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REVIEW

Biosensors based on immobilized insects fragments

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Abstract The application of insect-sensing organs as parts of advanced biosensor devices with an electronic reading of the signals produced by the biological component is briefly reviewed, and some future applications are discussed.

Introduction

Biosensors always include two major parts: (1) a biological part which is responsible for sensing chemical or physical changes in the environment, and (2) an electronic part, which performs transduction of chemical signals generated by the biological counterpart into electronically readable signals (i.e., current and voltage changes) [1]. Most current biosensors are based on reactions biocatalyzed by enzymes or biorecognition processes in the presence of complementary biomolecules (e.g., antigen–antibody, DNA–complementary DNA, biotin–avidin). It should be noted that even the most advanced biosensors are still based on biomolecular assemblies that are relatively simple compared with natural biological sensing organs. Thus, the approaches used to develop these biosensors are mostly chemical rather than biological. The sensing biomolecular systems are usually immobilized on the interface of a chemical/physical transducer that converts chemical signals generated by the biomolecules in the presence of analytes into physical, electronically readable signals. These signals can be further processed by an electronic amplifier and a computer to yield a final analytical output. Different transduction methods can be used in biosensors depending on the applied biomolecular systems, including commonly used electrochemical tools (amperometry, potentiometry, and impedance spectroscopy) [2, 3], field-effect transistors (FET) [4], quartz-crystal microbalance (QCM) [5], optical methods (absorbance or fluorescence spectroscopy, surface plasmon resonance (SPR), etc.) [6]. Thus, the development of biosensors is based on the state-of-the-art in biomolecular science and electronics, and it requires knowledge of biochemistry and chemistry/physics/engineering to put together the biosensoric part and the electronic/optoelectronic transducer.

On the other hand, there are methods to electronically read signals from whole organisms (including human bodies). Signals can be obtained from the brain (electroencephalograms) [7], heart (electrocardiogram) [8], skin [9], etc. These signals report about the entire organism's condition and its interaction with the environment. To some extent, the systems reading the electronic/optoelectronic signals from whole organisms might be considered super-advanced biosensors. However, the complexity of the processes does not always allow simple and quantitative interpretation of the signals. The high level of complexity requires knowledge of biology, physiology, medicine, and psychology (instead of simple chemistry) to interpret the signals. Despite the fact that some work has been done to use whole simple organisms as biosensors (e.g., for sensing of pheromones or volatiles [10]), the idea of the electronic transduction of natural senses to electronic signals is still far from being practically achievable. For example, dogs sniffing for explosives can not be directly linked to electronic transducers to read out the sensing process electronically. Human sensing is even more complicated and strongly affected by brain operation.

Dedicated to Professor Dr. Yakov I. Tur'yan on the occasion of his 85th birthday.

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The compromise between the “simplicity” of chemical systems and “smartness” of biological organisms could be achieved by application of individual-sensing organs cut from primitive organisms and connected to electronic transducers. In this review, we focus on one of the most challenging and intriguing type of biosensors–detectors that implement fragments of living species, specifically, parts of insects. This type of biosensor possesses a number of useful properties and features: (1) Insects fragments, due to their size, are easy (in comparison to individual cells or proteins) to assemble on electronic devices (e.g., an antenna of a beetle can be glued to a silicon wafer with the help of regular micro tweezers). (2) Specific fragments by their nature have sensory selectivity to specific analytes (e.g., a Colorado beetle’s olfactory receptor senses volatiles from potato tubers [11]). (3) Different fragments of primitive organisms are developed by evolution to solve narrow and specific problems such as detection of volatiles and odors (e.g., beetles with olfactory receptors detect different plants volatiles that are of vital importance for them [11, 12]), recognition of geometrical objects (e.g., an eye of a dragon fly can sense horizon, speed, and size of the surrounding objects [13]), and sensing physical parameters (e.g., bees detect temperature inside a hive, spiders can discriminate vibration generated by a trapped object from vibration generated by the wind).

The general scheme of a biodetector, based on the natural-sensing organs taken from insects, consists of an analyte, receptor, signal amplifier or transducer, and a data recorder (Fig. 1). A sensing device based on this scheme works in the following way: Molecules that are of interest to an insect are detected with olfactory receptors, attached to a transducer, and generate an electrical impulse. Then, the signal propagates via neural cells to the transducer where it is amplified and modified for further processing on a computer. It should be noted that the primary transduction of the chemical signals generated by the natural-sensing receptors into the electrical signals proceeds in the entire organ, and the electronic devices are needed only for the amplification of the biologically generated electrical signals.

