Biosensors and Bioelectronics 25 (2010) 2212–2217

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Extracellular potentials recording in intact olfactory epithelium by microelectrode array for a bioelectronic nose

Qingjun Liu[a,b], Weiwei Ye[a], Lidan Xiao[a], Liping Du[a], Ning Hu[a], Ping Wang[a,b,*]

[a] Biosensor National Special Laboratory, Key Laboratory of Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, PR China
[b] State Key Laboratory of Transducer Technology, Chinese Academy of Sciences, Shanghai 200050, PR China

ARTICLE INFO

Article history:
Received 4 January 2010
Received in revised form 11 February 2010
Accepted 23 February 2010
Available online 1 March 2010

Keywords:
Bioelectronic nose
Cell and tissue based biosensor
Olfactory epithelium
Microelectrode array (MEA)
Olfactory receptor neurons

ABSTRACT

Human beings and animals have sensitive olfactory systems that can sense and identify a variety of odors. The purpose of this study is to combine biological cells with micro-chips to establish a novel bioelectronic nose system for odor detection by electrophysiological sensing measurements of olfactory tissue. In our experiments, 36-channel microelectrode arrays (MEAs) with the diameter of 30 μm were fabricated on the glass substrate, and olfactory epithelium was stripped from rats and fixed on the surface of MEA. Electrophysiological activities of olfactory receptor neurons in intact epithelium were measured through the multi-channel recording system. The extracellular potentials of cell networks could be effectively analyzed by correlation analysis between different channels. After being stimulated by odorants, such as acetic acid and butanedione, the olfactory cells generate different firing modes. These firing characteristics can be derived by time-domain and frequency-domain analysis, and they were different from spontaneous potentials. The investigation of olfactory epithelium can provide more information of olfactory system for artificial olfaction biomimetic design.

1. Introduction

Biological olfactory system has high sensitivity and specificity to discriminate different odors. As an excellent gas detecting system, the olfaction has become an interesting study object for its wide potential applications of environment monitoring, food safety, and medical diagnosis (Gilbert and Firestein, 2002). To achieve these goals, the electronic nose systems, which mimic the biological olfactory working process, are studied intensively. And, many achievements for gas detection have been made using absorbability or catalysis property of the sensing materials to special odors (Gopel et al., 1998; Rock et al., 2008). However, the electronic nose system is not so perfect as the biological olfaction in the performance of sensitivity and specificity, which mainly attribute to the well-evolved structure of the biological tissue and information coding mechanism.

The first stage of olfactory sensing occurs in the neurons of olfactory epithelium, where odorants interact with the olfactory receptors specifically, and chemical signal is converted into action potential of the neurons then electrical signal is delivered to the olfactory bulb to encode and decode, ultimately the smell information forms in the brain (i.e. Buck and Axel, 1991; Laurent, 1999; Narusuye et al., 2003; Liedo et al., 2005; Kleene, 2008). Nowadays, scientists have proposed the concept of bioelectronic nose to realize artificial olfaction biomimetic design (Gopel et al., 1998; Gopel, 2000; Minic-Vidic et al., 2006). Combining the biomolecular function units with sensors to construct the bioelectronic nose has become a new branch of olfactory biosensors. Using bioactive units directly, bioelectronic nose can promote the development of gas sensing, and establish a research platform for olfactory mechanisms.

In the recent research, cell and tissue based biosensors, which treat living cells as sensing elements, can collect the functional information of bioactive analytes (Bousse, 1996; Rudolph and Reasor, 2001; Wang and Liu, 2009). Neural cells and tissues can be extracted from primary sources and cultured *in vitro*. The electrical activities relating to cellular functions can be detected by microelectronic sensor chips. This novel biosensor technology, characterized with high sensitivity, excellent selectivity and rapid response, has been applied in environmental monitoring and biomedical diagnosing. In the previous study, we have tried to explore the cultured olfactory cells as sensing elements to fabricate the cell-based biosensor as a first step towards a neurochip of bioelectronic nose (Liu et al., 2006; Wu et al., 2009). The biosensor composed of light-addressable potentiometric sensor (LAPS)

* Corresponding author at: Biosensor National Special Laboratory, Key Laboratory of Biomedical Engineering of Education Ministry, Department of Biomedical Engineering, Zhejiang University, Zheda Road No. 38, Hangzhou 310027, Zhejiang, PR China. Tel.: +86 571 87952832; fax: +86 571 87951676.
E-mail address: cnpwang@zju.edu.cn (P. Wang).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.02.024

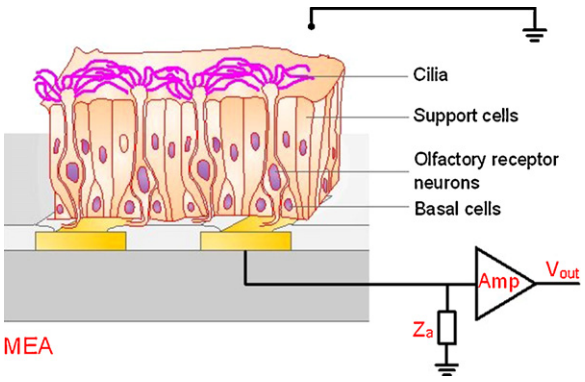

Fig. 1. Recording extracellular potentials of olfactory receptor neurons in intact epithelium by microelectrodes.

and olfactory neurons can monitor the extracellular potentials, and was sensitive to odorous changes. The most significant advantage is light addressable, which enables it to detect the potential changes on any site of the chip. However, the culture of olfactory cells on LAPS was relatively difficult. The living environment for cells changed from the original system. Besides, LAPS was a single channel recording system.

