенсорів, аналіз хімічних і електричних інформаційних сигналів на його вході та виході, можливості кодування інформації біосенсором, а також детальний аналіз можливостей інкорпорування цього біотехнічного пристрою до електронних інформаційних систем як їхнього елементу, функціонально пов'язаного завдяки електричним сигналам на виході біосенсора. Застосовано методи компаративного аналізу характеристик вхідних і вихідних хімічних та електричних інформаційних сигналів біосенсора, розроблено його фізичну та математичну моделі. Наведено концепцію біосенсора, надано їхню загальну характеристику та розглянуто відповідні прототипи. Розроблено фізичну модель біосенсору та наведено деякі результати його випробування. Розглянуто нейроподібний біосенсор як абстракцію у послідовній єдності його функцій: приймач сигналів — фільтр — аналізатор — кодер/декодер. Наведено коротке математичне описання функціонування біосенсора та відповідний алгоритм. У результаті виконаних робіт обґрунтовано можливість інкорпорування нейроподібного біосенсора у біоінформаційні електронні системи та надано приклад такої системи «ЕкоІС».

Ключові слова: біосенсор, фізична модель, математична модель, електронна інформаційна система, ввід даних.

## BIOSENSOR CONCEPT AND DATA INPUT TO BIOMEDICAL INFORMATION SYSTEMS

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**Background.** In present publication we generalized and analyzed deeply the experience of some biosensors studying in biophysical experiments with aim to incorporate them further to electronic information systems. Output biosensor electrical signals were input ones to electronic information system making their connection into joined bioinformation system.

**Materials and methods.** Methods of comparative analysis of the characteristics of input and output electrical information signals of biosensor were applied; its physical and mathematical models were developed. For biosensor properties studies the methods of transmembrane electric currents recording in voltage-clamp mode as well as patch-clamp on hippocampal neuronal membranes were used.

**Results.** Biosensor concept and their general characteristic were given, corresponding prototypes were observed. The physical model of biosensor was developed and some test results of this device were suggested. The biosensor was examined as abstraction in consistent unity of its functions: signal receiver — filter — analyzer — encoder/decoder. A brief mathematical description of biosensor functioning was given as well as corresponding algorithm. As a result of performed works the possibilities of this biosensor incorporation to bioinformation electronic systems were substantiated and the example of such system «EcoIS» was observed.

**Conclusion.** In conclusion following results of the works were summarized. The detailed description of technical devices — biosensors as elements of biomedical information systems were done as well as analysis of electrical information signals at output of biosensor, its ability to encode information and detailed analysis of the possibility to incorporate this biotechnical device into electronic information systems due to biosensor output electricals signals.

**Key words:** biosensor, physical model, electronic information system, data input.

## КОНЦЕПЦИЯ БИОСЕНСОРА И ВВОД ДАННЫХ В БИМЕДИЦИНСКИХ ИНФОРМАЦИОННЫХ СИСТЕМАХ

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В статье представлено детальную характеристику технических устройств — нейроподобных биосенсоров, анализ химических и электрических информационных сигналов на его входе и выходе, возможности кодирования информации биосенсорами, а также детальный анализ возможностей инкорпорирования этого биотехнического устройства в электронные информационные системы как их элемента, функционально связанного благодаря электрическим сигналам на выходе биосенсора. Применено методики сравнительного анализа характеристик, входящих и выходящих химических и электрических информационных сигналов биосенсора, разработанные его физическая и математическая модели. Представлена концепция биосенсора, дана их общая характеристика и рассмотрены соответствующие прототипы. Разработана физическая модель биосенсора и приведены некоторые результаты его испытания. Рассмотрен нейроподобный биосенсор как абстракция в последовательном единстве его функций: приемник сигналов — фильтр — анализатор — кодер/декодер. Приведены короткое математическое описание функционирования биосенсора и соответствующий алгоритм. В результате выполненных работ обосновано возможности инкорпорирования нейроподобных биосенсоров в биоинформационные электронные системы и рассмотрен пример такой системы «ЭкоИС».

**Ключевые слова:** биосенсор, физическая модель, математическая модель, электронная информационная система, ввод данных.

**Introduction.** The combination of artificially created technical systems and natural systems based on the latest information and computer technologies (ICT) is one of progressive directions of modern science and technique. The invention of such hybrid systems allows to overcome numerous problems that do not find their solution within the limits of one branch knowledge; and Moore's Law is only one example of such restrictions [1]. According to classic definition, the term «information system» (IS) means any system that is able to receive, process, record and transmit information [1, 2]. In concordance with classical ideas of cybernetics, we distinguish two types of information systems [1, 2]. According to them, «information system» can mean: 1) technical information systems (tIS) and 2) living systems in the Nature («open» biological information systems), we propose to call them «natural information systems» (nIS). Hybrid information systems (ISs) have also been developed over the last decade; they combine the characteristics of nIS and tIS [1-4]. In this publication we suggest the authors data about such information systems.

Specific direction which has become increasingly important in recent years suppose a solution of the problem of creating electronic information systems combined with biosensors (BS) — elements of biological origin or their artificial analogs. In previous publications on biomedical ISs [1-4], the author has already discussed the issue of high quality biomedical input data for such ISs [1, 5, 6]. The number of requirements important for biomedical ISs input data was described in those publications [1, 5]. Present publication is devoted to the input data that come to the IS from biosensors in the form of electrical signals and biosensors, able to output such data. Basing on previously obtained data [5, 6, 8, 9, 12, 13, 15-17], the author formulates one of the concepts of biosensor, which is based on decades of experience in studying the properties of brain neurons in biophysical experiments with registration of transmembrane electric currents. The input of such biosensor receives information that is encoded in: a) the structure of chemical substances acting on the biological object (or biological fragment of the cell or tissue), and b) the characteristics of the input electrical information signals. The characteristics of electrical signals at the input and output are always different. Studying electrophysiology of neurons we used the term «neuron-like» biosensor or its element, if they demonstrate some «neuron-like» properties. The «neuro-like» element in the biosensor is characterized by the set of characteristics that can be actually recorded

digitally during biophysical experiments. This type of biosensor is called also «neurobiosensor» (NBS). The data suggested in present article are real experimental results of brain neurons studying, obtained by the author with colleagues in O. O. Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine: these data were processed and analyzed further at the National Aviation University (Kyiv).

The junction of technical information systems (tIS) and biological (open) information systems (nIS), such as a biosensor, in one complex system is an extremely powerful technique, because the formed information complex combines the capabilities and advantages of each of the components. In our case, which will be presented below, the element of nIS is neurobiosensor NBS, described in our previous publications [1, 12]. Neurobiosensor NBS consists on biological fragment (BF) connected with electronic system (Figs 1, 2). Combining the NBS biosensor with contemporary tIS, a complex information system with databases (DB) is formed, which is network-based, often with access to global Internet. At the same time, the neurobiosensor NBS demonstrates by itself the possibilities of information encoding, which are given it by the Nature.

Below in the article, the materials were presented in following order:

- 1) Biosensor — a general concept. General characteristics of biosensors;
- 2) Biosensor NBS: prototypes;
- 3) Biosensory fragment in the form of neuronal membranes in some biophysical experiments;
- 4) Biosensor as abstraction, its 4 main functions;
- 5) Overview of the sum of biosensor functions: a) information signals receiver (chemical and electrical signals acceptor), b) signal filter, c) analyzer (membrane local analyzer, e.g. electrical signals and/or chemicals at the input), d) encoder/decoder; the encoder encodes the input information signals;
- 5) incorporation of this biotechnical device into electronic information systems as its element.

**Purpose of the work** is to make detailed description of technical devices — neurobiosensors, analysis of its input and output chemical and electrical information signals, biosensors ability to encode information, as well as detailed analysis of the possibility to incorporate this biotechnical device into electronic information systems due to biosensor output electrical signals.

