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REVIEW

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Cell-based biosensors

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Abstract Different classes of cell-based biosensors are introduced. These include devices to measure cell-cell contact and set-ups to determine metabolic products. Main emphasis is put on sensors based on nerve-cell networks which are able to detect neuro-active compounds. The different experimental set-ups are explained and examples for typical applications are given. A main point concerns new achievements and prospects for future developments.

1 Introduction

Until now, the practical applications of biosensors have been almost entirely limited to very few specific examples including, in particular, glucose and pH measurements. Exciting results may be achieved by expanding the field of biosensors to the adoption of complex biological recognition elements such as cells. There are at least three main advantages using intact cells or tissue slices: First of all, "group effects" such as toxicity, mutagenicity, or pharmacological activity become accessible to measurements using sensor technology. Secondly, internal amplification cascades can be used to increase the sensitivity of the device. Thirdly, whole cells are the smallest biological entity which is self-sustaining. If non-neural cells are used the sensors are inexpensive because the preparation costs of culture growth are low.

Cell-based sensors can be divided into several classes. The first distinction may be made by the type of cells utilized in the system. For the past 25 years cells from bacteria and algae have been used, mainly in amperometric and potentiometric devices. In these sensors a cell layer is immobilized on the surface of an electrode. Oxygen electrodes can be used to probe the quality of water by measuring the biological oxygen demand (BOD), and such sensors are already comercially available. New developments aim at fully automated systems [1]. Another parameter to monitor is the presence of herbicides which can be measured by using cyanobacteria together with a redox couple such as $[Fe(CN)_6]^{3-/4-}$. If the photosynthetic activity of the bacteria is reduced by the presence of herbicides the reduction of the iron complex is also reduced and hence the response of an amperometric sensor device [2]. Other applications make use of the fact that some bacteria contain large amounts of a certain enzyme. The metabolic product is measured, e.g. by an ion-selective electrode (see, e.g., [3]) or an amperometric device (see, e.g., [4]). Examples also exist where the bioluminescence or a fluorescence signal were measured as a response to an enzymatic reaction (for a recent example see [5]).

Because biosensors based on simple cellular organisms are well-established, only biosensors based on cells from complex organisms will be considered in this review.

Another distinction may be made by the kind of connectivity the cells keep in the sensor system. One can either use single cells, cell layers, cell networks, tissue, or even whole animals/plants. The first three principles are the main subject of this review. Tissue-based sensors are often based on a classical sensor system such as a glucose sensor but in which the active biological entity, i.e. in the case of glucose sensors glucose oxidase, is not embedded into an artificial environment but taken in its natural tissue environment. These sensors based on the pioneering work of Rechnitz are not reviewed here, see instead, e.g., [6]. The utilization of neural tissue and whole animals for sensing purposes will be sketched briefly in Section 4.2.

Last but not least the measurement principle may be used to classify cell-based sensors. Three different approaches for whole cell sensors have mainly been applied (Fig. 1): In the first, the mechanical contact between cells and between cells and substrates is measured *via* a.c. conductivity measurements in which the cells act as resistor. In the second, (bio-) chemical sensors are used to measure metabolic products delivered from cultured cells to the medium. In the third approach the direct electrical response of electrogenic cells (neural cells, heart muscle

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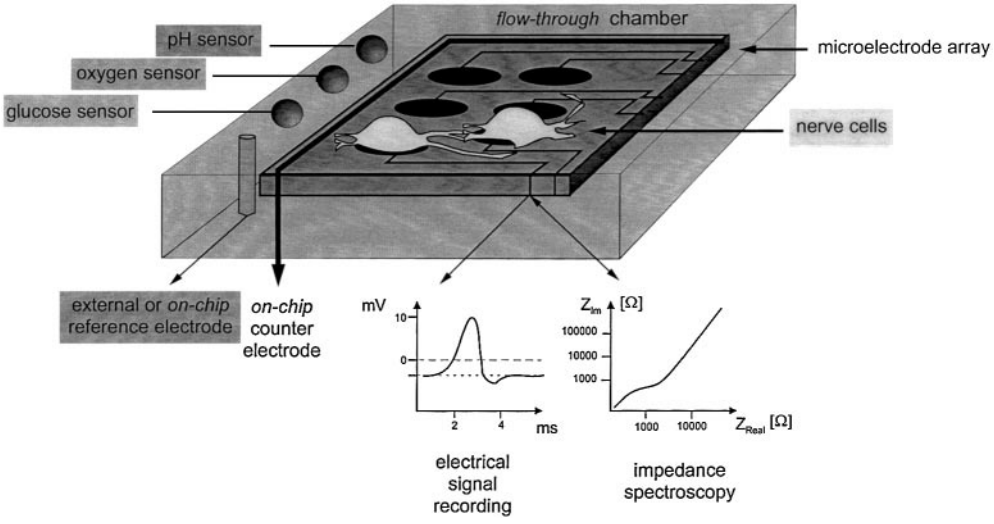


