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Short communication

Taste receptor cell-based biosensor for taste specific recognition based on temporal firing

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ABSTRACT

Taste receptor cells are the taste sensation elements expressing sour, salty, sweet, bitter and umami receptors, respectively. There are cell-to-cell communications between different types of cells. Nevertheless, the mechanism of taste sensation and taste information coded by taste receptor cell is not well understood at present and it is a long-standing issue. In order to explore taste sensation and analyze taste-firing responses from another point of view, we present a promising biomimetic taste receptor cell-based biosensor. The temporal firing responses to different tastants are recorded. Meanwhile, we investigate the firing rate and temporal firing of taste receptor cells. The experimental results are consistent with that from patch clamp and molecular biology experiment. Firing rate is dependent on the concentration of stimulus. PCA analysis (principal component analysis) of the temporal firing responses shows that the responses from different types of taste receptor cells can be distinguished. Furthermore, exogenous ATP is applied to mimic the effects of transmitter ATP (adenosine triphosphate) released from type II cells onto type III cells. Both enhanced and inhibitory effects on spontaneous firing are observed. This novel biomimetic hybrid biosensor provides a potential solution to investigate the taste sensation and coding mechanisms in a non-invasive way.

1. Introduction

Benefited from the application of molecular biology, patch clamp, genetics and behavior assays, the study of taste have made great progress in the last decade. Previous studies revealed that taste receptor cells can be categorized into four types based on ultrastructural features: Type I, II, III and IV cells. Type I cells function as supporting cells. They cannot produce action potentials because of the intrinsic lack of voltage-gated inward Na^+ currents. Type IV cells are the progenitor cells that can differentiate into the other types of the cells. Type I and IV cells will not be considered in this article because their roles in taste sensation and taste coding are uncovered at present.

Sweet (T1R2/T1T3) (Nelson et al., 2001), bitter (T2Rs) (Adler et al., 2000) and umami receptors (T1R1/T1R3, mGluR4) (Nelson et al., 2002; Chaudhari et al., 2000) are expressed in distinct subset of type II cells (Fig. 1(a)). However, evidences show that they have no classical ultrastructural synaptic connection with nerve processes (Clapp et al., 2006). Type III cells express sour receptors (ASIC and PKDL channels) (Ishimaru et al., 2006; Ugawa et al., 2003), which is the only type of cells that forms conventional synapses with nerve fibers. Both type II and III cells can fire action potentials in response to tastants. However, how the information of taste quality and taste density is presented and coded by taste cells at periphery is not well understood and is in an endless debate.

Another long-standing issue is how taste signals from type II and III taste cells is transmitted to the afferent nerves and finally to the brain. Recent evidences show that there should be communications between type II and type III cells (Roper, 2006; Huang et al., 2007). In response to taste stimuli, type II cells secrete the neurotransmitter ATP onto type III cells, then, serotonin is released from type III cell that indirectly leads to the output of taste signals. The mechanism of taste sensation and information transduction was shown in Fig. 1(a). Patch clamp electrophysiological recordings showed that ATP either depolarized or hyperpolarized taste cells (Hayato et al., 2007).

Patch clamp is the golden standard technology for electrophysiological recording, however, with some intrinsic limitations like complicated operation. Based on MEMS (Micro Electro-Mechanical systems) technology and benefited from optical electronics, cell-based LAPS (Light Addressable Potentiometric Sensor) chip is used as the platform for the research of taste sensation and firing responses. By scanning the light-pointer along the LAPS surface, the surface potential at any desired position can be recorded, which greatly enhance the flexibility and feasibility of cell-based

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chips compared with other biosensors. Meanwhile, this convenient operation of moving light-pointer can overcome the problem of low-density culture of taste cells.

In this study, we develop a biomimetic taste receptor cell-based biosensor to explore taste sensation and taste cell-to-cell communication. We cultured taste cells on LAPS chip as the sensing elements. The temporal firing responses were recorded upon different taste stimuli. Exogenous ATP was applied to mimic the effects of transmitter ATP released from type II cells onto type III cells. We observed the enhanced and inhibitory effects of ATP on spontaneous firing of taste cells, which were evaluated by means of peristimulus-time histograms (PSTHs) and interspike interval hisograms (ISIH).

