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Short communication

A novel biomimetic olfactory-based biosensor for single olfactory sensory neuron monitoring

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ABSTRACT

This paper presents a novel biomimetic olfactory biosensor for the study of olfactory transduction mechanisms on the basis of light addressable potentiometric sensor (LAPS), in which rat olfactory sensory neurons (OSNs) are used as sensing elements. Rat OSNs are cultured on the surface of LAPS chip. To validate the origin of the electrical signals recorded by LAPS, the inhibitory effect of MDL12330A to the olfactory signals of OSNs is tested, which is the specific inhibitor of adenylyl cyclase. The enhancive effect of LY294002 to the responses of OSNs is also investigated, which is the specific inhibitor of phosphatidylinositol 3-kinase (PI3K). The results show that this hybrid biosensor can record the responses of OSNs to odours efficiently in a non-invasive way for a long term, and the responses can be inhibited by MDL12330A and enhanced by LY294002. All these results demonstrate that this hybrid biosensor can be used to monitor electrophysiology of OSNs in a non-invasive way and suggest it could be a promising tool for the study of olfactory transduction mechanisms.

1. Introduction

The mammalian olfactory system has an exquisite capacity to recognize and discriminate thousands of distinct odours that are present in the environment. Nowadays, much effort has been invested to the research of olfactory transduction mechanisms and some obvious achievements have provided deep insights into the mechanisms underlying the sense of olfaction. However, it is still far from the complete understanding of olfaction due to the complications of olfactory mechanisms and the lack of powerful tools for the study of olfaction.

Patch clamp, as the golden standard of cellular electrophysiology, contributes greatly to the studies of olfactory mechanisms. However, its intrinsic limitations such as cost, invasion and sophistication hamper its applications in cellular physiology. The emergence of biosensors may provide novel solutions to these problems. By combining cells with various secondary sensors, such as field effect transistor (FET) and microelectrode array (MEA) (Denyer et al., 1998; Yeung et al., 2001), many potential detection biosensors have been fabricated in order to provide novel methods for cellular electrophysiology. These biosensors offer non-invasive, easy operating, low cost and long-term methods to measure the extracellular potential of excitable cells, but their potential measurements are restricted to discrete active sites, such as the gate-electrode of individual FET and the tip of individual microelectrode. Light addressable potentiometric sensor (LAPS) is a silicon-based surface potential detector that overcomes the above limitation (Hafeman et al., 1988). It has been reported that LAPS as a cell-semiconductor hybrid system can addressably monitor the changes of extracellular potentials (Ismail et al., 2003; Stein et al., 2004; Xu et al., 2005; Liu et al., 2006).

The purpose of this study is to develop a novel biomimetic olfactory-based biosensor for the research of olfactory transduction mechanisms. The structure of LAPS–olfactory sensory neurons (OSNs) hybrid biosensor is constructed on the ground of LAPS by using rat OSNs as sensing elements. For validating the origin of the recorded electrical signals, the inhibitory effect of MDL12330A to the olfactory signals of OSNs is tested, which is the specific inhibitor of adenylyl cyclase (Guellaen et al., 1977). To further testify the capability of this hybrid biosensor for the research of olfactory transduction mechanisms, the enhancive effect of LY294002 to the olfactory signals of OSNs is also tested, which is the specific inhibitor of phosphatidylinositol 3-kinase (PI3K) (Vlahos et al., 1994). If this hybrid biosensor can record the responses of OSNs successfully, it could be a valuable tool for the study of olfactory transduction mechanisms, especially for the electrophysiological or pharmaceutical characterization of OSNs.