Fig. 1 Different approaches to biosensors based on electrogenic cells combined within one measurement chamber [7]

cells, pancreas beta cells) or a neural cell network is measured.

The first and second approaches have two main advantages: Established sensors and cell lines can be used. In contrast, studies of neuronal cells are difficult to perform for several reasons: Neuronal cells cannot be produced in continuous cell lines and they appear more fastidious in their choice of substrate. However, the unique advantage of high specificity through receptor-interaction can be made use of and receptors may be incorporated intentionally for "fine-tuning". Because of the high innovation potential of these sensors main emphasis will be put on this subject. The particular aspects of olfactory sensing, i.e. mimicking the human or animal noses, are summarized in an earlier review [7].

Finally, it has to be mentioned that a distinction is sometimes made between sensors measuring cell functions to understand cellular mechanisms, and sensors in which the cells act as a biological entity in a "normal" biosensor, i.e. to make a qualitative and/or quantitative analysis of molecules in the test analyte. However, if molecules are detected through their influence on the cellular behavior this distinction only depends on the personal view of the user. Therefore, all aspects of cell-based biosensors will be treated in this review, although main emphasis is put on the more technical biosensor applications.

This paper is not a complete review of the literature but presents a selection of important references. To make the review as useful as possible a lot of web addresses are also given where the reader may access the recent publications of the respective research groups or find information on commercial products.

2 Measurement of cell-cell contact

There are two main experimental set-ups used to measure cell-cell contact: In the first, an array of planar electrodes is placed onto a substrate, and the cells are cultivated on top of them. The array is either an interdigitated comb structure [8, 9] or another geometric arrangement of electrodes [10]. The impedance is either measured between two of the electrodes [8, 9] or between the electrode(s) and a large counter electrode on chip [11] or put into the medium [12] (Fig. 1). Alternatively, as in more classical experiments, the cells are cultivated on top of a water-porous filter membrane which separates two compartments [13, 14]. There are usually two large electrodes in each of the compartments (Fig. 2). This set-up gives the overall resistance of the whole cell layer on the filter. If inhomogeneities within the cell population play an important role or the cell layer is incomplete, e.g. in early days of cultivation, a new technique which allows the measurement of only small areas is needed [15]. This can be realized by a small area tube on top of the lower compartment. The first set-up has the advantage that the cells are cultivated as in normal cultivation dishes, i.e. on a solid non-porous support. However, the planar electrodes have to be prepared separately by thin film evaporation processes.

In all set-ups the impedance is measured as a function of time and changed environment which may influence the cell-cell contact. In the first set-up the cell-electrode contact also influences the resistance. These parameters are interesting for different applications: Firstly, pharmaceuticals can be tested. These may include anti-tumor drugs if tumor cells are used (see Fig. 5 and description below [22]) or drugs which have to pass the blood-brain

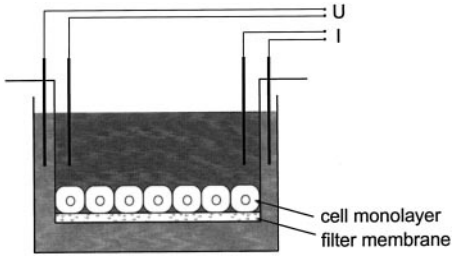


Fig. 2 Commonly used setup to determine cell layer resistances [10]

barrier if endothelial cells are utilized [16]. The second application concerns the toxicological influence e.g., of heavy metal ions [8]. In the third application, cell-cell contact, in most cases of epithelial or endothelial cells, is studied from a fundamental scientific point of view, e.g. to understand the tight junctions involved in the blood-brain barrier [12], to study the barrier function of the intestinal wall [17], or to study wound-healing by using damaged endothelial cell layers [18]. These measurements can also supplement those on neural networks (see Section 4 below).