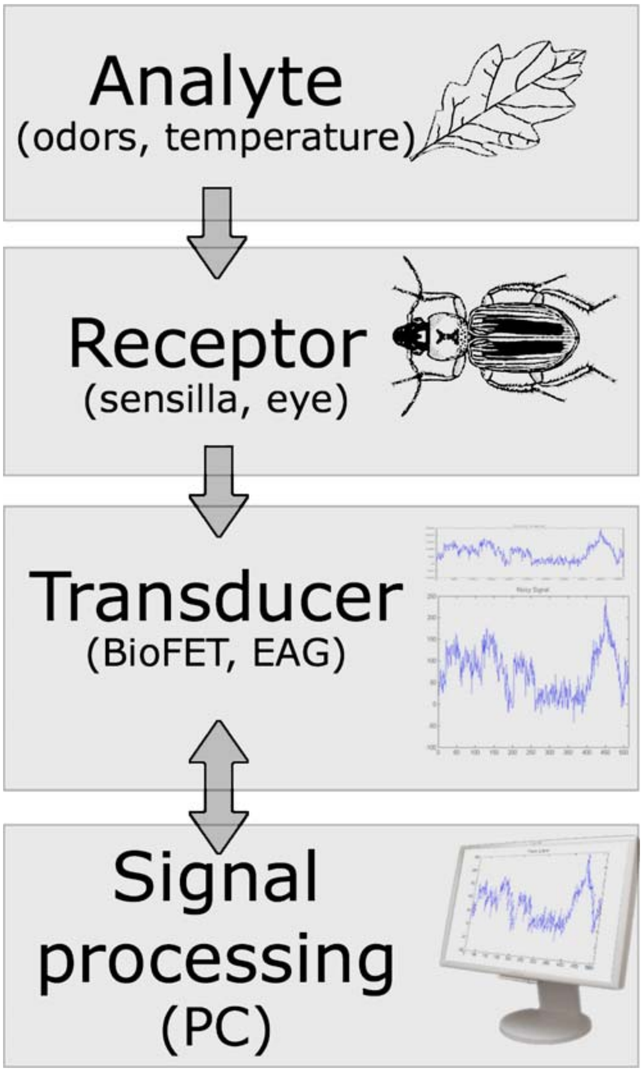

Fig. 1 General setup of an insect–based biosensor consisting of analyte, receptor, amplifier, and signal processing components

Intact chemoreceptors

The idea of biosensors based on natural biological receptors directly connected to electronic devices was originally implemented in the mid-1980s, in the experiments of Rechnitz [14] by fabrication of biosensors based on crab antennules. The bioelectronic system demonstrated the ability to detect an analyte; however, it had a short lifetime (about 48 h) and low detection range [15]. Nevertheless, this prototype initiated research devoted to biosensor detectors based on fragments of living species due to their potential capabilities (such as high sensitivity, naturally tuned for specific tasks, and relatively simple assembly). In the last decade, several major types of the biosensors based on insect fragments were developed using the following methods: (1) olfactory receptor neurons (ORN) method [16], (2) electroantennogram (EAG) method [12] as part of the ORN method, and (3) bio-field-effect transistor (BioFET) method [17]. The major contributions to this research field originate from the publications of M.J. Schöning, S. Schütz, and K.C. Park et al.

Principle of signal generation in olfactory receptor neurons

An insect antenna is a primitive organ that is covered with sensitive receptors called sensilla [18]. These receptors respond to physical, mechanical, and chemical signals, generating electrical signals in the antenna and transferring them to an insect nerve-knot for further reflex [17]. When an important odorant molecule reaches the sensilla, it penetrates and becomes trapped by odor binding proteins (OBP). As soon as a certain number of odor molecules become bound, OBP diffuses to the nerve cell membrane, depolarizes part of it, and this initiates electric impulse. The amplitude and width of the signal is correlated with the analyte concentration in the surrounding air. Thus, amplitude of a potential change generated by several sensilla may reach a range of millivolts [19], and rise/decay time for the response reaches 50–300 ms [10, 16, 17].

