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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.

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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 6×6 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 5×5 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:

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

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

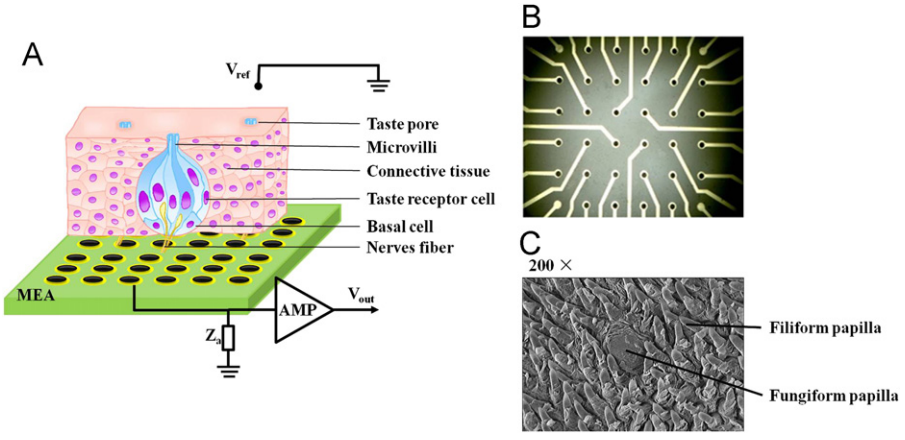

Fig. 1. The recording extracellular potentials of taste receptor cells in taste buds by microelectrodes. (A) Schematic diagram of the biosensor. (B) The pattern of 30 μm electrodes in 36 channel arrays. (C) Fungiform papillae of the taste epithelium observed by the scanning electron microscope.

$$\frac{dX_i}{dt} = \frac{X_{i\infty} - X_i}{\tau_{Xi}} \tag{3}$$

where i_x is the current of the ion channel. g_x is the maximum conductance of the ion channel. V represents the induced action potential. X_a represents the activation process of the ion channel while X_i describes the inactivation process of the ion channel. $X_{a\infty}$ is the steady-state activation constant. $X_{i\infty}$ is the steady-state inactivation constant. τ_{Xa} is the activation time constant. τ_{Xi} is the inactivation time constant. m, n are all constants, usually 1 or 2. E_X is the reversal potential, which can be expressed by the Nernst Equation below:

$$E_X = \frac{RT}{FZ} \ln \frac{[X^+]_o}{[X^+]_i} \tag{4}$$

where $[X^+]_o$ represents the concentration of the extracellular ions, and $[X^+]_i$ represents the concentration of the intracellular ions. T is temperature (K). R, Z, F are all standard constants.

In salt taste perception, ENaC is believed to be the main ion channel of receptor cells, which mediates the selective response to Na^+. Besides, the voltage-gated Na^+, K^+ currents and the leakage current are proposed to make contribution to the potentials of the salt taste receptor cells. Thus, combining the salt transduction with Hodgkin–Huxley theory, the action potential of the salt taste cell can be calculated by:

$$C_m \frac{dV}{dt} = i_{Na} + i_k + i_{ENaC} + i_l \tag{5}$$

where C_m is the membrane capacitance per unit area. i_{ENaC} is the current of ENaC. i_{Na} is the voltage-gated Na^+ current. i_k is the voltage-gated K^+ current, and i_l is the leakage current.

2.4. Record of taste signals and NaCl stimulation

We applied the USB-ME16-FAI system from Multichannel Systems (MCS, Reutlingen, Germany) to record 16-channel signals. The whole recording system was placed in a shielding box to avoid external disturbances. Noise of blank measurement was about 10 μV. The software of MC RACK (MCS, Reutlingen, Germany) and MATLAB were used to display and analyze the signals.

In the experiments, we used 100 mM, 300 mM, and 500 mM NaCl solutions (Sigma Aldrich) as salt stimuli to taste epithelium. Before stimulation, native electrophysiological activities of the epithelium were recorded for 5 min. After one concentration of the NaCl solutions was injected into the MEA chamber, the recording lasted for about 5 min. Then the stimulus was washed out from the chamber by fresh standard perfusate. In order to rule out the influence of residual tastants and make electrodes and tissue return to a stable state, the minimum interval between the taste injections was 5 min. All recordings were performed at room temperature (18–25 °C).