3 Measurement of metabolic products

The measurement of extracellular metabolic products is well established. The determination in direct and on-line studies of the metabolic activity and the functional analysis of living cells is possible which is of major importance for both, basic research and pharmacological applications. Metabolic rates in cells are due to receptor mediated events and can be defined, e.g., by the rate of acidification of the extracellular environment of cells, or by the change in oxygen, glucose, or lactose content. Depending on the metabolite of interest, well-established chemical or biochemical sensors can be used for detection. These include simple glass electrodes, pH-sensitive ISFETs (ion-sensitive field effect transistors with Si_3N_4, Al_2O_3, or Ta_2O_5 as gate insulators), or light addressable potentiometric sensors (LAPS) [19, 20] for monitoring pH-changes, Clark electrodes for oxygen, and enzyme-based amperometric devices for glucose and lactose measurements. Furthermore, ions other than H^+, e.g. Na^+ or Ca^+, can be detected by ion-sensitive electrodes or ISFETs with ion-selective membranes on top of the gate. Figure 3 shows the different transducer principles. All the different sensors can be used alone, in combination, or in addition to the measurement principles discussed above and below in Sections 2 and 4, respectively. Because cells respond to external stimuli with the parallel activation of different signalling pathways, the combined measurement of different signals is very useful. If one has to work with small numbers of cells, the use of miniaturized sensors is required to minimize the size of the measurement chamber.

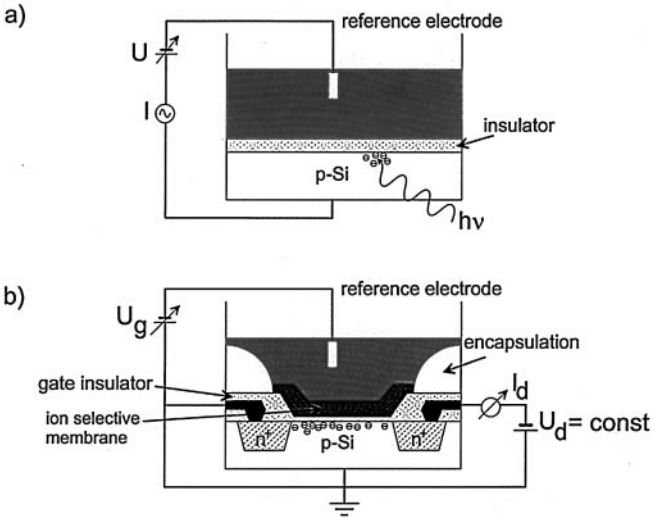


Fig. 3 a, b Transducer principles to detect cell metabolites. **a)** Light addressable potentiometric sensor (LAPS). The silicon chip is covered by a thin nitride or oxynitride layer and functions as an extremely sensitive pH sensor that depends on pH in a Nernstian fashion. It enables measurement of a voltage U that is linearly related to pH. Acidic metabolites, produced by the cells, change the surface charge of the silicon material and therefore alter this voltage. If the light is pulsed, an ac current is measured. Because electrons are only produced in the illuminated region, spatially resolved measurements are also possible; **b)** Ion sensitive field effect transistor (ISFET). U_g is the gate voltage determined by the surface potential influenced by the present ions, U_d and I_d drain voltage and current, respectively. If U_g is changed, the measurement signal I_d is also influenced. If pH is to be measured and the gate insulator is Si_3N_4, Al_2O_3, or Ta_2O_5 the ion-selective membrane is not needed

Figure 4 shows typical metabolic pathways in which pH-changes occur and demonstrates the importance of pH-measurements in a biosensor system. Miniaturized potentiometric glass-electrodes have the advantage of long-term stability (minimum drift) compared to miniaturized Si-based ISFET- and LAPS-devices [21, 22]. In a comparative study [23], LAPS and ISFETs showed a similar behavior wih respect to sensitivity, drift, and response to a pH change. However, the authors claim three main objectives to use LAPS devices in a commercial instrument:

- The LAPS sensing surface is flat and free of metal contacts. This makes it easy to be implemented in a measurement chamber. The flat surface is also present on backside-contacted ISFETs, but these are not as robust as the LAPS.
- LAPS manufacturing is simple and cheaper than ISFET production.
- LAPS lifetimes are longer, in particular, because the long-term stable encapsulation of ISFETs is still a problem.