Sensors based on the olfactory receptor neurons method

Each olfactory sensillum contains receptor neurons [18], which adsorb chemicals from the surrounding air and generate an electrical signal. The main feature of the ORN method is data collection from a single sensillum. Huotari [16] and Huotari and Mela [20] experimentally demonstrated such a possibility by being able to recognize and record signals from a single sensillum of a blowfly (*Callifora vicina*). Reference and working microelectrodes were inserted into the blowfly antenna from different sides of the sensillum and connected to an electronic amplifier (Fig. 2a). A sample air tube and an amplifier were then connected to the data collecting setup, which was connected to a computer for further data visualization and recording. It was shown that such ORN system based on the blowfly antenna implementation could be extremely sensitive for specific chemicals and totally mute for others, thus demonstrating impressive selectivity [16]. For example, this particular system was able to selectively sense 1,4-diaminobutane and 1-hexanol in the large concentration ranges: 1 ppb–100 ppm and 8 ppm–500 ppm, respectively [16]. In spite of the fact that this method shows very good response and sensitivity characteristics, it is extremely difficult to setup assemble, tune, and operate. On one hand, care should be taken so as not to damage olfactory cells while inserting microelectrodes into the sample, but on the other hand, electrodes should be close enough to the nerves to sense electric signals of 50–2,000 μV [16]. Spontaneous signal changes and sometimes their complete absence were observed due to possible cell irritation with the microelectrodes [16]. Therefore, the biosensor device required constant calibration, and it was not a reliable detector. Therefore, some efforts were made to improve the system and to bring the biosensor to reliable performance levels. The techniques and methods for the fabrication, immobilization, and data characterization of artificial olfactory biosensors were improved, particularly by the immobilization of the olfactory receptor on a conductive substrate [21]. Still, it was concluded that EAG and BioFET methods had more promise for the development of biosensors based on insect receptors.

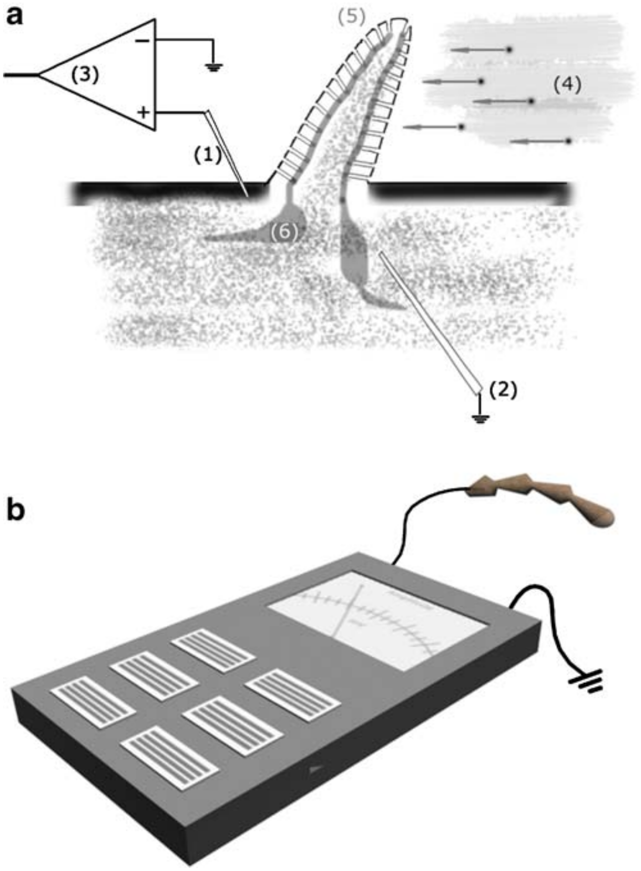

Fig. 2 **a** An olfactory sensilla structure with inserted microelectrodes: *(1)* measurement and *(2)* reference microelectrodes, *(3)* amplifier, *(4)* exposure airflow, *(5)* sensillum, *(6)* nerve cell membrane. **b** Components arrangement for EAG method