In contrast to LAPS, microelectrode array (MEA) can record the multisite potentials simultaneously and own the ability to long-term recording the firing of neural networks *in vitro* (Gross et al., 1995; Maher et al., 1999; Kovacs, 2003; Stett et al., 2003). In the present study, we managed to combine the intact olfactory epithelium with MEA for a bioelectronic nose of olfactory receptor neurons. Compared to the cultured olfactory cells, the intact olfactory epithelium can be obtained conveniently with the primary cell structure well preserved. Mimicking the *in vivo* process of gas sensing, it is a good candidate for the biological elements of bioelectronic nose. In our study, MEA can record the extracellular potentials of the olfactory receptor neurons in the epithelium. The multi-channel signal analysis might reveal some spatial and temporal information of early olfactory sensing for bioelectronic nose.

2. Theories

2.1. The structure and electrophysiology of the olfactory epithelium

In the olfactory epithelium, there are three types of cells: olfactory receptor neurons, support cells and basal cells (Fig. 1). The olfactory receptor neurons are sensory cells, which have axons and dendrites. Axons from neighboring receptor cells combine with each other, penetrate the basal membrane to form specialized nerve bundles and eventually connect to the brain. The cilia of these neurons, covered with olfactory receptors, are believed to be the odor's initial receptive field. Odor molecules interact with the olfactory receptor specifically, trigger the intracellular signal cascades and induce the action potential. The electric signal is finally transmitted to the brain. The support cells form the upper layer of the olfactory epithelium and connect to the basal membrane. The basal cells, which locate towards the basal side of olfactory epithelium, usually extend through the lower layer of the epithelium. Besides, there are a lot of glands to secrete the mucus, and the mucus layer forms humid environment to promote interaction between odorants and olfactory receptors.

Isolated neurons allow better control of the extracellular environment and odor delivery, however, the intact epithelium preserves native state of the neuron population and can be obtained easily (Reisert et al., 2005; Nickell et al., 2007). Olfactory neurons have different kinds of olfactory receptors and response to different odorants uniquely. The spatial and temporal coding is very important for olfactory systems. As a multi-channel recording device, MEA will greatly promote analyzing the coding information of the olfactory epithelium.

2.2. Theories of the tissue electrophysiological recording on MEA

MEA is a useful multi-channel recording device and has been extensively employed to record the tissue electrophysiological signals and study the spike firing mechanism. One successful example is spike recording in explanted retina by MEA (Grumet et al., 2000; Stett et al., 2003; Chen et al., 2003; Segev et al., 2004). By designing the electrode site area comparable in size to the ganglion cell, MEA can record from nearly all of the ganglion cells in a patch of the retina. This novel design provides a new way to understand neural circuits of retina *in vitro*.

In this paper, the intact olfactory epithelium was isolated and fixed on the surface of MEA. Fig. 1 illustrates the experiment of olfactory epithelium recorded by MEA. To record the electric signals from the epithelium efficiently, the basal membrane was contacted to the microelectrodes while the cilia were exposed. When the tissue is attached on the electrodes, a conductive cleft is formed between the tissue and the electrode, which is filled with electrolyte. A small patch of tissue is composed of many olfactory receptor neurons. Based on the H–H theory and the solid–electrolyte interface model, the transmembrane current of the cell–electrode junction is given by:

$$I_M = C_M \frac{dV_M}{dt} + I_{ionic} \quad (1)$$

where V_M is the transmembrane potential, C_M is the membrane capacitance per unit area, and I_{ionic} is the current due to ions flow through ion channels in the cellular membrane.

The I_{ionic} of the olfactory receptor neurons is defined as:

$$I_{total} = -(I_{CNG} + I_{ClCa} + I_{NCX}) \quad (2)$$

where I_{CNG} represents the inflow of Ca^{2+} and other extracellular cations through the cyclic nucleotide-gated (CNG) channels; I_{ClCa} represents the oulflow of Cl^- through Ca^{2+}-gated chloride (ClCa) channels; and I_{NCX} represents outflow of Ca^{2+} via the Na–Ca exchange protein.

The extracellular potential monitored by MEA is due to the ions flow through the cell membrane. The total field potential of the receptor neuron is given by:

$$\frac{V_i}{R_{seal}} + \frac{V_J}{Z_{electrode} + Z_a} = C_M \frac{d(V_M - V_J)}{dt} + I_{ionic} \quad (3)$$

where V_J is the polarization voltage detected by electrode. d is the thickness of average patch-to-insular distance. V_i is the transmembrane voltage. The transductive extracellular potential V_J represents general extracellular potential detected by MEA. R_{seal} is the seal resistance, which can be defined as:

$$R_{seal} = \frac{\rho_{seal}}{d} \frac{l}{w} \quad (4)$$

where ρ_{seal} is the sealing resistivity, l and w are the length and width of the effective portion of electrode coupled to the patch of the tissue, respectively.

Therefore, the microelectrodes with diameter of 30 μm were designed to record the extracellular potentials of olfactory receptor neurons. In order to get a successful recording of the potentials, "seal" impedance (voltage division of the signal) was decreased by electrodepositing platinum black onto the microelectrodes, and the thickness d was decreased by improving the adhesion of the tissue