A number of disadvantages of LAPS have also to be stated:

- Due to the light sensitivity the measurements have to be performed in the dark and no parallel light microscopical control is possible.
- Due to the fabrication process and the measurement principle a parallel integration of LAPS together with other sensors is not possible or does not make sense.
- The miniaturization potential is limited
- A parallel operation of two or more LAPS on one chip requires structuring of the chip.

The basic scientific applications of cell metabolism sensors are widespread (see, e.g., [24] for a commercial LAPS instrument) and concern all aspects of metabolism-affecting pathways (cf. Fig. 4). Commercial aspects mainly concern pharmacological, immunological, or toxicological questions. Figure 5 shows the combined measurements of extracellular acidification, cellular respiration, and cell-electrode contact for the particular example of testing cytosta-

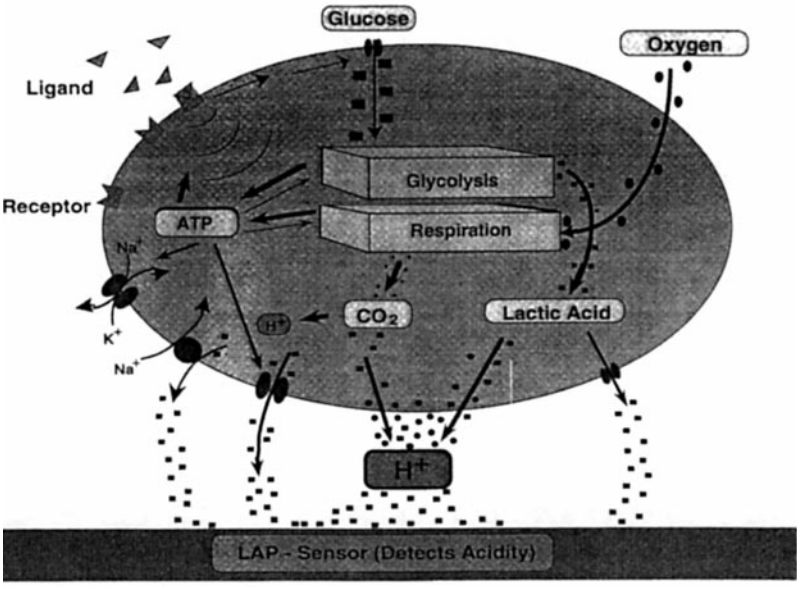
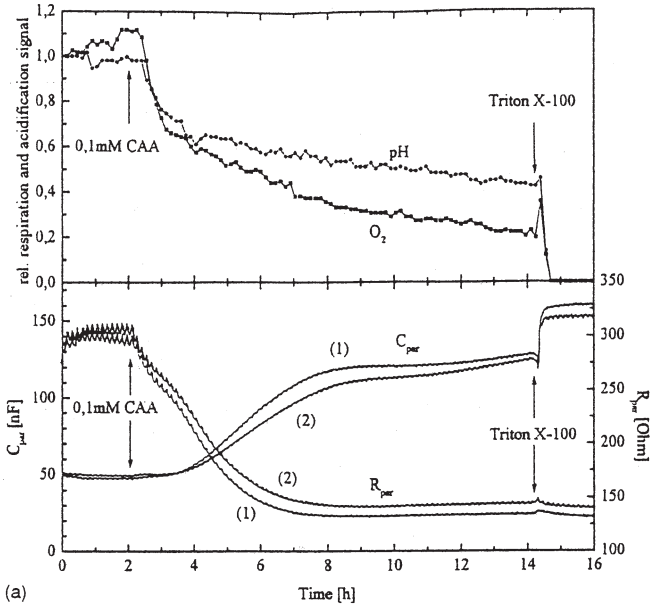


Fig. 4 Schematic representations of metabolic pathways in which extracellular pH-changes occur [7]