3. Results and discussion

3.1. Simulation results of action potentials in salt taste receptor cells

Based on the simulation model of action potentials in salt taste receptor cells, we pre-calculated the signal responses of taste receptor cells to NaCl solutions in concentrations of 100 mM, 300 mM and 500 mM. Using the data from the published physiological experiments (Sheng et al., 2003; Chen et al., 2009), we obtained the involved ion channel currents. Combining the ion channel results with the Hodgkin–Huxley model, which turned into first order ordinary differential equation (5), the action potentials V of taste receptor cell to NaCl stimuli of different concentrations were computed and plotted, with the spike firing frequency representing the intensity of the potentials. As is shown in Fig. 2, continuous spike signals were calculated during salt stimulations and the firing frequency presented a dose-dependent increase. The firing frequencies of spikes in 100 mM, 300 mM and 500 mM NaCl were about 21 Hz, 32 Hz and 36 Hz, respectively. In our simulation, the signal responses were all basic spike potential representations, with the firing frequency characterizing the signal intensity.

In the simulation, we calculated the salt sensing responses of taste receptor cells, which basically revealed the functional activity of single activated taste receptor cell. The extracellular tissue signals, however, are usually local field potentials (LFPs) which reveal the coherent activities within a population of receptor cells. That means the general experimental LFP is a summation of simulated spikes within the activated receptor cells. Therefore, the differences of spike results to NaCl stimuli in simulation predicted the likely results of taste experiments. Actually, the dose-dependent firing in simulation has already been found in consequence of some previous physiological experiments (Treesukosol et al., 2007; Shigemura et al., 2007; Yoshida et al., 2009).

3.2. Mult-channel recording of the extracellular potentials of the taste buds

MEA has the benefit of detecting signals of many cells synchronously, which is convenient to comparatively analyze recorded information in parallel. Taste buds have different populations of taste receptor cells which mediate different patterns of signal responses. Therefore, the multi-channel signals in our experiment can properly reveal the coherent activities of different taste receptor cells in taste buds. Fig. 3 shows the multi-channel signals in different stimulations. The stimulations of NaCl solutions activated different populations of taste receptor cells. When stimulated by 100 mM NaCl, only taste receptor cells in channel 5, 6, 7 and 8 groups (marked in red) were activated and fired continuous potentials. When under the stimulation of 300 mM NaCl, the red groups still responded and released signals with

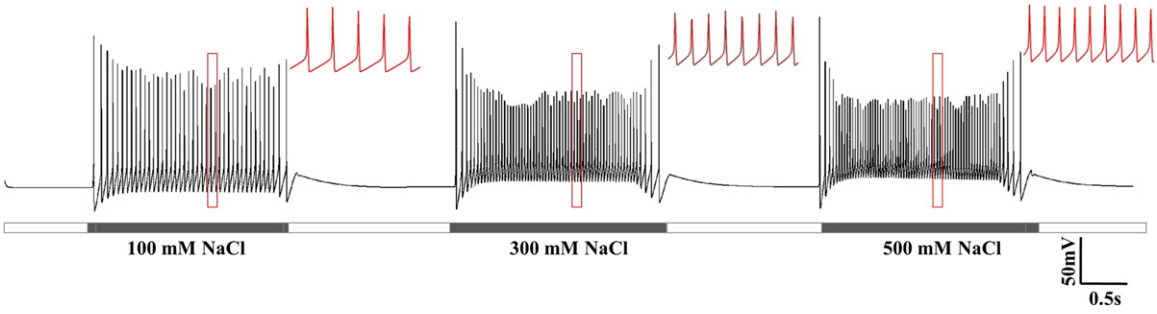

Fig. 2. The simulated action potential of taste receptor cells to salt stimuli. The red marked parts are magnified in timeline shown beside the original spikes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