**Materials and methods of investigations.** Methods of comparative analysis of the characteristics of input and output electrical information signals of biosensor were applied; its physical and mathematical models

were developed. For biosensor properties studies the methods of transmembrane electric currents recording in voltage-clamp mode as well as patch-clamp at hippocampal neuronal membranes were used.

**Results and their discussion.** Biosensor — a general concept. In the literature one can find several biosensor definitions and formulations of biosensor concept. This happens due to great variety of biosensor types and the tasks they perform, as well as broad range of their applications. Thus, the definition of the author corresponds to own experience of biosensor (neurobiosensor) study in biophysical experiments described below.

The author considers the biosensor as analytical device (as well as bioinformation system), which includes a neuro-like element(s) with its(their) properties, with the set of its functions as an acceptor (receiver of information signals), filter, bioanalyzer and encoder/decoder of these signals. Electronic subsystem may be included as a part of biosensor; it receives output electrical signals from incorporated biological fragment (BF).

Some other authors have suggested different definitions of biosensor too. These definitions do not contradict to our, but complement the abovementioned one being more or less close to it. Here are some of the most usable and close to our definitions of biosensors and relevant brief information about them.

*The first definition.* «Biosensor is an analytical device that uses enzyme-catalyzed reactions, immunochemical reactions, or reactions going in organelles, cells, or tissues to determine chemical compounds. In biosensors, the biological component is combined with a physicochemical transducer».

This definition differs from our abovementioned ones because it was formulated for biosensors that demonstrate only physicochemical and biochemical reactions; other details of both definitions are more or less coincided.

The second definition. There is another definition of biosensor, which is coincided more with one formulated by the author, because it reflects the conversion of information at biosensor input («biological reaction») into the output electrical signals. So, «Biosensor is analytical device that converts a biological reaction into electrical signals. Biosensors must be highly specific, independent on physical parameters such as pH and temperature, and must be reusable. A biosensor is an analytical device used for detection of chemical substances that combine a biological component with a physical and chemical detector» [7].

Concerning the structural and functional analysis of biosensor described in the literature, it is often possible to find the opinion that biosensors consist on three parts [7]:

- bioselective element (material of biological origin or element that mimics it). The sensitive element can be constructed using bioengineering;
  - transducer or converter (it works basing on physicochemical principles: optical, piezoelectric, electrochemical, etc.). This part of device converts the signal that appears as a result of analyte interaction with bioselective element, into another signal that is easier for measurement;
  - connected electronics, which is responsible, first of all, for displaying the results in user-friendly form [7].
- In our case, described below, such electronic systems have to be responsible for the maintaining of biological object vital functions.

The author proposes a functional analysis of biosensor (according to its functions). According to this approach, the first two parts of the above list can be detailed:

- the bioselective element corresponds to the sequence of 3 functions: a) acceptor, or receiver of information signals (chemical or electrical signals), b) filter of input signals, c) local analyzer (of input acting chemicals or input electrical signals);
- the converter (transducer) corresponds to the functions of encoding/decoding of input signals.

*Classification of biosensors.* Electrochemical biosensors. Depending on the type of transducer, biosensors can be classified into optical, acoustic, calorimetric, thermal and electrochemical. Electrochemical biosensors, consequently, are divided into potentiometric, amperometric and conductometric [7].

Among the above types, we are the most interested in electrochemical biosensors, because the biosensors we invented belong to this type. The functions of electrochemical biosensors are based usually on the enzymatic catalysis of reaction in which electrons are released or absorbed. Such biosensor includes usually three electrodes: reference electrode, working electrode and auxiliary electrode. The biological material is applied on the surface of working electrode, which specifically reacts with the agent to be analyzed (analyte). The charged products of reaction make a potential at working electrode, which is subtracted from the potential at reference electrode to obtain the output signal. Current measurements are also used; in this case, the electron flux intensity is proportional to the analyte concentration. This procedure has to

be done at constant potential, or the potential can be measured at zero current (this gives a logarithmic response). Direct electrical determination of small peptides and proteins by their characteristic charge is possible, using biologically modified ion-selective field-effect transistors (ISFTs) [7].

**NBS biosensor: prototypes.** Developing the concept of neurobiosensor (NBS), the author proceeded from the results of biophysical experimental researches with the registration of electric currents (signals) in voltage-clamp mode (or patch-clamp) on the cell membranes of living organisms (brain neurons as well). On the examples of such objects further work has been done — the incorporation of the NBS biosensor as element, as subsystem to electronic information systems (IS). The results of this work are presented in this article, they are protected by the patents of Ukraine [8, 9]. Accordingly, the following foreign works served as prototypes for biosensor NBS.

The first prototype was a biosensor [10] which comprises a substrate having a buried electronic sensing element and a substrate surface above the buried electronic sensing element; a structured top layer covering the substrate surface, having a top surface above the substrate surface, and comprising at least one stimulation and/or sensing electrode and a channel for holding the biomolecule by means of suction through said channel arranged between the top surface and the substrate surface, the sensing electrode being electrically coupled to the electronic sensing element; wherein the top surface is provided for placing a biomolecule present in a sample solution thereupon, the sensing electrode is provided for sensing electrical variations in and presence of the biomolecule [10].

The disadvantages of this method are that such biosensor was formed by a set of artificial objects, but not natural ones, including laboratory-synthesized molecules. So, it does not reflect the real mechanisms of influence of chemicals on natural objects, including electrical sensory processes in the Nature. As a result, it will not be enough successful to use this method to record chemicals effects on the properties of living objects (including electric currents of biomedical object, as well as the action of harmful and toxic substances of natural and artificial origin). In the prototype [10], it is impossible to track rapid changes in the characteristics of electric currents in natural objects. The electrical signals by themselves are almost impossible to subdivide against the background of noise. So, this calls into a question the quality and

efficiency of such system [10].

The second prototype, the most close to the authors invention, is the invention [11] that relates to a method for detection of receptor antagonists comprising the following steps: (I) a sample containing the receptor antagonist is fractionated by use of a liquid-based separation means, preferably capillary electrophoresis, (II) a fraction containing the receptor antagonist or modulator is fed directly to a biosensor which is activated by an appropriate receptor agonist and, as a result of this activation, is generating a measurable response, said agonist being fed to the biosensor through the liquid-based separation means together with the antagonist or modulator, said activation of the biosensor being pulsed by delivery of the receptor agonist to the biosensor for short period of times, said periods being separated by other periods when no agonist is delivered to the biosensor, and (III) the change of the response resulting from deactivation of the receptor agonist-activated biosensor by the receptor antagonist or modulator is measured, preferably by means of a patch clamp electrode. It is further possible to resensitize the biosensor desensitized as above by use of pulsed superfusion of the biosensor. This invention also relates to an apparatus usable for practicing the above mentioned method [11].

The disadvantages of prototype device and method [11] are that the biological fragment BF before its use is pre-treated in an imperfect way, which leads to: 1 — death of biological fragments, 2 — in case of survival of biological fragments electrical signals from them can not be registered well because of noise. As a result, accordingly, it is impossible to obtain an electrical signal of satisfactory quality at BF output, therefore, it will not be successful to use such a device and relative method for recording the effects of chemicals on electric currents of biomedical object (including harmful and toxic substances and artificial origin). This calls into a question the quality and efficiency of prototype system [11] and quality and reliability of measurements [9].