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2. Theories

OSNs can respond to odours and convert the chemical signals of odour molecules into electrical signals (Buck and Axel, 1991). Fig. 1a is the schematic diagram of olfactory transduction pathway of mammalian OSNs. Olfactory sensation is initiated by the specific binding of odour molecules to olfactory receptors (R), which can activate specific G proteins (G) (Ache and Young, 2005). Then G protein activates adenylyl cyclase (AC), leading to an increase in intracellular cyclic adenosine monophosphate (cAMP) levels. The increased cAMP activates an olfactory-specific cyclic-nucleotide gated ion channel (CNG), which is a non-selective cation channel that increases intracellular calcium and secondarily activates a calcium-activated chloride channel (Cl^- channel). This cascade of enzymatic reactions ultimately leads to the generation of action potentials. Hence, MDL12330A, which is the specific inhibitor of adenylyl cyclase (Guellaen et al., 1977), can be used to inhibit the responses of OSNs to odours. It has also been demonstrated that LY294002, which is the specific inhibitor of PI3K, can enhance the responses of rat OSNs to a complex odours (Spehr et al., 2002).

LAPS can detect the changes of extracellular potential of OSNs cultured on the surface of the silicon chip. Fig. 1b shows the basic detection mechanism of the LAPS–OSNs hybrid biosensor. LAPS has the structure of electrolyte–insulator [SiO_2]–semiconductor [Si] (EIS) (Xu et al., 2005). When the light with a certain wavelength illuminates the LAPS chip, the semiconductor absorbs energy and leads to the generation of electron–hole pairs. Due to the rapid recombination of electron and hole, the photocurrent is unable to be detected by peripheral circuit. If the LAPS is biased in depletion, the width of the depletion layer is a function of the local value of the surface potential. The local value of the bias voltage can be read out with ac photocurrent. When the extracellular potential of OSNs cultured on LAPS is changed, the photocurrent can generate corresponding fluctuation. Therefore, the changes in the extracellular potential can be monitored by the measurement of photocurrent fluctuation.

3. Experiments

3.1. Fabrication of LAPS chip and detection chamber

The upper side of n-type silicon wafer (⟨100⟩, 10–15 Ω cm) is thermal oxidized with a layer of SiO_2, thickness about 30 nm. The back of silicon wafer is grind to 100-μm thick. Then Al is evaporated on the back side of silicon wafer to create an ohmic contact and is used as working electrode. Micro-chambers for cell culture and detection are made by polydimethylsiloxane. LAPS chip is fixed on the bottom of the chamber. Pt wire immersed into the solution in detection chamber is used as reference electrode.

3.2. OSNs isolation and culture

OSNs are prepared from the olfactory epithelium of 2–3-day-old Sprague–Dawley (S–D) rats. The rat is decapitated and the nose is dissected. The entire intact olfactory epithelium is gently peeled away from the underlying bone. Then the olfactory epithelium is incubated in the enzyme solution of 0.25% trypsin (pH 7.4–7.8) at 37 °C for 20 min and transferred to mammalian Ringer's solution (138 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, pH 7.4) for gentle blowing with pipette. Dissociated cells are subsequently filtered with sieve of 200 holes per inch into the suspension of fresh Dulbecco's modified Eager's medium (DMEM) with 10% fetal bovine serum (FBS). Then the suspension is added into the chamber of LAPS and cells are allowed to settle and attach, at 37 °C under standard conditions of humidified air with 5% CO_2. To improve the biocompatibility of the silicon device, poly-L-ornithine and laminin mixture (100 μg/ml poly-L-ornithine and 8 μg/ml laminin mixed by the rate of 1:1) is coated on the surface of LAPS chip prior to seeding cells (Ismail et al., 2003). On the third day, OSNs are mature and the measurement experiments begin to perform.