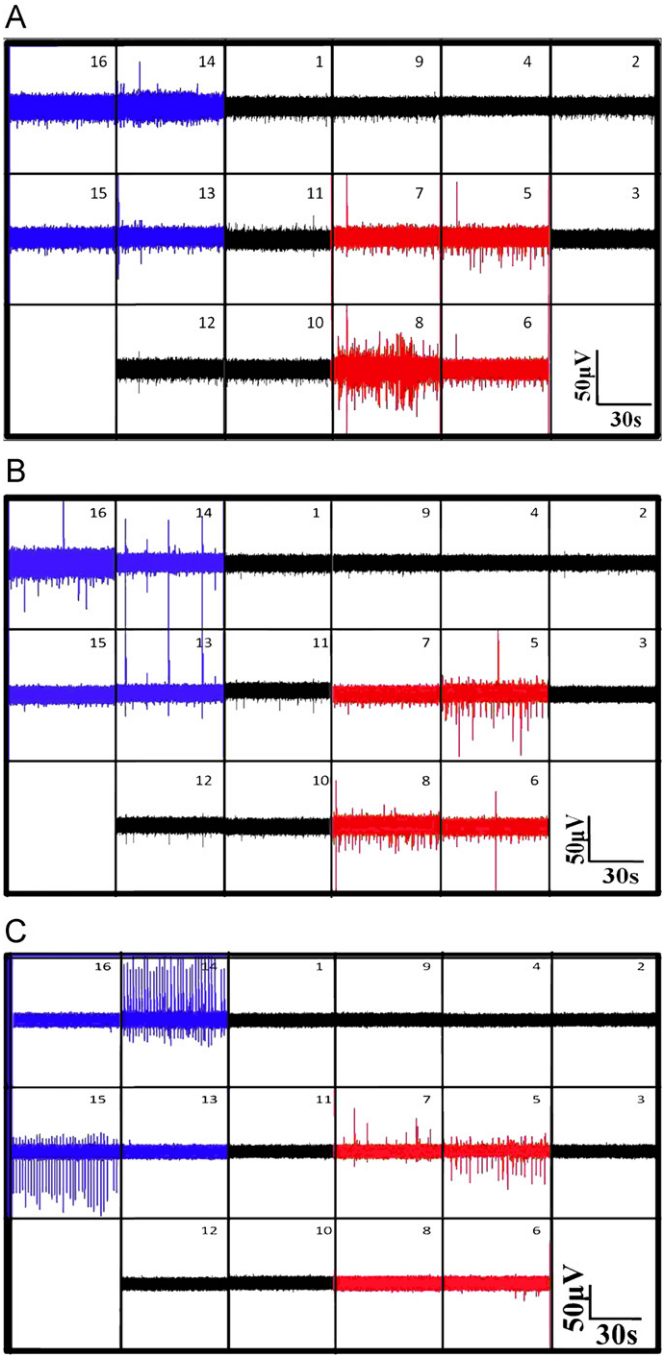

Fig. 3. The multi-channel signals in different NaCl concentrations. The red and blue marked channels represent two populations of activated taste receptor cells. The different signal responses of two functional populations of taste receptor cells in stimulus of 100 mM NaCl (A), 300 mM NaCl (B), and 500 mM NaCl (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

increased intensity, while the blue ones, including channel 13, 14, 15, 16, covered by another population of taste receptor cells, released sporadic big signals. Finally, when it came to the stimulation of 500 mM NaCl, the blue groups, especially channel 14 and 15, displayed intensive potential firings, while the red groups still responded with increased continuous potentials. That meant a population of taste receptor cells in red group channels responded to all NaCl stimuli, while the taste receptor cells in blue group channels would not respond until in 300 mM stimulation, and released intensive potentials in high concentration of 500 mM. Therefore, from the multi-channel results, we found two non-overlapping populations of taste receptor cells in taste buds were activated by salt stimuli. One responded to all concentrations, whereas another responded only to high concentrations.