2. Materials and methods

2.1. LAPS chip and measurement system

LAPS chip with an electrolyte-insulation-semiconductor (EIS) structure was employed. N-type silicon wafers (φ = 1.5 in.) with specific resistance of 10–15 Ω cm were used as the LAPS chip. The upper side of the chip was insulated with a layer of 30 nm SiO_2 and 60 nm Si_3N_4. A 1 μm layer of aluminum was sputtered on the backside of the wafer to create an ohmic contact. A separated platinum wire attached to the inside of the culture dish, serving as a ground reference (Fig. 1(b)). The details could be seen in our previous paper (Xu et al., 2005).

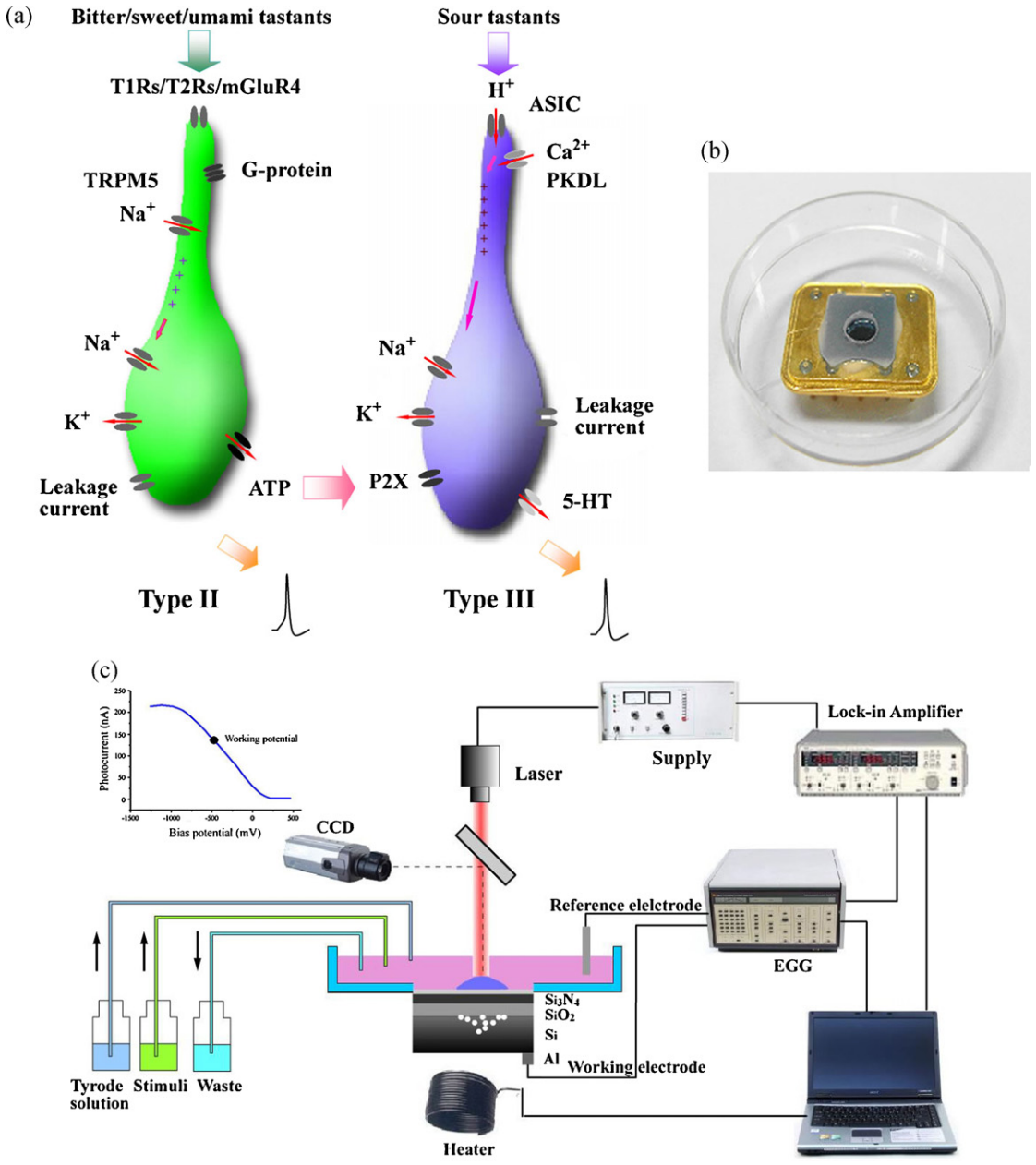

Fig. 1. Scheme of taste transduction and LAPS measurement system. (a) Type II cells are tuned to bitter, sweet or umami stimuli. The inward current from TRPM5 channels depolarizes membrane potential. Voltage-dependent Na^+ and K^+ respond to the membrane potential changes and produce action potentials. Meanwhile, the neurotransmitter ATP is secreted onto type III cell. Sour taste receptor is expressed in type III cells, which can also fire upon corresponding stimuli. (b) The LAPS chip with a chamber. (c) The LAPS measurement system for extracellular electrophysiological recordings of taste receptor cell.

As shown in Fig. 1(c), a modulated light (wavelength 543.5 nm, power 5 mW, 4 kHz) was focused on the surface of a single taste receptor cell cultured on LAPS ($\varnothing \approx 10\,\mu m$). The characteristic *I–V* curve of LAPS chip was obtained. The working potential was set to the point that was most sensitive to the surface potential changes. After taste stimuli were applied, LAPS detected the surface potential changes corresponding to the responses elicited from taste cell. A corresponding photocurrent was recorded by the electrodes of potentiostat (Model 273A, EG&G Princeton Applied Research) and lock-in amplifier (Model SR830 DSP, Stanford Research Systems). The cut off frequency of low pass filter (LPF) in lock-in amplifier was set to about 200 Hz. The phase shift of LPF was eliminated to ensure the phase of the signals after lock-in amplifier would be the same with the extracellular signals from taste receptor cells. A 16 bit data acquired card and the software of LABVIEW were employed for data collection, analysis and storage. The sampling rate was 40 kHz. All measurement was performed at room temperature about 22 °C. Data were analyzed in Matlab (MathWorks). Standard error (SE) and Student's *t*-test were calculated for statistical analysis.