*Biosensors receiving input information encoded in electrical signals or chemical structures.* Fragments of living organisms — «biological fragments» (BF) can act as biosensors by themselves [12, 13, 18-20]. Many of them are able to interact with chemical elements and compounds. Changes in electrical properties of these objects, including occurrence of electric currents in them are often consequence of such interaction. So, according to the definition, such BF can be sensitive (sensory) part of biosensor. The study of such objects

is within the competence of electrophysiological, biophysical researches, for example, with the use of the methods of transmembrane electric currents recording under the voltage-clamp conditions, patch-clamp, others. It is extremely important that in the process of such experiments, the numerical data obtained on the brain neurons can be digitized and input further to information systems. These numerical data characterize the real processes in living systems. Thus, thanks to such improvements in biological experiments, important biological research has been transferred from the descriptive field to the fields of exact sciences [1]. The complex of BF with experimental electrophysiological setup (EPS), and with electronic information systems (ISs) can be considered as united information bioelectronic complex: BF — EPS — ISs.

Here is a brief description of devices and methods, which in this article will be considered as basic ones for the experimental study of electric currents activated in NBS by chemicals [12, 13]. The experiments were performed on internally perfused

neurons of the brain, using the techniques of voltage-clamp and patch-clamp [12, 13, 18-20]. The membrane potential was recorded using an Ag-AgCl microelectrode, and the grounding electrode with Ag-AgCl was in solution external to BF. A standard electronic circuit was used for single-electrode recording in voltage-clamp mode [12, 13, 18-20]. Both currents and voltages were monitored under the computer control; the results were recorded in its memory for further analysis. All values and their changes were recorded in the form of digital values with great accuracy: the lower limit of the registration of electric currents amplitudes was 0.1 nA, and for potential changes was 0.1 mV. The scheme of experimental setup used for electrophysiological study of transmembrane ionic currents in voltage-clamp mode is shown on Figs. 1 and 2 (B).

Fig. 1 demonstrates a block diagram of technical biosensor system developed and applied by this research group successfully during several years. Fig. 2 illustrates the operation of biosensor, including its electronic measuring circuit.

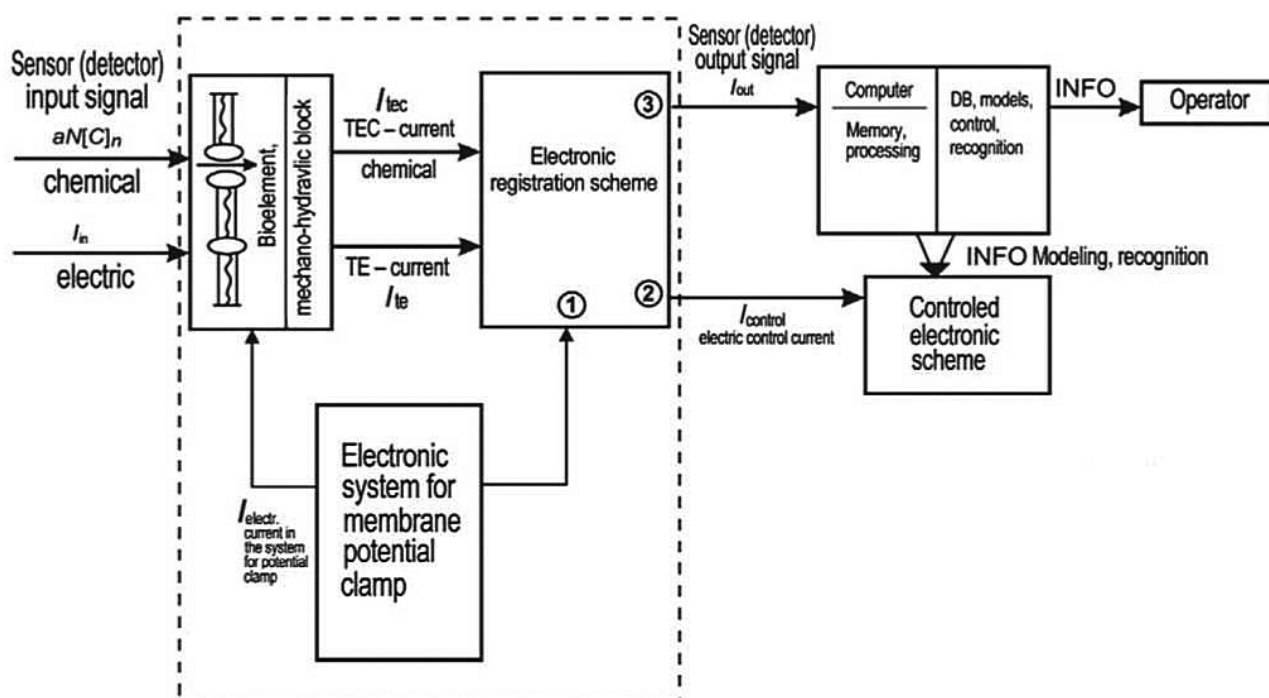


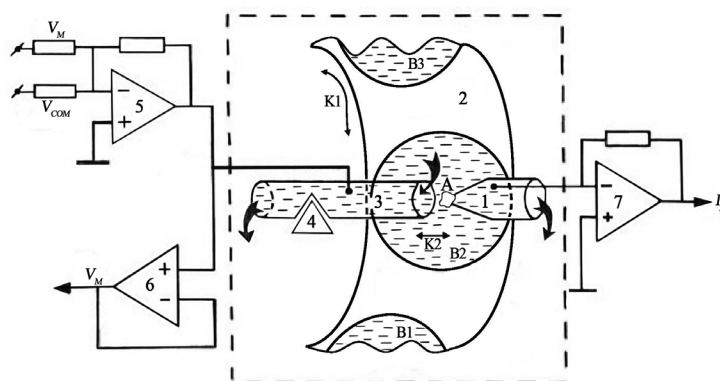
Fig. 1. Block diagram of the technical biosensor system

Invention of experimental methods was based on methods previously developed by biophysical research groups under the leadership of Academicians of the USSR Academy of Sciences and the National Academy of Sciences of Ukraine, Profs. Kostyuk P. G. and Kryshchal O. A. in one of scientific groups with

collaborators Ph.D. Tsyndrenko A. Ya., Kiskin N. I., and Klyuchko O. M. The last author has worked long with such techniques and objects [12, 13, 18-20], namely: a) membranes of brain neurons as objects (BF); b) the study of transmembrane ion currents in voltage-clamp mode, testing of different substances



I)



II)

Fig. 2. I) the object (BF): dissociated neurons of rat hippocampus (in 1 sm — 10  $\mu$ m); II) the scheme of experimental setup that was used for electrophysiological study of transmembrane ionic currents in voltage-clamp mode. Significations at the scheme II). A — Neuron at the pore of glass micropipette. 2 — Micropipette was filled with solution for intracellular perfusion; mobile cassette with experimental chambers with different solutions for the application (B1, B2, B3) in three different chambers; arrow K1 indicates directions of chambers with these solutions movement. 3 — Tube in which cell A was moved from one chamber to another; applications of substances to the surface of neuronal membrane were done in this tube according to following procedures. Cell A on the micropipette 1 was inserted into the tube 3 (arrow K2 indicates the direction of movement). When electromagnetic valve 4 was opened, a quick application of solution B2 was done; this solution was sucked into the tube due to the negative hydrostatic pressure. The dark arrows indicate the directions of the solutions flow during their application in tube 3, and flows' directions in the micropipette 1 during the cell fixation at the pore. The dotted line limits the mechanical part of experimental setup. 5 — Amplifier of holding potential  $V_M$  and command  $V_{COM}$ . 6 — Device for the measurement of potential. 7 — Amplifier of registered transmembrane currents [13]

influences on BF; c) combination of a) and b) with electronic technical information systems (tIS) [12, 13, 18-20].

**Biosensor as abstraction and its four main functions.** Considering the biosensor NBS as abstract issue, regardless of its molecular structure, what substances initiate electric currents in it, and etc., one can notice a set of important features common to many NBS. These common features are functionally determined and characteristic for this type of NBS. Let's list these four NBS functions and, respectively, four NBS elements (Fig. 3, 4).