Sensors based on the electroantennogram method

The EAG method is based on imbedding an electrode into an insect's antenna (Fig. 2b) so that the potential generated in the antenna can be electronically readable. In other words, it is a multiple ORN method, where a microelectrode is inserted into the base part of the antenna allowing sensing of a superposition of the action potentials from all proximal neurons. The EAG method demonstrated advantages over the parent ORN method, showing a faster recovery time (less than a second) and a high electrical sensitivity (fractions of mV). At the same time, the EAG method is easier to assemble, and as a result, it has been successfully developed to identify volatiles [12] and embedded into a number of devices [22, 23]. After years of research, there was a great improvement in operating parameters of EAG-based devices (e.g., the operational lifetime of the devices was increased up to several days [15]), the sensitivity to odorants was enhanced into the order of ppb [12], and the signal-to-noise ratio was significantly improved [24]. However, still there are some problems in detection, caused by the receptor degradation due to natural aging processes in the natural organ (especially during the first hour after the antenna is cut from an insect [12]). This results in the need for frequent re-calibration of the biosensor. Despite of the fact that EAG sensors allow for multi-component odor analysis, it is still difficult to discriminate individual compounds from a complex mixture using a single insect antenna. To solve this problem, an array of receptors from five different species was assembled for EAG responses to 20 different compounds. Figure 3 shows the EAG response profiles of five sensing species to 20 different odorants [25]. As one can see, there are some similarities in the profiles between some species and differences between others. For example, the responses of *Heliothis virescens* almost replicate each other and, at the same time, differ from *Ostrinia nubilalis* significantly. This data become more presentable after construction of an odor map and rearrangement of each compound across all five species. For example, Fig. 4 shows the responses of the five different species to specific odorant components [25]. Such five-component arrays may easily recognize 20 or more different odors. Remarkably, the realization of neural network concepts [26] allows for such systems to be interactive and "smart." Indeed, a memory device (e.g., a flash drive) can store all patterns obtained during testing mode with the ability in future either to distinguish unknown profiles or recognize those that are in a database.