3.3. LAPS system and the detection of extracellular potentials

Fig. 2a shows the schematic drawing of the LAPS testing set-up, which is similar to the system we have reported (Xu et al., 2005; Liu et al., 2006). During experiments, LAPS chip with cultured OSNs is mounted under a microscope object lens (numerical aperture: 0.4) in the set-up. The light source is a He–Ne semiconductor laser (Coherent Co.), with 543.5 nm wavelength and 5 mW power. Chopper (C-995, Terahertz Technologies Inc.) modulates the beam of laser and generates modulated pulse light with 4 kHz frequency. Then the modulated light is focused to less than 10 μm by a lens and highlights on the desired neuron. A bias voltage of 2.0 V is applied to the LAPS chip. When neuron produces action potential under stimulations, LAPS can detect the changes and give corresponding photocurrent in a few microseconds, which is transmitted into peripheral equipments through working electrode. The electrodes of potentiostat (EG&G Princeton Applied Research, M273A) are used to detect the current. We use a 16-bit data collection card and the software of LABVIEW to control the collection, analysis and storage of data. Culture medium or stimulator is pumped alternatively by a peristaltic pump and passes through a degasser, a selective valve, and flows into the detection chamber. PC controls the on–off of pump and the temperature of chip. All measurements are performed at 37 ± 0.2 °C.

4. Results and discussion

4.1. Results of cell culture

Three types of cells dominate the mammalian olfactory epithelium: the OSNs, the supporting cells and the basal cells (Buck and Axel, 1991). OSNs have the features of neuron, which are slender and bipolar. The apical pole of OSNs gives rise to dendrites that extend toward the mucosal surface. The end of dendrites forms a number of specialized cilia, where is the location of the olfactory receptors. The basal pole of OSNs projects an axon to the olfactory bulb to establish contact with the secondary neuron, such as mitral cells and tufted cells, which are the output neurons of the olfactory bulb and can relay olfactory signals to the olfactory cortex (Mori et al., 1999).

When the mammalian olfactory epithelium is enzymatically dissociated and cultured on the surface of LAPS chip, due to the neuronal features of OSNs, we can distinguish OSNs from other types of the olfactory epithelium. Fig. 2b shows the results of cells cultured on LAPS chip for 3 days, OSNs are still maintaining their bipolar shape and obviously different from other round shaped cells.

4.2. Inhibitory effect of MAL12330A to the olfactory signals of OSNs

OSNs can respond to the stimulations of odours and convert the chemical signals of odour molecules into electrical signals. In mammalian, each OSN expresses only one type of olfactory receptor and can respond to a few distinct odour molecules (Ache and Young, 2005). To validate the response capacity of OSNs cultured on the LAPS chip to odours, we first use odour mixture to stimulate OSNs. The odour mixture is prepared by mixing five compounds

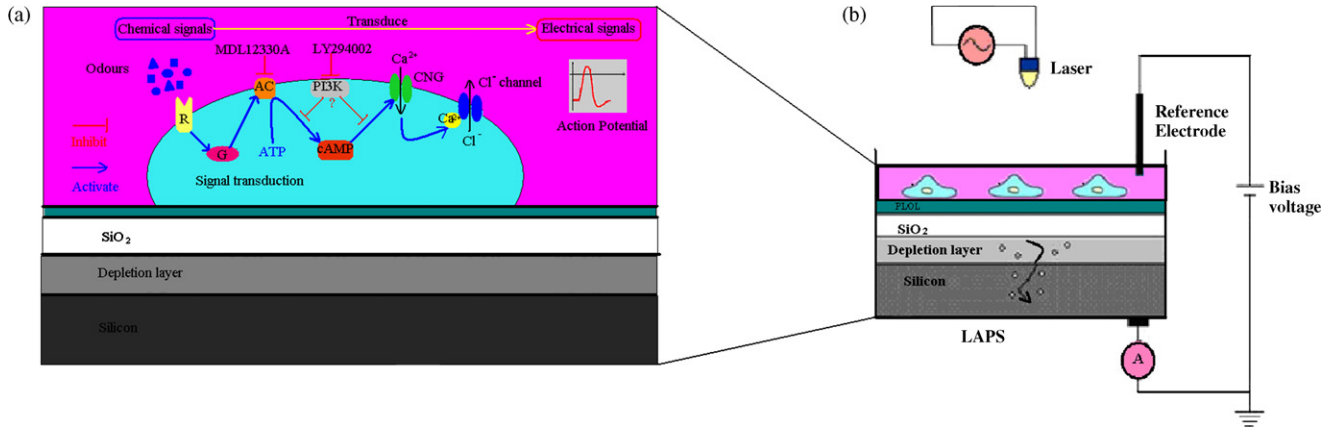

Fig. 1. (a) Olfactory cAMP-signaling pathway in mammalian OSNs. Note some additional regulatory pathways are omitted from this diagram for simplicity. (b) Detection principle of the LAPS–OSNs hybrid biosensor.

