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# Bioelectronic tongue of taste buds on microelectrode array for salt sensing

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ABSTRACT

Taste has received great attention for its potential applications. In this work, we combine the biological tissue with micro-chips to establish a novel bioelectronic tongue system for salt taste detection. Before experiment, we established a computational model of action potential in salt taste receptor cell, simulating the responsive results to natural salt stimuli of NaCl solution with various concentrations. Then 36-channel microelectrode arrays (MEA) with the diameter of 30 μm were fabricated on the glass substrate, and taste epithelium was stripped from rat and fixed on MEA. When stimulated by the salt stimuli, electrophysiological activities of taste receptor cells in taste buds were measured through a multi-channel recording system. Both simulation and experiment results showed a dose-dependent increase in NaCl-induced potentials of taste receptor cells, which indicated good applications in salt measurements. The multi-channel analysis demonstrated that different groups of MEA channels were activated during stimulations, indicating non-overlapping populations of receptor cells in taste buds involved in salt taste perception. The study provides an effective and reliable biosensor platform to help recognize and distinguish salt taste components.



## 1. Introduction

Taste, as one of the basic sensory systems, in charge of evaluating the nutritious contents of food and preventing the ingestion of toxic substances, has received great attention for its potential applications in food safety, pharmaceutical industry, and environment monitoring. To achieve these goals, the electronic tongue systems, which mimic the biological taste working process, have been intensively studied in recent years (Gilbert and Firestein, 2002; Escuder-Gilabert and Peris, 2010; Woertz et al., 2011). The general concept of the electronic tongue involves application of an array of nonspecific or low-selective sensors in order to produce analytically useful signals during the analysis of multi component matrices. Many achievements have been made in liquid substances detection by means of different artificial membranes and electrochemical techniques (i.e., Vlasov et al., 2000, 2005; Legin et al., 1999; Winquist et al., 2000). However, there still exists a certain gap in electronic tongue systems and biological taste, which mainly lies in the biological receptor structures and information coding mechanisms.

In biological taste system, the initial sensing organ lies in the taste epithelium, which contains different types of papillae: filiform papillae acts as an abrasive coating in tongue, while fungiform, foliate and circumvallate papillae, which contain taste buds, are responsible for chemosensory perception of basic taste modalities of sweet, bitter, salt, sour, and umami (Avenet and Lindemann, 1991; Gilbertson et al., 2000; Bear et al., 2006; El-Yassimi et al., 2008). Taste bud is the sensory end organ for taste, comprising a collection of 50–100 taste receptor cells, each of which has microvilli that poke through taste pore to the top of the bud. Chemicals from food termed tastants dissolve in saliva and contact the taste receptor cells through taste pores, where they interact either with taste receptors or with ion channels. These interactions trigger the intracellular signal cascades and induce the action potentials of the cells. The electric signals are finally transmitted to the brain via nerve fibers. Therefore, if taste buds are employed as sensitive materials to develop electronic tongues, the bionic design will have high performance in taste detection.

Among basic taste modalities, salt taste has recently drawn a great deal of attention for its crucial effects on health. As is known, moderate salt is vital for health, by regulating blood pressure and assisting with muscle and nerve function, but too much of it can lead to diseases, such as hypertension, heart disease and stroke (He and MacGregor, 2009; Strazzullo et al., 2009; Stolarz-Skrzypek et al., 2011). So, the salt evaluation has become one of the important issues in taste researches recently. Traditionally, salt taste is naturally evoked by sodium chloride (NaCl), which apart from water, is the major component of blood and ensures the proper dietary electrolyte balance. It is evident that the taste receptor cells can generate action potentials, usually

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repetitive spike trains, by $Na^+$-stimulation (Chen et al., 1996; Miyamoto et al., 1996b; Furue and Yoshii, 1997, 1998). When stimulated by NaCl, the $Na^+$ concentration rises outside the receptor cell, and the gradient for $Na^+$ across the membrane is made deeper. $Na^+$ then diffuses down the concentration gradient and the resulting inward current causes the cell membrane to depolarize (Bear et al., 2006; Chandrashekar et al., (2010)). The epithelial sodium channel (ENaC), a kind of dedicated $Na^+$-selective channel, is believed to contribute to this process.

Cell and tissue based biosensors, which treat living units as sensing elements, can collect the functional information of bioactive analytes (Bousse, 1996; Rudolph and Reasor, 2001; Wang and Liu, 2009). In our previous works, we have established the bioelectronic nose system for odor detection by electrophysiological sensing measurements of olfactory cells (Liu et al., 2006, 2010). Compared to the cultured cells, the intact tissue can be obtained conveniently with the primary structure well preserved. And, the microelectrode arrays (MEA), as a long-term and non-invasive method, can record the network potentials of the cells in intact tissue (Gross et al., 1995; Kovacs, 2003; Stett et al., 2003; Biran et al., 2007; Guo et al., 2010). In taste system, every taste bud has a collection of taste receptor cells and can be well preserved in taste epithelium. Therefore, combining the taste buds in epithelium with MEA, we tried to establish a novel bioelectronic tongue system to realize *in vitro* detection of the neural potentials of taste receptor cells to salt stimuli. Mimicking the *in vivo* process of liquid sensing, taste buds are good candidates for the biological elements of bioelectronic tongue. In this study, we distinguished the different discharge modes of salt stimulation in different concentrations. And the multi-channel analysis provided a powerful support within salt sensing pathways.