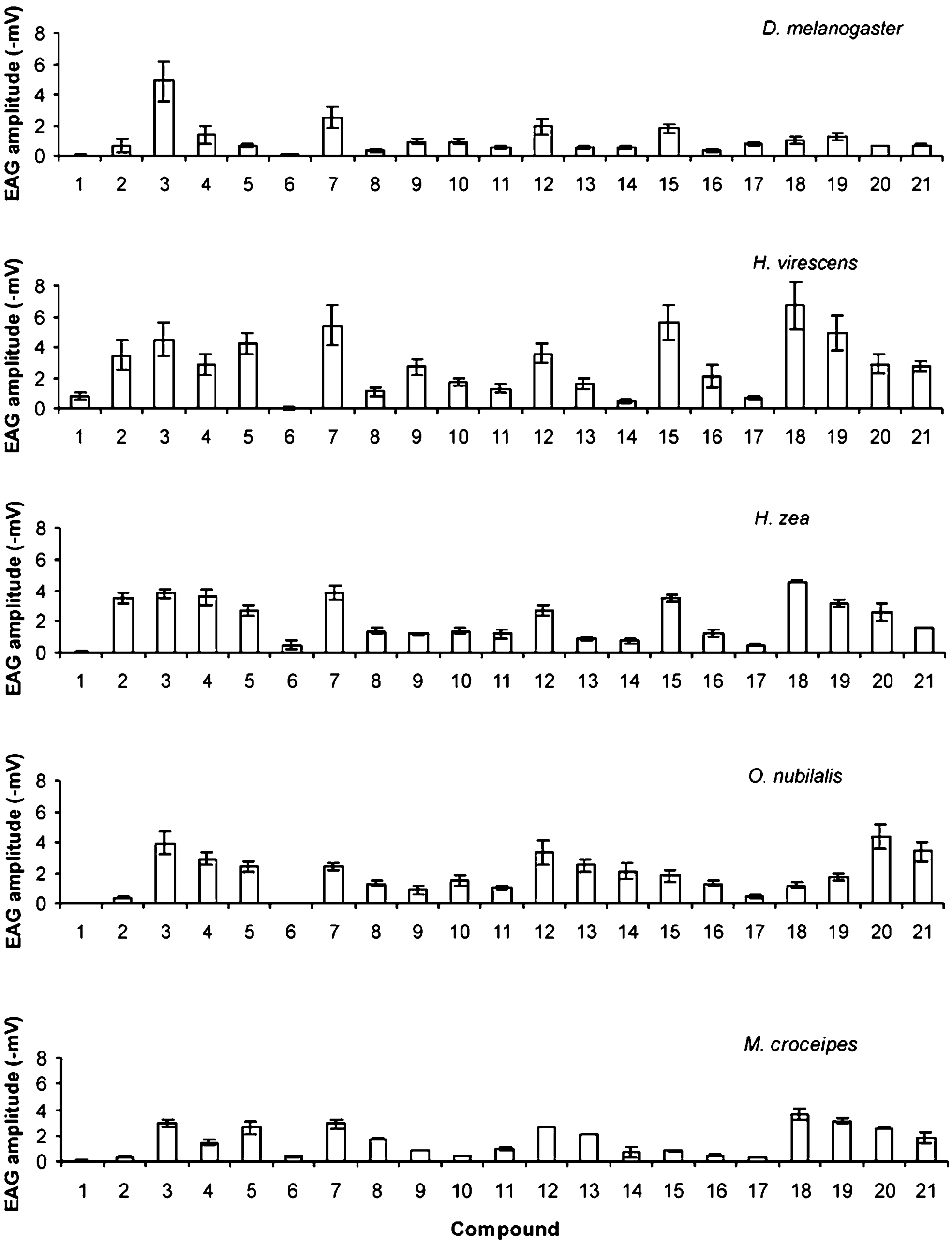

Fig. 3 EAG response profiles of five different insect species (male *Drosophila melanogaster*, male *Heliothis virescens*, male *Helicoverpa zea*, male *Ostrinia nubilalis*, female *Microplitis croceipes*) to 20 different volatile compounds: *(1)* control blank, *(2) cis*–11–hexadecenal (Z11–16:Ald), *(3) cis*–3–hexenol (Z3–6:OH), *(4)* hexanioc acid, *(5)* benzyl acetate, *(6)* 2–metyl–5–nitroamine, *(7)* cyclohexanone, *(8)* α–pinene, *(9) cis*–nerolidal, *(10)* trans–nerolidal, *(11)* β–caryophyllene, *(12)* β–ocimene, *(13)* (*R*)-(+)–limonene, *(14)* methyl jasmonate, *(15)* 2–diisopropylaminoethanol, *(16)* indole, *(17)* 2,2–thiodiethanol, *(18)* 1–heptanol, *(19)* 1–octanol, *(20)* 1–nonanol, *(21)* 1–decanol (adopted from [25] with permission)

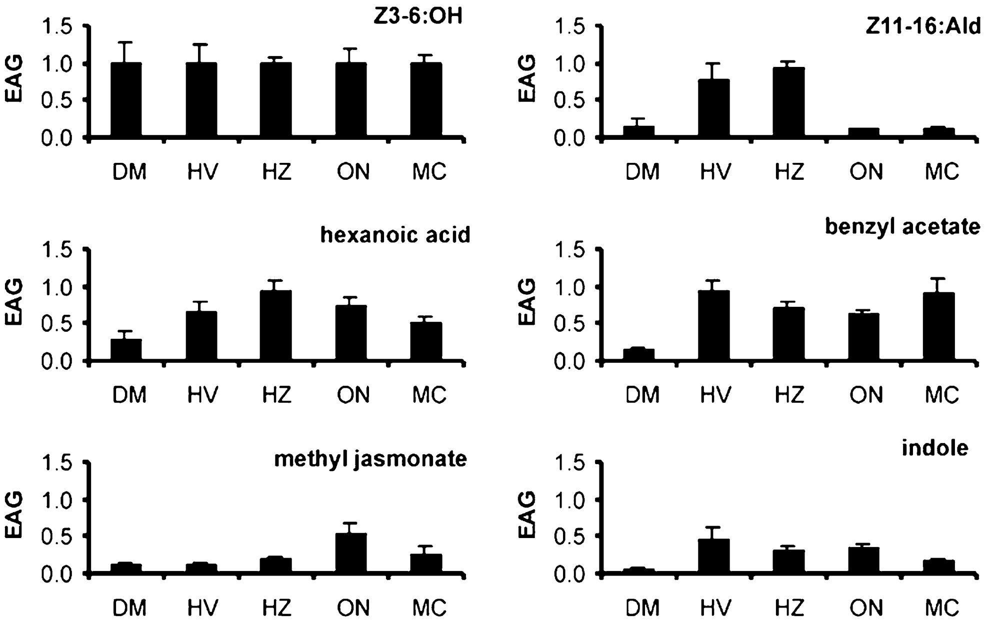

Fig. 4 EAG response spectra across five different insect species (*DM* male *Drosophila melanogaster*; *HV* male *Heliothis virescens*; *HZ* male *Helicoverpa zea*; *ON* male *Ostrinia nubilalis*; *MC* female *Microplitis croceipes*) to six different categories of volatile compounds: *cis*–3–hexenol (Z3–6:OH), green leaf, volatile alcohol; *cis*–11–hexadecenal (Z11–16:Ald), sex pheromone compound of *H. zea* and *H. virescens*; hexanoic acid, aliphatic carboxylic acid; benzyl acetate, aromatic compound; methyl jasmonate, commonly occurring plant volatile; indole, nitrogen–containing animal odor (adopted from [25] with permission)

The present example illustrates the possibility to design more complex sensing systems based on multi-sensing arrays allowing for multi-target analysis. Indeed, there is no physical and engineering limit that can prevent the use of more than five antennae; if spectra-based response from five species gives an ambiguous profile, one can add or exchange one or more antennae from different species and hence resolve ambiguities.