1) *Biosensor NBS as a receiver of information signals (acceptor)* (Fig. 3, unit 2). It is well known that the input of the NBS information comes in two ways, in the form of signals of two types.

*Type 1.* Encoded in the chemical structures of substances acting on BF membranes. In this case, the electrical signals that have been registered at NBS output have characteristics co-related directly with the signals at the input. In other words, the characteristics of such output electrical signals correspond to the structures of substances that interact with BF surface

membranes (BF of biosensor). Thus, at BF level of biosensor, input chemical signals were converted to electrical ones. In this case, the term «biosensor» refers often to the surface neuronal membrane and corresponding phenomena and scenarios of chemicals interaction with it.

*Type 2.* The input NBS information is received in the form of electrical signals with certain characteristics, and these signals are perceived by the membranes structures of BF. At NBS output the signals of electrical nature are also registered, but with characteristics different from input signals characteristics. In this case, at BF level of biosensor, electrical signals with some characteristics have to be encoded to electrical signals with other characteristics.

2) *Biosensor NBS as filter of input information signals* (Fig. 3, unit 3). It is well known that NBS does not receive all information through the surface input membrane (because it often receives «white noise» of signals). The input information is perceived by NBS selectively, i.e. «significant» at the input are such signals that carry chemicals

of a very specific structure and electrical signals with well-defined characteristics. The «filtering» of input signals happens because they must interact only with well-defined molecular structures in

membranes. In fact, abovedescribed functions 1) and 2) determine the NBS role as a code key in information perception and further transmission [14] (Fig. 3, 4 A).

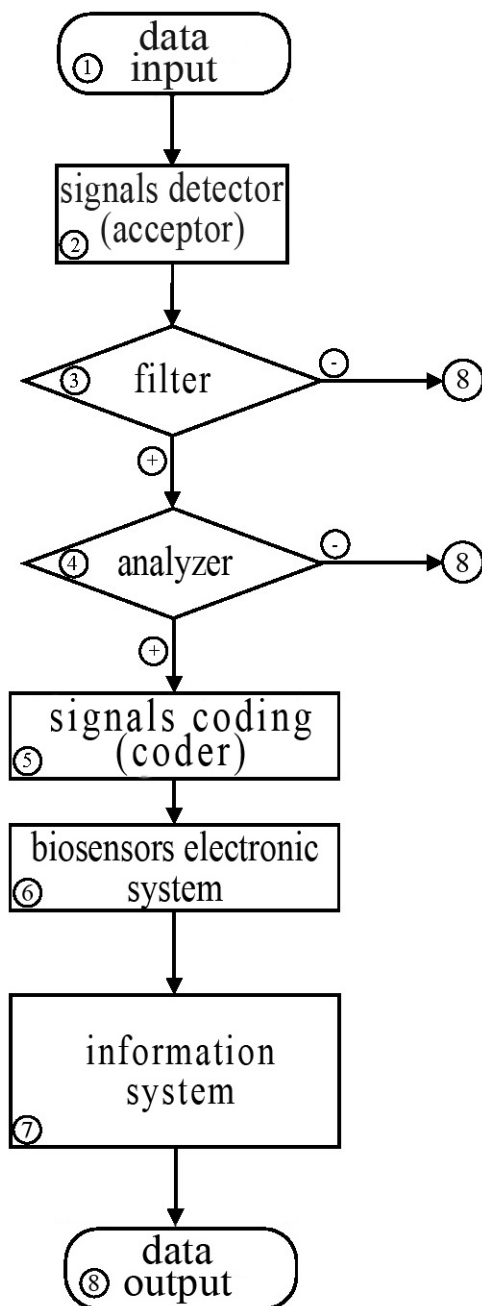


Fig. 3. Algorithm of NBS biosensor functioning: 1) Data input; 2) NBS as receiver of information signals (acceptor). The NBS input receives information in the form of signals of two types: a) encoded in the chemical structures of substances acting on BF, and b) in the form of electrical signals with certain characteristics; 3) NBS biosensor as a filter of input information signals. Functions 2) and 3) define the role of NBS as a code key in information transmission; 4) NBS as primary analyzer of input information signals. The set of NBS properties and corresponding scenarios 2), 3), 4) leads to phenomena that biosensor «distinguishes» which chemicals interact with it (and to some extent, in what quantity) [12]; 5) NBS as encoder/decoder of information; 6) Electronic system as part of NBS; 7) Information system (tIS) to which electrical signals from the NBS output are input; 8) Data output (including ones to the monitor)



3) *Biosensor NBS as primary elementary analyzer of input information signals* (Fig. 3, unit 4). The structure of the surface NBS membrane (its composition of certain chemicals, their conformation, their relative position in space, etc.) in combination with a) transformations that occur in the membranes due to the chemical molecule approaching a surface, and b) those chains transformations (including chemical reactions) that follow this, determine the NBS function as

«analyzer» of input information signals. In other words, the set of phenomena and properties of NBS 1), 2), 3) leads to biosensor «distinguishing» of what chemicals interact with it, as well as, to some extent, in what quantity [12]. New methods of qualitative and quantitative analysis, which were proposed by Klyuchko O. M., are based on these properties of membranes and corresponding effects; four patents of Ukraine were obtained for these methods; some details were published in [12] too.

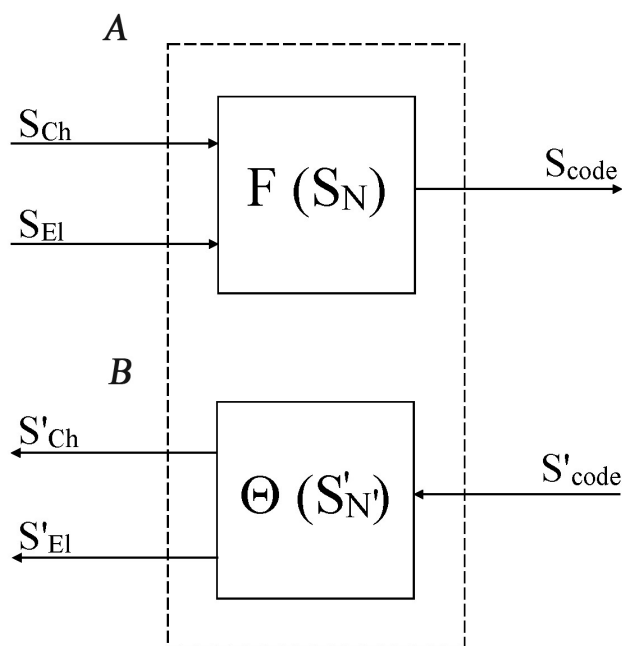


Fig. 4. Abstract representation of NBS biosensor functions as encoder (A)/decoder (B) of input information signals. The dashed line shows that the encoder/decoder elements are located within one membrane structure of biosensor. Significations at the scheme 4:  $F$  — coding function,  $\Theta$  — decoding function,  $S_N$ ,  $S'_{N'}$  — signals in quantities  $N$ ,  $N'$ , as arguments of functions respectively for encoding/decoding,  $S_{X_M}$ ,  $S'_{X_M}$  — information signals encoded in chemical structures,  $S_{El}$ ,  $S'_{El}$ , — electrical information signals

4) *Biosensor NBS as encoder/decoder of information* (Fig. 3, unit 4 and Fig. 4). The phenomenon of information encoding by biosensor and its implications for technique are extremely important. The algorithm of information encoding by biosensor is represented on Fig. 3 (unit 4), and relative functions — on Fig. 4 A, B. As it was shown above, the natural biosensor NBS performs too the functions of devices, which in technique are called encoders/decoders of information. In numerous experiments there was registered that the NBS receives information in the form of information signals — ions or molecules of chemicals, or electrical signals with certain characteristics. At the NBS level, this information is recoded into electrical signals with other characteristics. Accordingly, this process is going in opposite direction too (Fig. 4 B). Phenomena and

processes of signals coding that going in the direction «left-right» (see Fig. 4 A) can occur due to the sequence of phenomena described in 1), 2), 3) plus phenomenon of some chemical changes at «output» of NBS biosensor (Fig. 4 A, B). These sequence of phenomena coincide with ones happened in neuron during mediator release into the synaptic cleft after the influence of transmitted information signal in electric form. On the other hand, phenomenon and the process of signals coding in the direction «right-left» [8] can occur due to the reverse sequence of phenomena described above, which, however, have their own specifics. In the Nature, this direction of signal coding can be illustrated by the example of mediator reuptake in the synaptic cleft by the end of the axon, from which this mediator was previously released during

the transmission of information signal through the synapse and subsequent events (Fig. 4 A, B) [8].