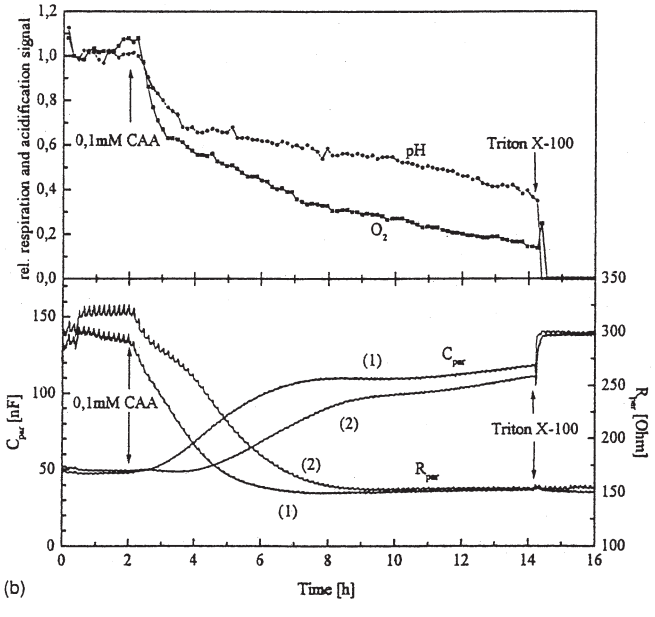


Fig. 5 a, b Combined measurements of extracellular acidification (pH), cellular respiration (oxygen), and electrical parameters (parallel capacitance C_{par} and parallel resistance R_{par}, each recorded with two interdigitated electrodes (1) and (2)) upon treating a confluent monolayer of cells from a human colon carcinoma cell line (LS 174 T) with 0.1 mM chloroacetaldehyde and the detergent Triton-X-100. To demonstrate the reproducibility the experiments were performed in parallel inside both channels (**a**) and (**b**) of a 2-channel version of the culture-sensor unit [22]

tic agents [22]. Here, chloroacetaldehyde (CAA), an alkylating agent originating *in vivo* from the cytostatic agent ifosfamide was used. It can be noticed that all three sensor types respond fast to the addition of CAA. From the signal not only the activity but also hints for the mechanism of cytostatic activity can be found: Particularly mitochondrial enzymes seem to be damaged since the oxygen content and hence the respiration rapidly decrease. Also, the morphological changes are rapid as reflected in the decreasing resistance measured by the interdigitated electrodes, whereas in light microscopy a rounding up of the cells can be detected only after more than one hour.

There exist also measurements of intracellular activities, in particular intracellular Ca^{2+} and pH changes, which can be measured by fluorometric assays. For a commercial high-throughput device see [24].

4 Extracellular signals of electrogenic cells and cell networks

4.1 Current state of the art

Nerve cells and nerve cell networks are very sensitive to a large variety of neuroactive compounds added to the culture medium. Neuroactive compounds can be defined as water-soluble molecules that can influence the sensitive electrophysiological mechanisms of nerve cells. These influences may be classified as done by Gross [25]:

- direct metabolic effects that may increase, decrease, or stop activity (e.g. c-AMP, cyanide);

- specific synaptic effects (all neurotransmitters and neuromodulators, e.g. strychnine);
- transmission effects that stop action potential propagation (tetrodotoxin, which blocks the voltage-gated Na^+ channel, or ouabain, which blocks the sodium pump, preventing maintenance of the membrane potential); and
- generic membrane effects mediated through non-synaptic Ca^{2+} or K^+ channels or by the generation of new channels (ionophores).

Nerve cells or nerve cell networks grown in culture on microelectrode arrays [26–28] or on field effect transistors [29] show effects on such neurochemicals. These effects can be measured by the change of membrane potential during an action potential. This potential has a direct influence on the gate of the field effect transistor, and it influences the capacity between a microelectrode and the axon which can be measured with a.c.-coupled amplifiers with high input impedances.

Single neural cells do not give reliable signals because 1) they do not usually become spontaneously active and have thus to be excited externally leading to a short life time, and 2) they lack their natural environment which leads to non-natural responses. In contrast, neural networks respond to neuroactive compounds with changes in their activity patterns. These changes are often substance and concentration specific and, most important, histiotypic. As in nature, networks are relatively fault tolerant concerning, e.g., changes in synaptic connections. The effects of an altered environment can be studied by detecting changes in the spontaneous native activity patterns which are present in the *in-vitro* cultures after several days. Alternatively, a network oscillation can first be induced as a function of blocking the inhibitory synaptic receptors, e.g. by adding bicuculline, and then the changes in these oscillations are followed. The advantage of the latter approach is a more defined initial signal, but the disadvantage is a less sensitive system, a non-natural behavior, and an induced stress of the neural cell culture and hence a much shorter lifetime. However, the shortening of the lifetime may be avoided by adding bicuculline just before a measurement, but this will in turn cause long preincubation steps before an analysis.