Continuing with the idea of applying multiple insect antennae, a multi-array sensor was successfully used to improve the signal-to-noise ratio in EAG responses [24]. The uniqueness of this particular study is in the assembling of multiple antennae of the *same* insect specie (male *Helicoverpa zea*) into one array either in series or in parallel. Figure 5 shows the EAG responses of different numbers of the male *H. zea* antennae, connected in series or in parallel, to various doses of a major female sex pheromone component (Z)-11-hexadecenal (Z11-16:Ald). To assemble the antennae in series, the base of the first antenna was connected to the reference electrode, and the tip was linked to the base of the second antenna. The tip of the second antenna was connected to the base of the third and so on, until the tip of the last antenna touched the working electrode. For parallel construction, all antennae were connected to the reference electrode at one end and to the working electrode with the other one. Up to four antennae were used in the "series" experiments (Fig. 5a), while only two antennae were used in the "parallel" experiments (Fig. 5b). Both systems have demonstrated significant improvement in signal detection compared to single-antenna devices (resulting in an almost twofold amplification of the active potentials for the series connection). The EAG response and the noise level were both increased when the antennae

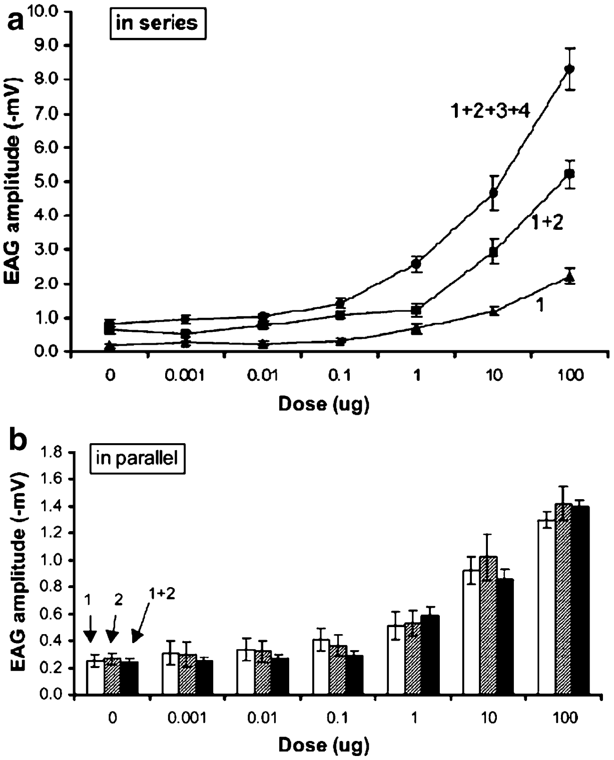

Fig. 5 **a** EAG dose–responses of male *Helicoverpa zea* to various doses of *cis*–11–hexadecenal (Z11–16:Ald) with different number of antennae connected in series. The EAG responses were measured from one antenna (*1*), two antennae connected in series (*1+2*), or four antennae connected in series (*1+2+3+4*). **b** EAG dose responses of male *H. zea* to various doses of Z11–16:Ald with different number of antennae connected in parallel. The EAG responses were measured from each of two different antennae (*1* or *2*), or two antennae connected in parallel (*1+2*) (adopted from [24] with permission.)

were connected in series, but the signal-to-noise ratio was significantly improved due to the relatively low noise enhancement [24]. In contrast, the parallel setup did not show any significant improvement in signal amplitude or noise level; however, the signal-to-noise ratio improved here as well. Thus, we can see that employing of arrays of insect antennae can bring essential improvements into EAG-based biosensor measurements. Use of multiple antennae of different species provides unambiguous results for monitoring and discrimination of chemical compounds; simultaneously, the use of multiple antennae of the same species, connected in series, may dramatically enhance sensitivity of a detector and specificity of a signal. Such combinations of these techniques may bring EAG-based detectors to a new level of biosensing.