Basing on the current level of knowledge and results obtained in our experiments, it can be proved that the functions of NBS biosensor for encoding/decoding can be expressed in two ways: 1) in tabular form (Fig. 5, and tables 1, 2), and 2) in analytical form

as a function or system of functions. The functions of information encoding and decoding by NBS biosensor are presented on Fig. 4. The next section provides an adequate mathematical description (model) of this physical model, as well as reveals the physical meaning of these functions and related phenomena.

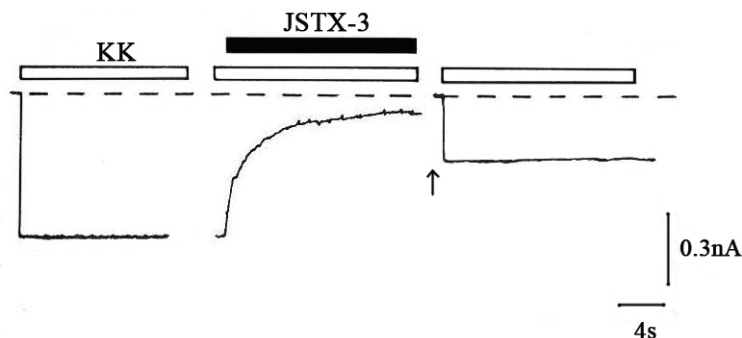


Fig. 5. Information signals from biosensor registered during electrophysiological experiment. At biosensor input is a substance kainat (KK), which initiated a steady electric current, and after some time the substance JSTX-3 was applied against the background of this current; KK reduced the current amplitude with certain kinetic characteristics (left and middle parts of the record to the arrow). At biosensor output a stationary electric current was received with a smaller amplitude (recording right after the arrow). The action of JSTX-3 is characterized by a number of characteristics, such as kinetic constants of currents amplitudes decrease, the rate of currents amplitudes decrease and a number of other numerical values (see also Table 1). Holding potential —100 mV

In previous publications, the author has already published data on the encoding of information in NBS biosensor [12], namely on the transcoding of chemical information into electrical signals. Table 1 demonstrates the results obtained by the author in biophysical experiments on the registration of electrical transmembrane chemoactivated currents in voltage-clamp mode for different tested substances. In these experiments, the glutamate- and kainate-activated currents through the membranes of rat hippocampal pyramidal neurons and the effects of a number of toxins with known chemical structures on these currents were investigated. The Table 1 demonstrates the co-relations between the chemical structure of the chemical compound acting on BF membrane and electrical signals registered at biosensor output, in this case — kainat — activated electric currents (KK-activated currents) (see Table 1) [12]. The table shows that after the influence on KK-currents of such substances with different chemical structures, the obtained records of output electric currents are similar, but differ in a number of characteristics specific to each active substance. Moreover, such differences are reliable, repetitive, uniquely relevant, and this permits to argue that the conversion of chemical information into electrical form has occurred and the result of this coding demonstrates also unambiguous compliance.

The data shown on Fig. 5 and Table 1 are, in fact, an example of the practical application of NBS biosensor to encode information about one of chemicals structure in the form of corresponding electric currents recordings. Currently, there are known about several thousand such examples (and they can be cited). When this information ordering into appropriate databases, it can be used to encode the information on relevant chemicals. The general ways of transmission, transformation, coding, all chains of information processing during and after the biophysical experiment, from the input of initial data to the biosensor (left) to the creation of models (right) are represented on Fig. 6.

Kinetic characteristics of KK-activated ionic currents block by integral venom from *N. clavata* and toxin JSTX-3 in comparison with toxins argiopin and argiopinines 1,2 from *A. lobata*. At biosensor input: substances—antagonists with certain chemical structure (far left column); at output: changed characteristics of electrical signals (data in all other columns of the table). Comments.  $\tau$  — is time constant of K-currents recovery. KK-kainate,  $k_1$ ,  $k_2$  — constant rates for velocity of blocking (direct reaction),  $v_-$  — velocity of recovery of currents' amplitudes,  $\tau_-$  — time constant of currents' amplitudes recovery,  $K_d$  — dissociation constant [12].

Table 1

Numerical characteristics of substances-antagonists action on kainat (KK)-activated transmembrane electric currents

Effect	Antagonist	Decrease of electrical currents' amplitudes (%)	Recovery of electrical currents' amplitudes (%)	Constant rates for velocity of blocking (direct reaction)		Velocity of recovery of currents' amplitudes $v=1/\tau_*$	Dissocia-tion constant Kd
				k1	k2		
JSTX-V		34,0	34,0	$4.4 \times 10^3 \mu\text{L}/\text{units} \cdot \text{s}$	—	—	—
JSTX-3		6,0	39,0	$2.1 \times 10^3 \text{ L}/(\text{mol} \cdot \text{s})$	—	$1.3 \cdot 10^{-2} \text{ s}^{-1}$	$6.2 \cdot 10^{-6} \text{ mol/L}$
argiopin (AR)		14,4	34,0	$1.6 \times 10^3 \text{ L}/(\text{mol} \cdot \text{s})$	$0.85 \times 10^4 \text{ L}/(\text{mol} \cdot \text{s})$	$4.2 \cdot 10^{-2} \text{ s}^{-1}$	$2.5 \cdot 10^{-6} \text{ mol/L}$
argiopi-nin 1 (ARN-1)		44,0	56,0	$3.3 \times 10^3 \text{ L}/(\text{mol} \cdot \text{s})$	$1.6 \times 10^4 \text{ L}/(\text{mol} \cdot \text{s})$	$7.9 \cdot 10^{-2} \text{ s}^{-1}$	—
argiopi-nin 2 (ARN-2)		22,0	47,0	$2.9 \times 10^3 \text{ L}/(\text{mol} \cdot \text{s})$	$0.59 \times 10^4 \text{ L}/(\text{mol} \cdot \text{s})$	$3.1 \cdot 10^{-2} \text{ s}^{-1}$	—