(acetic acid, octanal, cineole, hexanal and 2-heptatone) with equal volume at the same concentration in Ringer's solution, and the ultimate concentration of each compound is 0.1 mM. The responses of OSNs to the mixture are recorded by LAPS measurement system. Fig. 3a shows the typical responses of OSNs to the mixture. After treatment with the mixture for 10 s, the number of recorded firing spikes increases significantly.

In order to confirm that the recorded firing spikes are originated from the olfactory signals of OSNs, MDL12330A, which is the specific inhibitor of adenylyl cyclase, is used to inhibit the olfactory transduction pathway in OSNs and subsequently inhibits the responses of OSNs to the stimulations. On the same OSN mentioned above, 10 s prior to and during the application of the mixture, the OSN is incubated in 50 μM MDL12330A. We can see from Fig. 3a that the number of recorded firing spikes decreases dramatically, which implies the responses of OSN to the mixture are inhibited by MDL12330A. The results proved that the recorded electrical signals by LAPS measurement system are originated from the olfactory signals generated by OSNs cultured on the LAPS chip. We have tested the responses of other 83 OSNs to the same mixture and the same protocol, 26 OSNs' responses can be recorded and the inhibitory effect of MDL12330A can be observed in all responsive OSNs to some extent. It may be due to the mixture we have used to stimulate OSNs is not enough to cover the possible chemical space of OSNs. Hence, not all the OSNs we have tested can respond to the mixture. Fig. 3c shows the statistical results of the number of the recorded firing spikes. The number of firing spikes in response to the mixture is 55.2 ± 4.95 spikes/5 s (mean ± S.E.M.), which is significantly higher than that of control condition (10.3 ± 1.20 spikes/5 s) without any stimulation and inhibitor. After the treatment of inhibitor, the number of firing spikes in response to the mixture is 20.5 ± 4.80 spikes/5 s, which is significantly lower than that of, stimulated by the mixture only (55.2 ± 4.95 spikes/5 s).

The above results demonstrated that this hybrid biosensor can record the olfactory signals of OSNs efficiently and can be used to characterize the electrophysiological features of OSNs.

4.3. Enhancive effect of LY294002 to the responses of OSNs

It is believed that LY294002 can enhance the responses of OSNs to a complex odours by inhibit the activity of PI3K (Spehr et al., 2002). To further testify the feasible applications of this LAPS–OSNs hybrid biosensor in the study of olfactory transduction, the enhancive effect of LY294002 to the olfactory signals of OSNs is tested. Similar to the protocol above, the mixture is employed to stimulate the OSNs cultured on the LAPS chip and the responses of OSNs to the mixture are recorded by the LAPS measurement system. Then 10 s prior to and during the application of the mixture, OSNs are incubated in 10 μM LY294002 to enhance their responses to the mixture. 17/62 OSNs show responses to the mixture and the enhancive effect of LY294002 can be observed in all responsive OSNs. Fig. 3b is the typical responses of OSNs to the mixture and LY294002. The responses of OSNs to the mixture are obviously enhanced by the treatment of LY294002. Fig. 3d shows the statistical results of the number of the recorded firing spikes. The number of firing spikes in response to the mixture is