BioFET sensors based on insect-sensing organs

Numerous biosensors were developed using FET as transducing devices [27, 28]. For example, enzyme-FETs (biosensors based on immobilization of enzymes on gates of ion-selective-FETs) [29], gen-FETs (DNA-sensors based on DNA hybridization on gates) [30], immuno-FETs (immunosensors assembled on gates) [31], and cell-FETs (biosensors based on cells immobilized on gates) [32] have been developed in the last decade. Wide implementation of FET in biological applications provided ample material for insect-based BioFET investigations [17]. The main idea of BioFET is that the voltage generated in the antenna, caused by odor molecules, influences the current between the source and drain electrodes of the transistor—in other words, FET becomes an amplifier for the insect's receptor. As a result, insect antenna, immobilized on a sensitive gate surface of a FET, showed high sensitivity and selectivity, being able to detect concentrations up to 1 ppb of specific odors [33, 34].

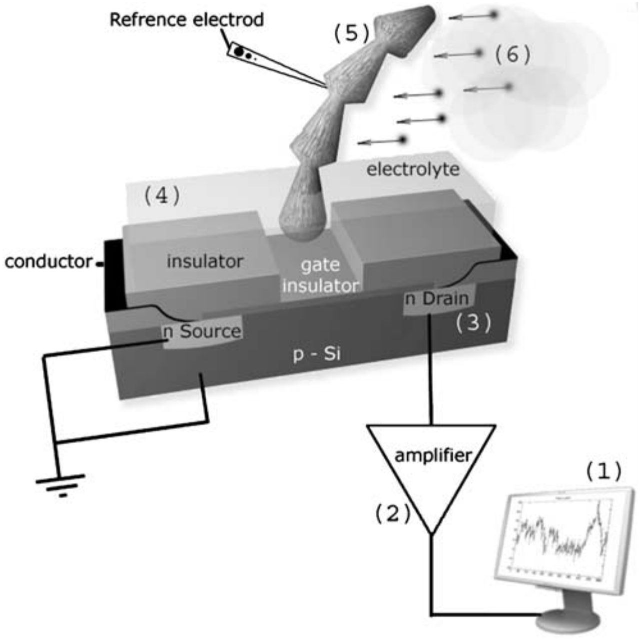

Fig. 6 BioFET setup. Fabricated according to semiconductor technology FET *(3)* connected to a computer *(1)* via electronic signal amplifiers and converters *(2)*. Due to response processes to odors *(6)*, insect antenna *(5)* changes potential near the gate insulator via electrolyte *(4)*, which influences current magnitude between the source and drain electrodes