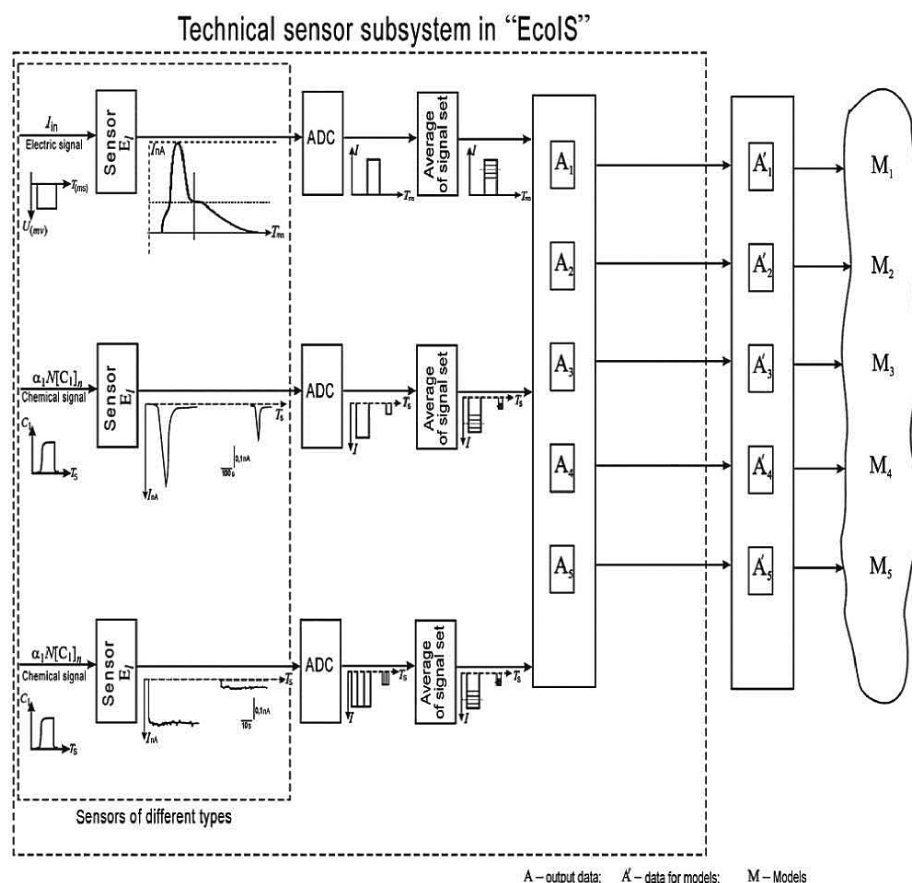
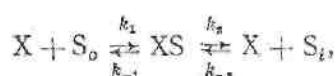


Fig. 6. Scientific bases of biosensor development: from electric signals to models. Signals to biosensor input can come both in electrical and/or chemical forms, which are converted into electrical output signals. In biosensor system they undergo further transformations when passing in circuits, when processed similarly to signals in technical systems (explanations see in text) [18]

*Mathematical description of the main phenomena and processes in biosensor.* Model in terms of Michaelis-Menten was used and developed for this description. When a molecule of chemical compound (agonist) is attached to its corresponding binding site on the surface of biosensor membrane, an electric current is initiated (due to the formation of channel-receptor complex (CRC)). In our case, such agonists that initiated chemo-activated transmembrane electric currents were molecules of sodium salt of kainic acid – (KK) 12. Lets assume that all ions penetrating through the channel, for one act of agonist molecule attachment, transfer the charge is  $z = S \cdot N$ . The ions transmission process can be written as:



where  $k$  are the rate constants of chemical reactions that depend on the voltage.

Basing on the laws of chemical kinetics, we can write an expression for a stationary value of output current:

$$I_s = zF \frac{k_{-1}k_{-2}[S]_i - k_1k_2[S]_o}{k_{-1} + k_2 + k_{-2}[S]_i + k_1[S]_o}$$

In our case, the KK agonist molecule is located on only one side of the membrane, and the equation can be simplified to a form corresponding to Michaelis-Menten expression for the enzymatic reaction with voltage-dependent rate constants. We introduce new parameters that depend on the voltage. There are the parameters: maximal current  $I_{max}(E)$  and imaginary Michaelis constant  $K_m(E)$ . In the presence of only internal penetrating ions:

$$I_s(E) = \frac{I_{max,i}(E)}{1 + K_{M,i}(E)/[S]_i}$$

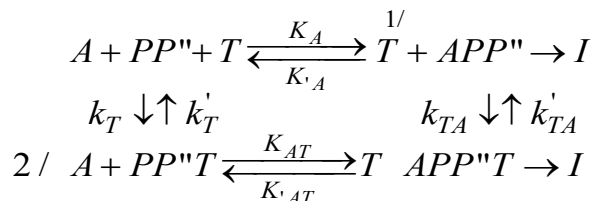
and taking into account the rate constants that depend on the voltage:

$$I_{max,i}(E) = zFk_{-1} = zFb_{-1} \exp(zFE\delta / 2RT)$$

and

$$K_{M,i}(E) = \frac{k_{-1} + k_2}{k_{-1}} = \frac{b_{-1} \exp[zFE(\delta - 0,5) / RT] + b_2 \exp[zFE(\delta - 1) / RT]}{b_{-2}}$$

When the agonist molecule is attached to the CRC, chemo-activated transmembrane electric currents ( $I_{Glu}$  or  $I_{KK}$ ) occur, which we block with antagonist molecule T. The blocking process proceeds according to the following scheme:



where  $I$  is chemoactivated current,  $A$  — is an agonist,  $P$  — is an agonist binding site on CRC,  $T$  — is antagonist (toxin), and  $PP''$  — is a toxin binding site on CRC.  $PP''$  forms a system of paired receptors.

*Electrical output signals of NBS biosensor as element of electronic information system.* In some previous publications the author has already published the recorded results demonstrating the co-relations between the chemical structure of molecules that interact with the surface of NBS membrane and electrical signals recorded at the output of biosensor [12, 13].

Some results of studies of the occurrence of electrical currents in brain neurons in response to electrical stimulus (electroactivated currents), or in response to chemical irritation (chemoactivated currents) are shown in Table 2 (see also [15]). From our point of view these results can be used for the solution of some problems of technical devices design, e.i. biosensors as elements of some components of computer, or as elements of some information systems tIS. Lets represent in generalized Table 2 («Signals...») the data that can be used for such purposes. There are the data on some effects recorded during biophysical experiments described in our and some other publications [8, 12-14, 20].

Table 2

Electrical signals at biosensor output, registered in biophysical experiments

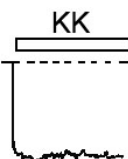

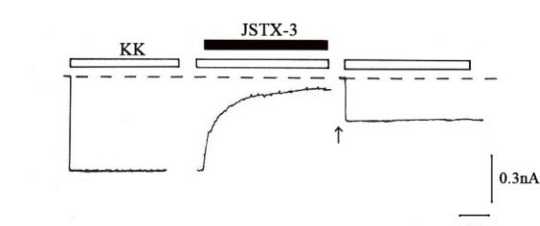
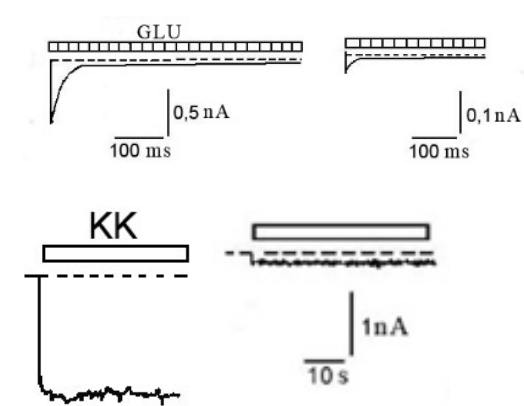