It has been reported that NaCl may trigger two divergent responses in mammals (Beauchamp et al., 1990; Lindemann, 2001). Some fluorescent studies demonstrated that two populations of taste receptor cells mediate distinct NaCl responses. A unique subset taste receptor cells respond to all concentrations of NaCl via Na^+-selective ENaC, while another subset of the receptor cells selectively respond to concentrations greater than 150 mM (Hettinger and Frank, 1990; Ninomiya, 1998; Yoshida et al., 2009). As was found in our experiment, there indeed existed two different functional populations of taste receptor cells mediating the salt taste perception. One population responded to all concentrations while another only activated by high concentrations. The two different transmission paths in salt sensing play a crucial way in regulating the salt intake, with the high concentration way serving as a warning mechanism against hyper-salinity.

3.3. Analysis of the electrophysiological signals for salt sensing

The evoked potentials in our experiments showed not only the activated taste receptor cells in taste buds, but also the different firing modes. To further explore the recorded potentials, we chose and plotted the typical long-time responses to NaCl solutions of different concentrations (Fig. 4). In stimulations of 100 mM and 300 mM NaCl, channel 5 released sustained signals, while in 500 mM NaCl stimulation, channel 15 became the firing centre. It can be seen that the taste buds delivered continuous signals with NaCl stimulation, and the induced signals were significantly suppressed with NaCl removal. Meanwhile, the potential responses of different concentrations showed distinct firing characteristics. The release of potentials after 100 mM NaCl stimulation was mainly sustained signals with an average amplitude of about 18 μV and firing frequency of about 0.6 Hz. During the stimulation of 300 mM NaCl, two different kinds of potentials were detected, mainly continuous signals with an average amplitude of about 31 μV and firing frequency of about 1.1 Hz. The other kind of the signals was sporadic negative potentials, with an average amplitude of about 56 μV. When stimulated by high concentration of 500 mM NaCl, the taste buds fired intensive signals with significant amplitude. The average amplitude of the signals was about 60 μV

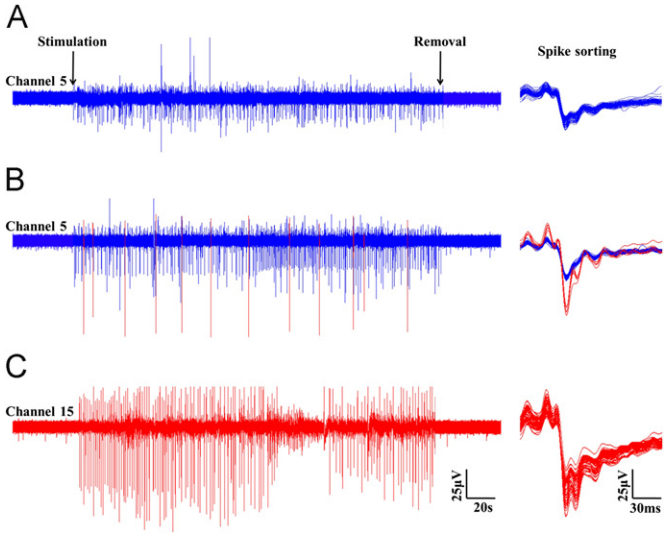

Fig. 4. The recorded extracellular potentials to salt stimuli. The spike sorting maps display the complete waveform of recorded potentials.

and the firing frequency was about 1.4 Hz. It is evident that the signal firing frequency and amplitude increased with the concentrations during the stimulations, displaying an obvious dose-dependent property. The spike sorting map displayed the complete waveforms of recorded potentials of same type. As shown, the responses of 300 mM NaCl demonstrated two different signal patterns. The mainly potentials in first two stimuli displayed similar firing appearances, while the sporadic signals in 300 mM NaCl looked more like signals in high concentration. As mentioned earlier, it was another functional population of taste receptor cells began to respond in 300 mM NaCl, which may lead to the second sporadic signals. As the extracellular potentials of taste buds in our experiment revealed the coherent activities within a population of taste receptor cells, the difference in signal waveforms may well come from different activated populations of taste receptor cells in stimulations. So, the two different signal waveforms also provided a powerful support for the two distinct salt sensing paths.