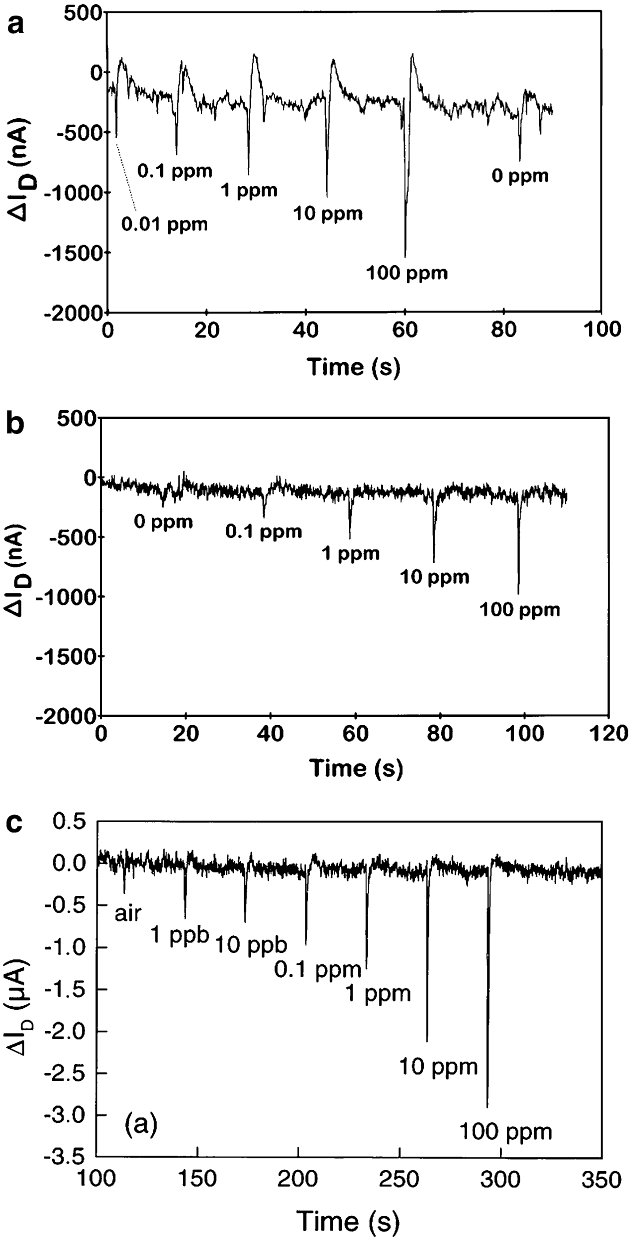

Fig. 7 Typical sensor response: variation of I_{SD} of the whole–beetle BioFET (**a**), isolated–antenna–BioFET (**b**), and isolated–antenna–BioFET with optimized parameters (V_{GR}=2 V, V_{SD}=2 V) (**c**) by changing *cis*–3–hexen–1–ol concentration in air (adopted from [35, 37] with permission)

There are two ways to set up antennae immobilization on FET. One possibility is to place a whole beetle on a FET gate. In such a setup, the antenna is dipped into a hemolymph Ringer's solution [33], which is in contact with a FET gate. In this case, the reference electrode was inserted into a beetle body that provides an electrical contact between the gate and the reference electrode. In another setup, the antenna was cut from the beetle body with microscissors and immersed into an electrolyte solution connecting the gate surface and the reference electrode. Figure 6 shows the standard setup where the insect's receptor is immobilized on the gate surface being dipped into an electrolyte solution. Potential changes, generated in the antenna in response to odorants, propagate to the gate surface, thus controlling the current value between the source and drain electrodes in the FET. A pioneering design for coupling of an insect antenna and FET was developed by Schöning et al. [33] using a potato beetle and a FET to sense different concentrations of *cis*-3-hexen-1-ol in air. Single insect-antenna-BioFET and whole-beetle-BioFET were reported on by Schöning et al. [35] and tested for the analysis of volatiles released by diseased potato tubers [36]. BioFETs typical responses, depicted in Fig. 7a and b, clearly correlate to the concentration of the gas in air (between 0.01 and 100 ppm). Optimization of the voltages applied between the gate and the reference electrode (V_{GR}) and between the source and drain electrodes (V_{SD}), improved the antenna-BioFET arrangement, and a special BioFET signal-interface development resulted in further increase in sensitivity for *cis*-3-hexen-1-ol up to 1 ppb (Fig. 7c) [11, 37]. Figure 7c also shows that the signal-to-noise ratio was significantly improved. The BioFETs based on the Colorado potato beetle and the steelblue jewel beetle were tested on sensing *cis*-3-hexen-1-ol (marker for diseased potato), gualacol (marker of coniferous wood fire), and 1-octen (marker for coal fire) volatiles, and the resulting biosensors were suggested for the detection of plant damage and fire detection [38]. The operational lifetime of these biosensors varied between 2 to 6 h [37].

Future applications of insect-based biosensors

The application of insect-based biosensors opens a wide range of opportunities. For example, there are no limitations that can stop one from selecting different living species or fragments of living spices and using them for detecting various compounds (not necessarily odors) and physical inputs (e.g., temperature change, light, sound, etc.). However, more challenging and delicate objectives can be achieved with the implementation of these biosensors in areas that are not usually considered to be directly related to biosensors. One of the unique properties of biosensors based on natural sensors is the principle of signal generation: Specifically, bioreceptors generate active potential in response to parameter deviation (either temperature, or pressure, or concentration of chemicals). The insect antennae respond rapidly to peak concentrations of sensed chemicals [25] in contrast to artificial chemical sensors that usually react slowly and read only time-averaged mean concentrations. Thus, insect-based biosensor devices may be used as direct information transducers (the basic principle of such transducer operation is the ability to react on the slope of a signal change). Information may be encrypted by means of mechanical, physical, or chemical parameter selection and transmitted to a recipient, which is then able to convert it back into meaningful data. For example, some sensitive information or security stamp can be printed with transparent but odorant "ink." A recipient, owning a properly tuned biodetector, can scan the source and read sensitive data. More challenging devices may combine several biosensors that differ by type and properties for solving complex, even logical, operations. Ideally, it may be a prototype for a biorobot: variations of environmental media may be sensed by olfactory [10, 16, 37], optical [39], and mechanical [40] biosensors based on natural sensor organs. The power for biorobot operation can be generated by biofuel cells using the existing "gastrobot" as a prototype [41].

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