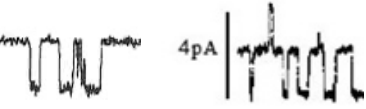
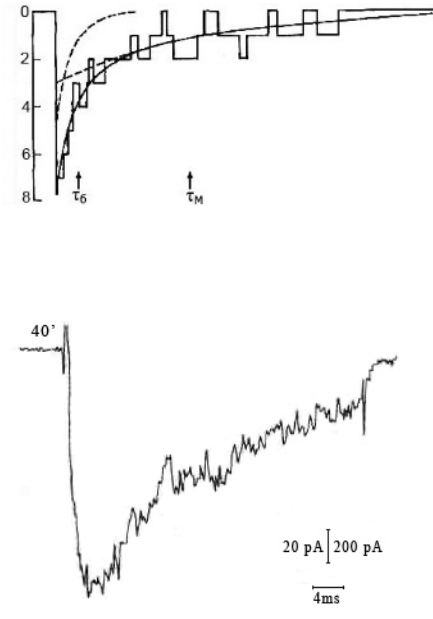
№	Object	Registered effect	Physical mechanisms in the base of effects
1	Neuronal mem-branes	<p>The pulses of electrical currents <math>I_{mem}</math> appear at neuronal membranes. The pulse shape can be set in advance by acting with different substances.</p> <p>1.a. kainate— activated current <math>I_{KK}</math>. – <i>rectangular step</i></p>  <p>1.b. glutamate — activated current <math>I_{Glu}</math> – <i>saw tooth triangular pulses</i></p>  <p>Pulses can be set periodical (<i>arbitrarily or purposefully</i>):</p> <p>1.c. kainite — activated current <math>I_{KA}</math>, and its further inactivation by Araneidae toxin (Tx) — rectangular step pulse <math>I_{KK+}</math> (Tx)</p> 	<p>Activation — deactivation of transmem-brane channels chemo-activated by various agonists, modification of transmem-brane currents <math>I_{mem}</math> by antagonists. Actions by chemical agents (agonists-antagonists) change the molecule structure of chemorecep-tor complex (CRC).</p>
2	Molecules of glutamate. channel receptor. complex (gCRC)	<p>Current pulses amplification of <math>I_{KK}</math>, <math>I_{Glu}</math> 11,8 times, noise reduction when signal occurs</p> 	<p>Reduction of gCRC damage by enzymes during membranes preparation for experiments</p>

Table 2

3	Currents $I_{KK}$ and $I_{Glu}$ : Araneidae toxins influence on them	Influence by toxins (Tx) on $I_{KK}$ and $I_{Glu}$ : «Exclusion» (if necessary) of transmembrane currents $I_{mem}$ , creation of pulses of the set forms (reversible / irreversible) 	Modification of the structure of CRC molecule during experiments
4	Currents through single channels	Obtaining miniature pulses of rectangular shape (pA) at patch-clamp registration [14] 	Single act of CRC molecule «work»
5	Currents through single channels	Statistical sum of currents through single channels (1) gives total current pulse $I_{mem}$ , pA (2), which was recorded experimentally [14] 	Total transmembrane current $I_{mem}$ formed by the sum of elementary currents through single channels

Analyzing the data in Table 2 («Signals...»), it should be noted that solving the problem of generating pulses of a given shape is one of the important tasks in the creation of computer technique and information systems in general. Our data obtained during studying of electrical properties of neuronal membranes allow us to create a model of technical device — biosensor that has the properties of encoding information and outputs pulses of various, predetermined shape and

with predetermined electrical characteristics [15]. The Table 2 for comparison contains the data of other authors obtained in experiments with other methods of biophysics [14].

As will be shown below, basing on some experimental data from Table 2, it is possible to demonstrate the biosensor capabilities to encode information and protect data in systems with biosensors. Let's examine the Table 2 in details; below we will give corresponding

explanations concerning the effects represented in it. The column on the left shows the name of biological object on which a certain phenomenon is registered. For example, a set of phenomena were registered on the membranes of biological neurons, in our case — on rat hippocampal neurons (for example, field 1 of Table 2. In the central column there is a list of the effects that were registered on certain object (for example, field 1 of Table 2 — 1.a, 1.b, 1.c). Because the effect — 1.a, 1.b, 1.c — we created purposefully in experimental conditions and they were repetitive, we can say that in the future they can also be repeated purposefully, as a result of the work of corresponding natural or artificial elements of future information systems with biosensors. For example, as in our experiments, such artificial elements of future biosensors in IS will be able to generate triangular pulses during the applications of substances — glutamate (IGlu), glycine (IGly) or GABA. Accordingly, such artificial elements will be able to generate rectangular pulses, if artificial gCRC would be activated by kainate (KK, IKK). In order to obtain a pulse of rectangular shape with given duration, at the right moment it is necessary either to stop the action of the KK, or to act with a specific substance — an electric current antagonist (fields 1.c and 3 in Table 2). The latter can be blocking agents from the set of specific Glu-R blockers (see Table 1). The column on the right shows the physical mechanisms in base of corresponding effect (if such mechanisms are currently known).

The above refers to the creation of single pulses. However, with the periodic repetition of these procedures, it is also possible to create periodic signals (meander, sawtooth series of pulses, and etc.) (field 1 of Table 2 — 1.c). Another example of periodic signal may be the opening of single channels in membrane that

transmit electrically charged ions, forming an electric current (field 1 (1.c) and field 4 of Table 2). An example of process control may be the field 5 of Table 2.

The possibility of modifying the signals from studied object — the brain neuro — is shown in field 2 of Table 2. Here are data on how to increase the amplitudes of output signals (IGlu, IKK) and to reduce the noise of this signal by perfection the chemical treatment of membranes at the stage of their preparation for experiment. We believe, and this has been proven by our numerous long-term experiments, that the treatment of neuronal membrane with proteases complex from *Aspergillus oryzae* is less harmful to Glu-R (in comparison with the treatment using the complex of pronase and collagenase), and enzyme treatment complex (and conditions) in this case are more close to the natural one [20]. Accordingly, the amplitude of registered signals of IGlu, IKK increases 11.8 times, the noise during the assignment of these signals is significantly reduced [8, 9, 12, 13].

**Bioinformation computer systems with incorporated biosensor.** The ability of NBS biosensor to generate and/or transmit and output electrical information signals (but not signals of other origin) is an extremely important property that allows to incorporate this object — NBS neurobiosensor — into the electrical systems (in our case — in setup for biophysical experiments), with the subsequent inclusion of the formed complex into the information systems tIS with access to networked Internet systems (Fig. 7). Different types of electrical signals that are actually registered at output of NBS biosensor, and which allow somebody to connect successfully this object with electrical devices and systems [15] are shown in Table 2. On Fig. 7 the NBS biosensor is also presented in the form of several functional blocks (description of their functions see above).

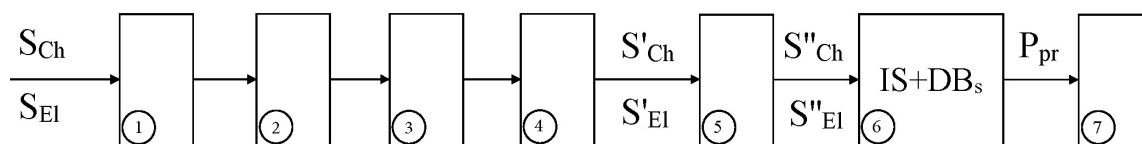


Fig. 7. Block diagram of the NBS biosensor and its incorporation into electronic information system: 1 — receiver of input information signals (acceptor), 2 — signal filter, 3 — elementary analyzer, 4 — unit with the function of input signals encoding (BF is represented by units 1-4), 5 — electronic unit for conducting of experiments with support functions of BF (NBS includes units 1-5), 6 — block of electronic information system with databases, in which the NBS is incorporated, 7 — data output, including output to the monitor

*Significations at the scheme 7.*  $S_{X_M}$ , — input information signals in which the information is encoded in the structures of chemical substances, and

$S'_{X_M}$ ,  $S''_{X_M}$  — their transformation in the biosensor; similarly  $S_{E_{El}}$  — are input electrical information signals, and  $S'_{E_{El}}$ ,  $S''_{E_{El}}$  — they are after the transformations in

the biosensor;  $P_{pr}$  — output information of complex system.