The signal waveforms revealed the activated populations of taste receptor cells in taste buds, while the basic characteristics of signals represented the response intensity to different salt stimuli. To further discuss the signals responses in different concentrations, we calculated and plotted a comparison diagram. Fig. 5A shows the normalized comparison result of basic characteristics of potentials in our experiment. It is evident that the signal firing frequency and amplitude increased with the concentration. This dose-dependent increase in potentials of taste buds was also found in our mentioned computer simulation spike results. As shown in Fig. 5B, a comparison diagram of normalized frequency in simulation and experiment was calculated, displaying the same rising trend. As has been mentioned, the experimental LFP is a summation of the spikes in simulation within the activated populations of taste receptor cells. Therefore, the increase of firing frequency in simulation is likely to result in the increase of amplitude and firing frequency in experiment.

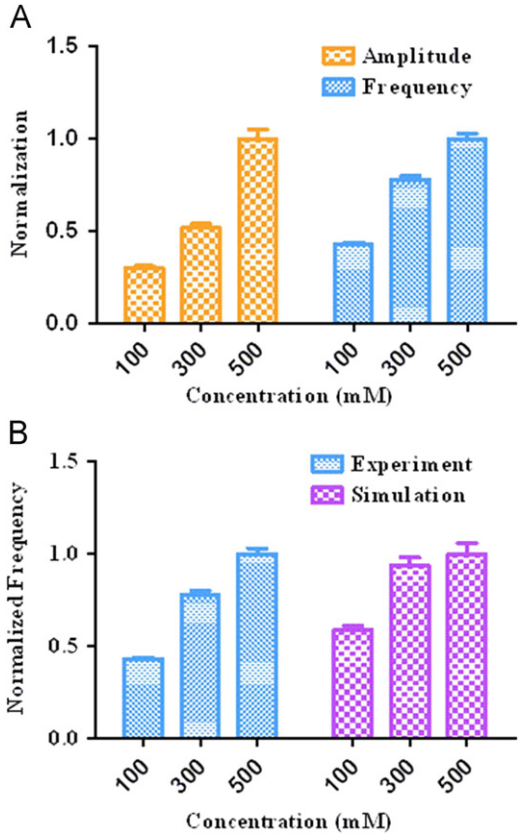

Fig. 5. The analysis of the potential characteristics. (A) The mean amplitude and firing frequency of experimental signals to salt stimuli of different concentrations. (B) The firing frequency comparison of potentials in simulation and experiment.

The mechanism of salt induced action potentials lies mainly in the interaction of Na^+ with taste receptor cells. The multi-channel MEA can detect and record the salt evoked potentials effectively. Therefore, it is easy to understand the dose-dependent changes in salt sensing of taste receptor cells in taste buds. When the concentration of NaCl stimuli increased, the Na^+ intake will elicit more action potentials in taste receptor cells as shown in simulation. Thus the MEA recorded potentials in taste buds, summation of responses within a population of taste receptor cells, are likely to be increased in amplitude and frequency. In this study, the tissue-MEA based bioelectronic tongue system provides an effective and reliable platform to help recognize and distinguish key salt components. Thus, the taste buds-based biosensor bridges the gap between conventional *in vitro* methods and complex *in vivo* experiments for taste mechanisms, making a good foundation for practical bioelectronic tongue system in the future.

4. Conclusions

Combining biological taste tissue with micro-chip technology, this study tried to design a novel bioelectronic tongue for salt taste detection by electrophysiological sensing measurements of taste buds. Extracellular potentials of taste receptor cells in intact taste buds were recorded through multi-channel MEA recording systems. When stimulated by NaCl of different concentrations, the recorded potentials presented different firing modes and demonstrated obvious dose-dependent changes in both simulation and experiment. And the multi-channel results indicated two non-overlapping functional populations of taste receptor cells in taste buds mediating the salt sensing. With function units well preserved and successfully recorded, the receptor cell-based biosensor technology is a promising platform for bioelectronic tongue with respect to tastes detection in the intact taste buds.

Acknowledgments

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