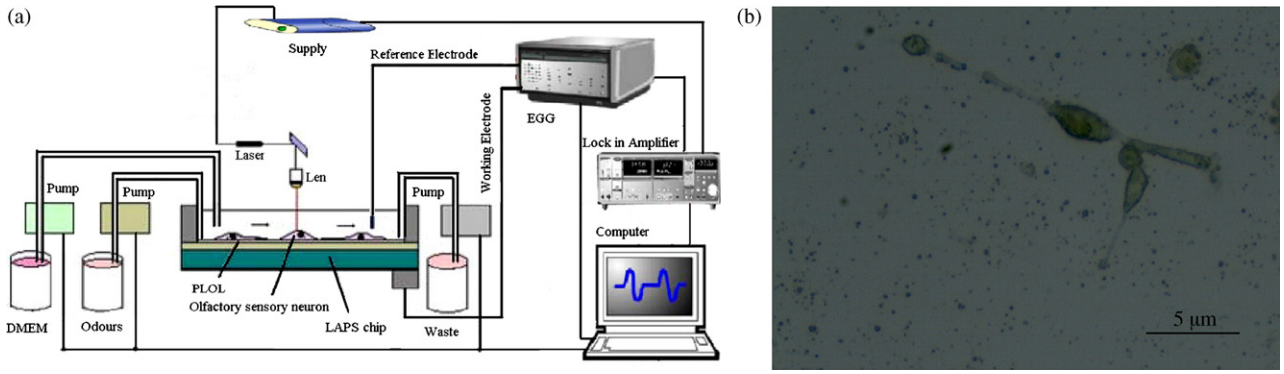

Fig. 2. (a) Schematic diagram of the LAPS measurement set-up. (b) Results of rat OSNs cultured on LAPS chip for 3 days.