As it is now possible to maintain networks in electrophysiologically active and pharmacologically responsive states for over 9 months *in vitro* [30, 31], such systems have become reliable candidates for the performance of certain sensory tasks. This indicates that cultured neuronal networks are practical systems that can be used for the detection and characterization of biologically significant effects of a great variety of chemical substances. An overview of tests performed till 1997 is given in [25, 31], a recent example (testing of cannabinoid agonists) can be found in [32]. Although still at an experimental stage, instruments for extracellular recording are commercially available [33, 34]. Reported applications are fundamental aspects of cell networks [35], as well as pharmacological [31, 32] and toxicological response studies [31, 36], qualitative [25, 26], and recently also quantitative analysis of neuroactive substances [37–39]. Pharmacological response has also been studied in heart muscle cells which give larger signals as compared to neural cells and are hence easier to study [40].

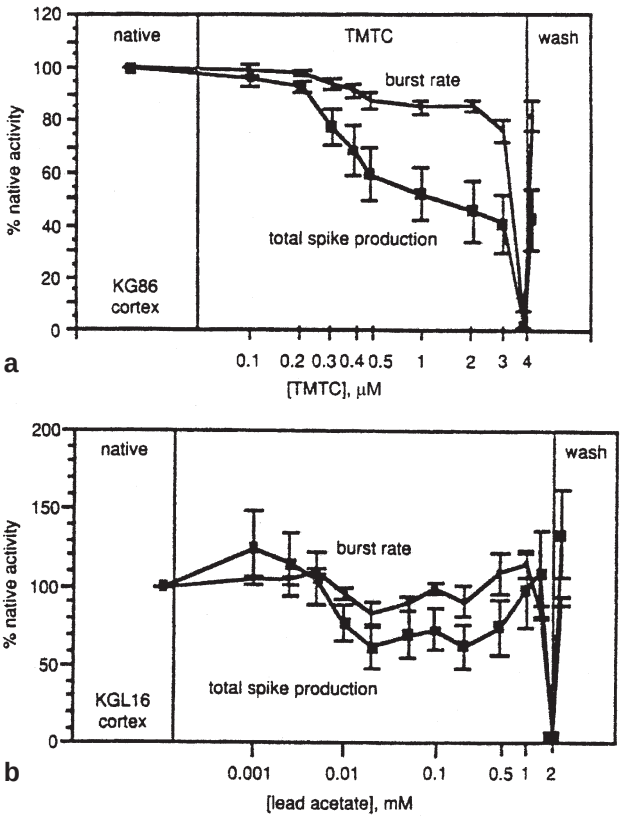


Fig. 6 Different recordings from networks of auditory cortex tissue upon application of trimethyltin chloride (TMTC) **(a)** and lead acetate **(b)**. For details see text and [31]

In the following a few examples will highlight the potential of neuronal networks for biosensing purposes. Figure 6 shows the toxicological effects of two neurotoxins, trimethyltin chloride (TMTC) and lead acetate (PbAc) [31]. The activity of the network is completely inhibited after addition of 4 μM of TMTC or 2 mM of PbAc, but is restored after washing. This figure also shows that taking different aspects of the network activity, i.e. the total spike activity (corresponding to the number of action potentials) and the burst rate (i.e. the number per minute of so-called bursts, in which high spike activity is recorded, for details of signal processing see [25, 30]), one may get fingerprints of the different substances. In Fig. 7 data on bicuculline and strychnine exposure are evaluated by principal component analysis [41]. Both molecules induce oscillatory network responses by blocking inhibitory synapses but at different concentrations. As can be seen from the figure, the two substances can easily be distinguished although the native activity before addition of the neurochemicals was widespread.

The many kinds of data from an extracellular recording cannot only be used for qualitative but also for quantitative biosensing purposes. Figure 8 shows the correlation of real concentration and the predicted concentrations from an artificial neural network (ANN) analysis of ex-

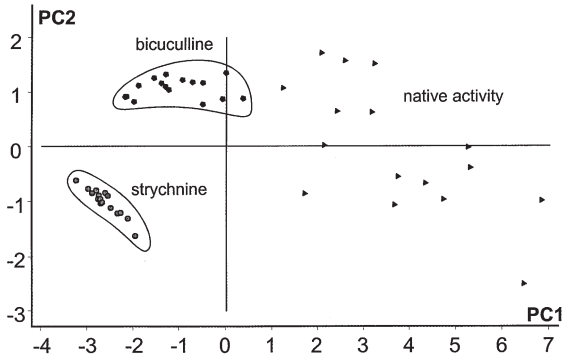


Fig. 7 Principal component analysis (PCA) of data recorded on embryonic mice spinal cord cultures in the native state, upon application of 60 μM bicuculline and, after a washing cycle, of 25 μM strychnine. PC1,2 are the principal components 1 and 2. Classification of data is best if data points are close together within one cluster and clusters are well separated. For details see text and [41]