The electrical nature of information signals at biosensor output is a necessary condition for biosensor incorporation into the information system; they can be functionally linked, because such linkage is a result of electrical connections. Under these conditions, both individual differences of biosensors and separate individual features, characteristics of information system become less important: there are many types of such systems in the World now [1-4]. For example, let's observe briefly a comprehensive hybrid bioinformation system developed by the author [18, 19], which unites tIS and described above a «neuro-like» biosensor NBS (nIS). The purpose of this work was to develop a new biotechnical information system for ecological monitoring in a broad time ranges — «EcoIS». «EcoIS» was constructed using contemporary information and computer technologies as well as knowledge about the latest electronic information systems with databases (Fig. 8). For this purpose, some modern methods of information security, the latest biotechnical and electronic information systems, as well as the possibilities of their application for environmental monitoring were analyzed. The following methods were used: comparative studies of the samples of technical devices, simulation and software modeling basing on numerical results obtained in experiments with registration of chemosensitive transmembrane electric currents in neurons in vltage-camp mode, patch-camp and other methods.

Applied biophysical methods allowed to reveal and identify substances dangerous for living organisms, and to make the first conclusions about their possible biological effects. As a result, an original system for environmental monitoring in broad time ranges was developed. It is combined with detector groups, databases, expert subsystem and interface; it is able to distinguish some types of chemicals at its input and to output the data of their identification and, if necessary, messages about their harmfulness. During such monitoring, it is possible to study the effects of substances during several periods of time, from the first moments of their exposure to single cells in organism — to months and years after such exposure to the whole organism. The first results of practical use of the developed technical system are generalized, some of such results cannot be obtained with the help of previously used devices and sets of methods. The results of the work were published in numerous publications, which provide some data

about the analysis of the developed technical system and its practical application, as well as some practical recommendations for environmental monitoring [1, 9, 16-19].

In the process of this long-term work, three types of biosensors were developed, which were proposed to be incorporated into several modifications of bioinformation system «EcoIS» (Fig. 8), developed related techniques; all developments were protected by the patents of Ukraine [8, 9, 15-17]. For all performed works the scientific base was developed, the accompanying laboratory, experimental researches, additional information on work of information system, namely processing of obtained experimental data, rights of access to the received experimental data, and etc. have been already published [16, 17].

Physical model of biosensor has been developed as part of technical bioinformation system, and appropriate software has been developed as well. Algorithms, mathematical and software approaches to database processing for the developed bioinformation system were proposed. The developed expert system with appropriate software, electronic automated working places (EARM), and etc. were developed for this «EcoIS» system.

Electronic automated working places (EARM) for this system have been developed on the basis of appropriate databases for the use by biologists of several specialties (ecologists, neurotoxicologists, zoologists, and etc.); they became the interface to «EcoIS» monitoring system. EARMs — their structure, functions — were developed on the basis of network technologies. The developed EARMs are easy in use and meet satisfactorily the requirements in experimental, theoretical data of the relevant experts.

During the development of biosensor as element of bioinformation system, a method of amplifying 11.8 times the amplitudes of biosensor output electric currents (in the membranes of brain neurons) was developed. This method improves the detection of electrical signals against noise. Using the developed method, the experimental recording of output electric currents became more perfect, so the further processing of IS data became more perfect too. Patents of Ukraine were obtained for all developed methods [8, 9, 15-17].

**Conclusions.** The results of research of biosensor (neurobiosensor — NBS) were described in present article. The general concept of biosensor was given as well as its definition, general characteristics and prototypes for invented BS version. The physical model of biosensor was represented; some test results



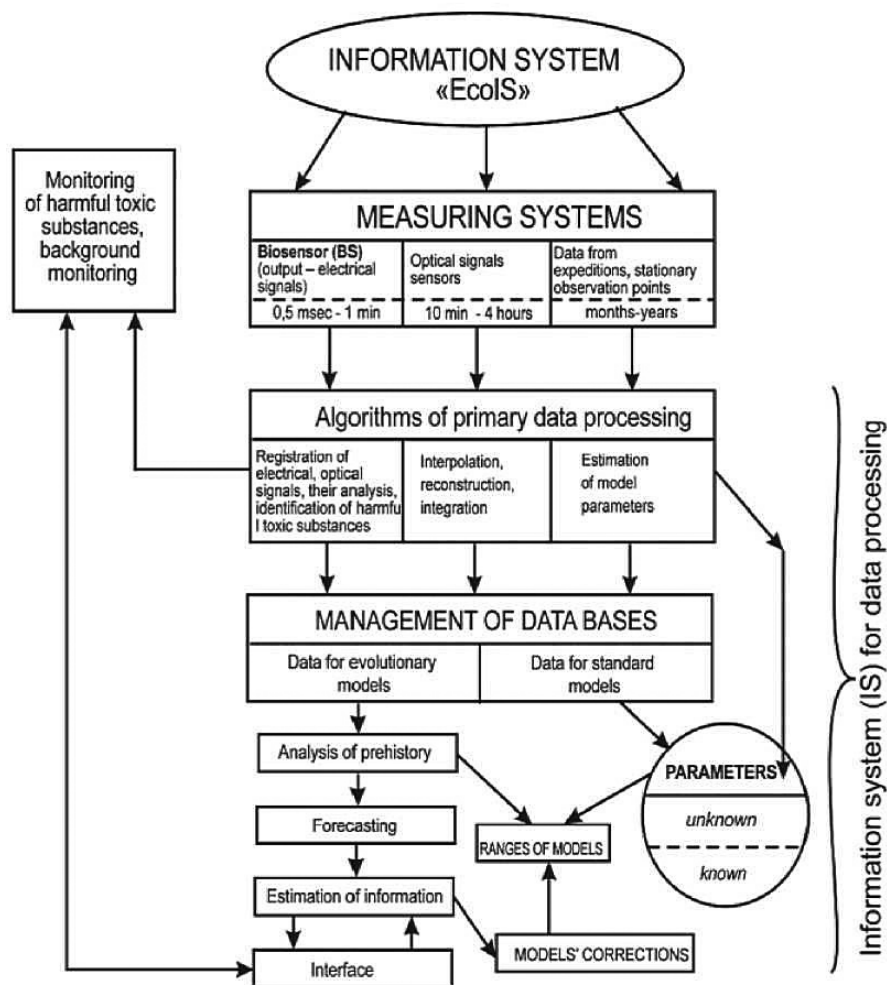


Fig. 8. Block diagram of information system «Eco-IS» with biosensors [18]

of this device were given. The neuro-biosensor is considered as an abstraction in consistent unity of its functions: signal receiver — filter — analyzer — encoder/decoder. A brief mathematical description of phenomena and processes in biosensor, its functioning and corresponding algorithm were suggested.

1. Possibilities of information conversion by biosensor within the following model were considered. At NBS input the information arrives being coded in the form of chemical structures of active substances or in the form of electrical signals with defined characteristics, after encoding the information is output in the form of electric signals with changed characteristics. It was shown that the reverse phenomenon — decoding of information is also possible both in the Nature and in technique.

2. An example of practical application of NBS biosensor for encoding of information about the structure of defined substance into the shape of corresponding electric currents was given. There are

more than several thousand of such examples were recorded for today. Being ordered into appropriate databases, such information on relevant chemicals will become available to users of electronic bioinformation systems, including ones with access to the Internet.

3. The incorporation of such biosensors, which transmit the information in the form of electrical pulses to electronic bioinformation system, was observed on the example of «EcoIS». This information system «EcoIS» with biosensors was developed by the author for ecological monitoring in broad time ranges: from the first moments of chemical substances influences on organism — to the months and years after it. This system was developed for the purposes of eco-monitoring of the impact of harmful technogenic pollutants on living organisms.

4. Some parts of performed work are theoretical. In order to produce industrial samples of biosensors as part of information systems, it is necessary to make some additional works in the future.

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