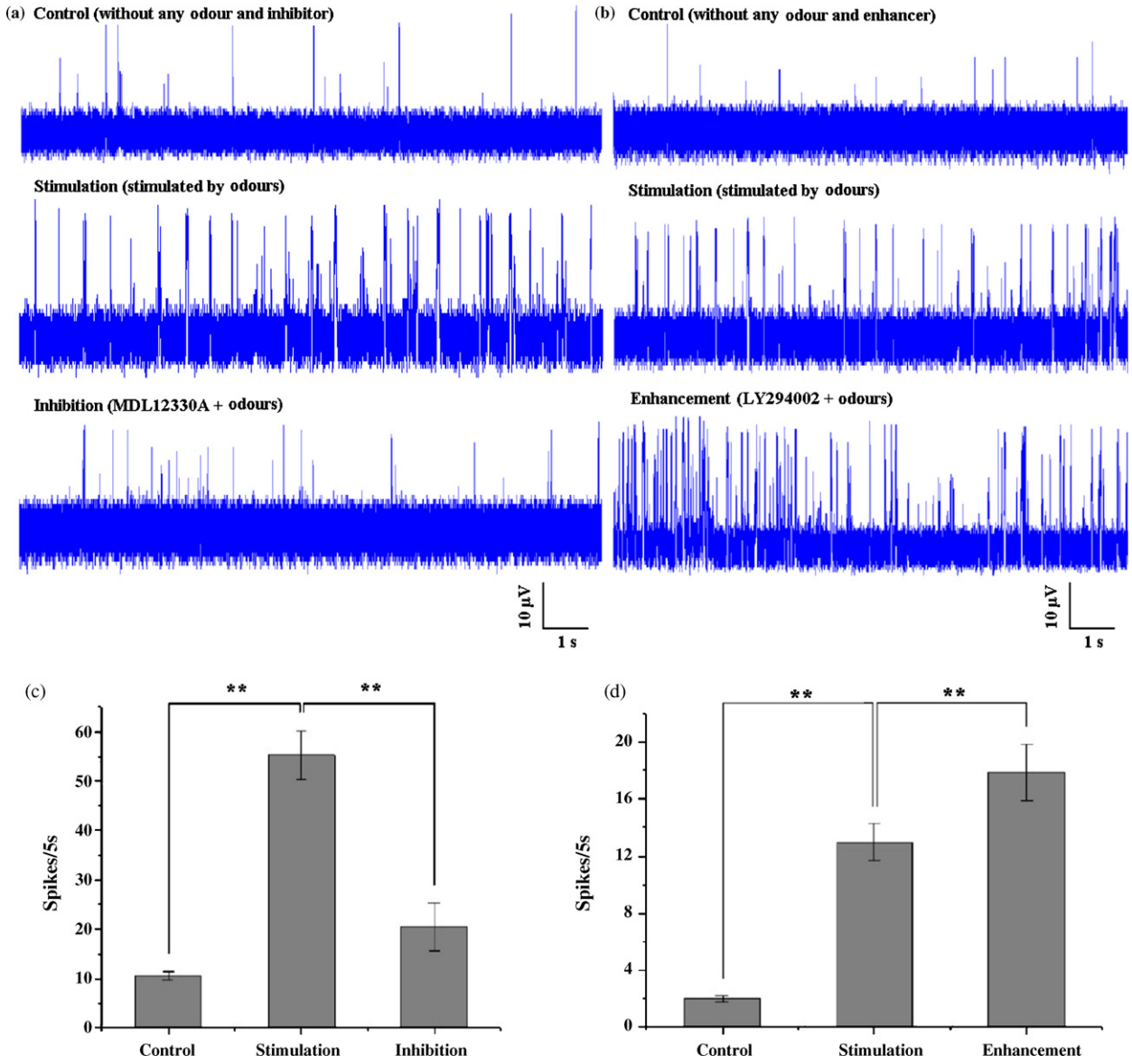

Fig. 3. Typical extracellular recordings from OSNs. The responses of OSNs to the odours and inhibited by MDL12330A (a) or enhanced by LY294002 (b). The statistical results of the number of the recorded firing spikes from the responses of OSNs to the odours and inhibited by MDL12330A (c) or enhanced by LY294002 (d). (c) The number of firing spikes in response to odours (55.2 ± 4.95 spikes/5 s) is significantly higher than that of control (10.3 ± 1.20 spikes/5 s, $p = 8.66 \times 10^{-9}$, $n = 26$). The number of firing spikes after treated by MDL12330A (20.5 ± 4.80 spikes/5 s) is significantly lower than that of odours (55.2 ± 4.95 spikes/5 s, $p = 3.41 \times 10^{-4}$, $n = 26$). (d) The number of firing spikes in response to odours (13.3 ± 1.30 spikes/s) is significantly higher than that of control (2.10 ± 0.65 spikes/s, $p = 4.78 \times 10^{-5}$, $n = 17$). The number of firing spikes after treated by LY294002 (17.9 ± 2.00 spikes/s) is significantly higher than that of odours (13.3 ± 1.30 spikes/s, $p = 3.40 \times 10^{-3}$, $n = 17$). All data are represented by the mean ± standard error of the mean (S.E.M.). $^{**}p < 0.01$, Student's *t*-test.

13.3 ± 1.30 spikes/s, which is significantly higher than that of control (2.10 ± 0.65 spikes/s) without any stimulation and enhancer. After the treatment of enhancer, the number of firing spikes in response to the mixture is 17.9 ± 2.00 spikes/s, which is significantly higher than that of, stimulated by the mixture only (13.3 ± 1.30 spikes/s). These results are consistent with the previous results obtained by calcium-imaging and may provide novel evidences to the olfactory transduction mechanism in OSNs (Spehr et al., 2002).

The signal-to-noise ratio of this hybrid biosensor is about 3. As compared to the FET system for cellular monitoring, which can achieve signal-to-noise ratios of up to 25 (Yeung et al., 2001), this hybrid biosensor still needs to improve its signal-to-noise ratio. The possible solution to this problem is to enhance the coupling of OSNs to the LAPS surface by improved surface modification.

5. Conclusion

In this paper, a novel LAPS–OSNs hybrid biosensor is developed and its applications in the research of olfactory transduction mechanisms are also investigated. The experimental results show that this hybrid biosensor can be used to monitor the extracellular potential of OSNs cultured on the LAPS Chip. It is a valuable tool for the study of olfactory transduction mechanisms, which has the advantages of non-invasion, cheapness and convenience. It may meet the specific study needs that are not met by existing technolo-

gies, such as the electrophysiological characterization of OSNs and the research on the neural networks.

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