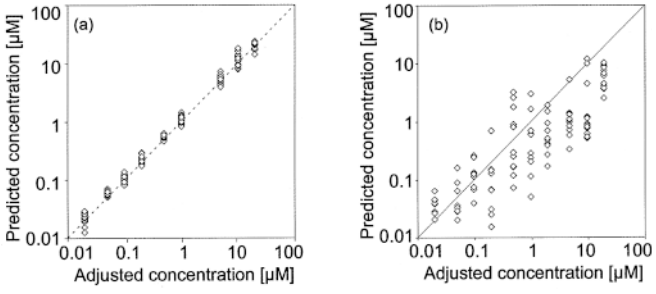


Fig. 8 a, b Predicted vs. adjusted (real) concentration of strychnine in embryonic mice spinal cord cultures, evaluated with a backpropagation artificial neural network [38, 39, 41]. **a)** The net was trained with 2/3 of the data and tested with the remaining 1/3 of the data. **b)** Training was performed with data from a first concentration series, test with data from a subsequent series performed 30 min after the first

perimental data of strychnine [37–39]. In the left part of the figure the good correlation within the same experiment is shown. However, for real biosensor applications the calibration and training of the ANN is done in one step. In a second step after days or weeks an unknown analyte is studied. The result of an experiment performed immediately after the calibration run is shown on the right side of Fig. 8. Although still correlated, the prediction error is now large. One reason is certainly the poisoning of the network by the high strychnine concentrations and a too short recovery time between the training and analysis sequences. But these results show that, in principle, a quantification of biosensor data based on natural neural networks and evaluated by artificial neural networks will be possible. Although many difficulties have to be overcome before (see Section 4.2) it can be of large practical use.

4.2 Trends

As can be seen from the data presented in Section 4.1, neural cell networks are interesting candidates for testing and development of new drugs and as sensitive biosensors. However, there are still a number of difficulties to be overcome before a wide commercial use is possible:

- The number of active electrodes is still low. There are two possible ways out: Either 1) smaller electrodes are prepared; but this gives a worse signal-to-noise ratio. Or 2) the “electrodes come to the cell”, if not the absolute number but the number of active electrodes which are covered by a firing cell are too low. This is possible by preparing light-addressable electrodes in which by selective illumination of a photoconductive material an active spot directly under the cell can be chosen. These systems are under current development [42].
- The signal-to-noise ratio is still low. One reason is the sealing resistance between the cells and the microelectrodes or field effect transistors. This problem may be overcome in two different ways: First of all, the cell-electrode coupling has to be optimized. The tighter the cell-electrode coupling, the smaller the loss of signal into the medium. For this purpose a variety of different solutions are tested. Two promising methods are the application of non-natural, positively-charged molecules such as aminosilanes [43], or of epitopes, i.e. small active peptide sequences from larger cell adhesion proteins such as laminin or fibronectin [44]. These approaches can be combined with efforts to pattern the surfaces and constrain cell development to the electrode area by either using molecules with electropolymerizable groups which only form layers on electrodes with applied voltage [45] or by using molecules with photoactive groups which can be modified by light and are therefore applicable for photomask processes [46–48]. Also anti-adhesive material can be used to pattern cell-attachment surfaces [49]. The best approach would certainly be to pattern the complete surface into areas with highly attractive and highly antiadhesive regions. The second possibility to increase the signal-to-noise ratio will be to minimize the signal loss due to horizontal electrical conductivity in the electrode [50]. The horizontal conductivity leads to signal loss into the electrolyte at electrode areas which are not sealed by the cell. This may be avoided by nanostructured electrodes which are only vertically conducting. This is possible, e.g. by preparing a mixed layer of conductive and non-conductive nanoparticles on top of a photoconductive material. The active electrode area is then chosen by illumination by the light-addressing approach discussed above.
- The third problem concerns the large amount of data which needs to be processed. If 50 electrodes are active and 10 data points are used to define an action potential, a typical firing rate of 1 kHz gives 0.5 MB/s. Therefore, an intelligent data reduction treatment has to be applied [25, 30].
- Most important is the lack of understanding of the complex neurobiological response: If one could predict from a microscopic picture of a cell-culture what electrical signal there will be, i.e. if one had an algorithm describing the total network behavior, then one could compensate drift effects caused by morphological

changes of the network and also transfer data processing from one network to the other. (For groups working on modelling of neuron response see [51] or for one particular program [52].)