## 2. Experiments and methods

### 2.1. Design and preparation of MEA

In taste buds, there are two types of cells, taste receptor cells and basal cells (Fig. 1A). The taste receptor cells are sensory chemoreceptors that send information detected by clusters of different receptors and ion channels to the gustatory areas of the brain via the nerve fibers. Taste pores, connected with taste receptor cells by microvilli, are believed to be the tastant's initial receptive fields. Taste substances interact with the receptors or ion channels, trigger the intracellular signal cascades and induce the action potentials for taste coding. As a multi-channel recording device, MEA will greatly promote the coding information analysis in taste buds.

Combining the taste buds with MEA, we fabricated a hybrid biosensor to detect electrophysiological properties of the taste receptor cells. MEA is composed of an array of electrodes where taste buds were fixed, and the fabrication and preparation of MEA were similar to our previous work (Liu et al., 2010). The micrograph of fabricated gold microelectrode with a $6 \times 6$ array pattern is shown in Fig. 1B. The electrodes were 30 μm in diameter with 200 μm center to center spacing, which could effectively avoid the electric interference between the neighboring electrodes.

### 2.2. Isolation and fixation of taste epithelium

Sprague-Dawley rats with weight of about 250 g were purchased from the Laboratory of Animal Research Center of Zhejiang Province, China. The rat was anesthetized by intraperitoneal injection of urethane. The tongue was wholly excised and immediately incubated in Ringer's solution. The epithelium with fungiform papillae was then peeled away from underlying tissue.

The isolated epithelium (about $5 \times 5$ mm) was rinsed with Ringer's solution and placed with taste pores side up on the surface of MEA. The epithelium was then fixed by a plastic ring-shaped frame covered with a tightly stretched piece of mesh. The enlarged fungiform papillae, where the taste buds located and taste receptor cells specialized for substances detection, were observed by the scanning electron microscope (SEM). As shown in Fig. 1C, the filiform papillae and fungiform papillae formed a dense meshwork on the epithelium surface. The native structures of the taste buds were well preserved, with the receptor cell populations intact.

### 2.3. Simulation of action potentials in salt receptor cells

We set up a computational model to pre-compute the salt responses of taste receptor cells with MATLAB (MathWorks, Natick, MA). Action potentials of taste receptor cells are due to ions flow through the cell membrane. To develop this model, we firstly calculated every activated ion channel by Markov model shown below:

$$i_X = g_X X_\alpha{}^m X_i{}^n (V - E_X) \tag{1}$$

$$\frac{dX_a}{dt} = \frac{X_{a\infty} - X_a}{\tau_{Xa}} \tag{2}$$

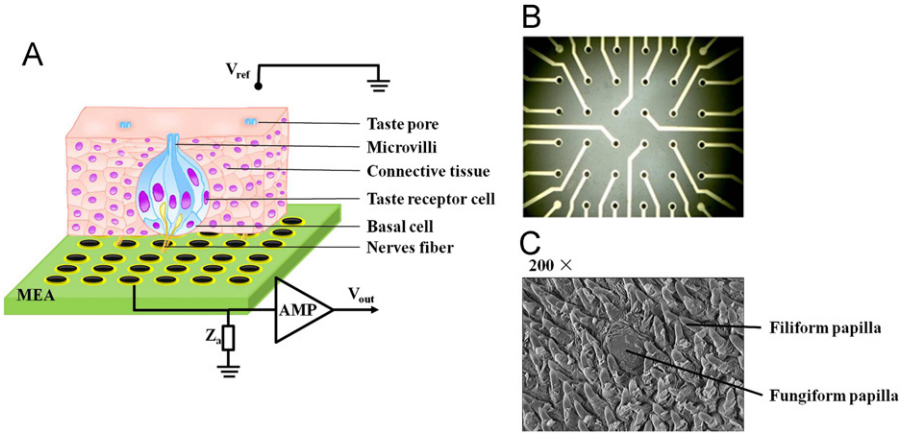


**Fig. 1.** The recording extracellular potentials of taste receptor cells in taste buds by microelectrodes. (A) Schematic diagram of the biosensor. (B) The pattern of 30 μm electrodes in 36 channel arrays. (C) Fungiform papillae of the taste epithelium observed by the scanning electron microscope.