- Another problem for "real" biosensors is the broad response of neural cells, including the response to physical parameters such as temperature, and gross parameters such as the nutritional state. If the most influential parameters are controlled this broad response can also be an advantage when testing pharmacological or toxicological activity. For detecting single species of molecules or ions, several cell networks with different receptor specificities have to be employed, as in gas sensor arrays for electronic noses [53, 54]. The fast growing field of gene technology and functional expression of gene products in heterologous systems may further increase the chances of obtaining highly specific neural cells. However, so far success with this approach has been very limited [55].
- Portable systems are required for biosensor applications. Therefore, miniaturized systems utilizing either conventional electrodes [56] or CMOS circuits [57] have been built.

Progress in this field depends on the cooperation of neurobiologists, genetic engineers, biophysicists, surface chemists, electrical engineers, and data processing specialists.

Other trends concern the utilization of whole tissue. Tissue may be even more organo-typic than *in vitro* grown cell networks. This is particularly important for drug tests in the pharmaceutical industry. However, tissue cultures are in most cases difficult to keep for longer time, although new culture techniques may help to promote longevity [58, 59].

Also taking whole animals or at least complete sensory organs such as insect antenna comprises a new trend although introduced by Rechnitz and coworkers already in 1986 [60]. New examples on potatoe beetles or their antenna show a sensitive response to broken green leaves [61], diseased potato tubers [62], or smouldering odorants [63].

5 Other measurement principles

There are only few examples for cell-based sensors which are not based on the above-mentioned three principles. One is the measurement of neurotoxins by inducing natural color changes in animal cells, i.e. chromatophore cells in the skin of fish, amphibians, and reptiles. These chromatophores change their color through enzyme-dependent movements of their colored subcellular organelles [64].

A completely different type of biosensor does not utilize biological matter to detect (bio)chemicals but infrared radiation. For this purpose the python pit organ, which is infrared-sensitive, may be used. So far, only preliminary experiments were made by using an infrared-sensitive camera to study the detection properties of the organ, which seems to be the most sensitive infrared detector known to date. Whether this will lead to a new biosensor or whether only the knowledge of the IR absorption mechanism will lead to better artificial detectors is still an open question [65].

6 Summary and outlook

Cell-based sensors comprise a lot of different setups, principles, and applications.

There are well established sensors such as those based on simple organisms such as bacteria to detect the biological oxygen demand. The future developments in this field are mainly technology- and market-driven and no completely new sensor principles are expected.

A second class of cell-based biosensors concern devices to measure cell-cell contact for different purposes. These include, for example, tests on anti-tumor drugs or fundamental studies on the blood-brain barrier. New developments which are expected in this field are mainly application-driven and concern devices which have been modified for specific measurement problems.

The measurement of metabolic products, in particular pH changes, is another well established field in cell-based biosensors. The future developments are therefore also mainly technology- and market-driven. However, the measurement of metabolic products is often used in fundamental research studies, in contrast to the microbial sensors mentioned above. Therefore, the market-drive is lower for metabolic sensing devices.

There are also very complex biosensors such as those based on neural networks which are still in a stage of development but show great promise for the future. Several trends were discussed which are technology- as well as science-driven. It will largely depend on the willingness and ability of researchers from different disciplines to communicate and work together on how and, in particular, how fast, this fascinating new field will evolve [66].

A general trend is to combine several transducer principles to get an as complete view of the analyte as possible. This has already been used for tumor cells [9] and will in the future also be implemented for other systems such as the nerve cell networks.

Acknowledgements This review is dedicated to Prof. Wolfgang Göpel who died unexpectedly after a severe car accident during the preparation of this manuscript. The work on neural network-based biosensors in Tübingen was started by him in a collaboration with Guenter Gross (Denton, Tx). Wolfgang Göpel's continuous support and his encouragement are not forgotten. We all lost an outstanding researcher, teacher, and a really good friend.

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