2.2. Cell culture

Taste receptor cells were isolated from Sprague-Dawley rat according to published procedures (Herness and Sun, 1995). Briefly, circumvallate papillae were excised and incubated in a cysteine-activated (1 mg/ml) papain/divalent-free bicarbonate-buffered solution (14 U/ml) for 3 h at 37 °C in 5% CO_2/95% air. Then, the tissue block was transferred to the extracellular solution. Taste receptor cells were dissociated from epithelium and cultured on the LAPS, which was coated with Cell-TAK (tissue adhesive from BD Biosciences). Taste cells were cultured about 2 h on the LAPS chip at 37 °C under standard conditions of humidified air with 5% CO_2 in DMEM before measurement. The taste cell density is 80–200 cells/cm^2 with the surface area of LAPS chip for taste cell culturing and recording around 12.56 mm^2 (diameter = 4 mm).

Tyrode solution (extracellular solution) contained (in mM) 126 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 5 NaHEPES, 1.25 $NaH_2PO_4{\cdot}H_2O$, 10 D-glucose, pH 7.5. Taste cells were stimulated by HCl (sour, pH 2 and pH 4), 0.03 M $MgSO_4$ (bitter), 0.3 M sucrose (sweet), 0.5 M monosodium glutamate (MSG) (umami) and 100 μM ATP.

Tyrode solution was applied to the LAPS chamber then switched to the acidic solution (pH 4). After recording for about 120 s, the acidic solution was washed out. Similar procedures were conducted to obtain the responses to the acidic solution (pH 2), the mixture of sweet, bitter and umami solution and exogenous ATP.

2.3. Data processing

We used peak value extraction method to obtain the firing spikes elicited by stimuli. The signals without the application of stimulus recorded by LAPS were taken as the reference signals. The mean value '*D*' of the reference signals was used to quantify the level of baseline noise. The threshold to discriminate the firing spikes from noise was set to 2.5*D*, which was chosen empirically based on the data we obtained from LAPS. In each sample of the signals, spikes with amplitude above 2.5*D* were extracted and analyzed.

An adaptive filter was utilized to improve the signal-to-noise ratio. The recorded signals were taken as the signal inputs into

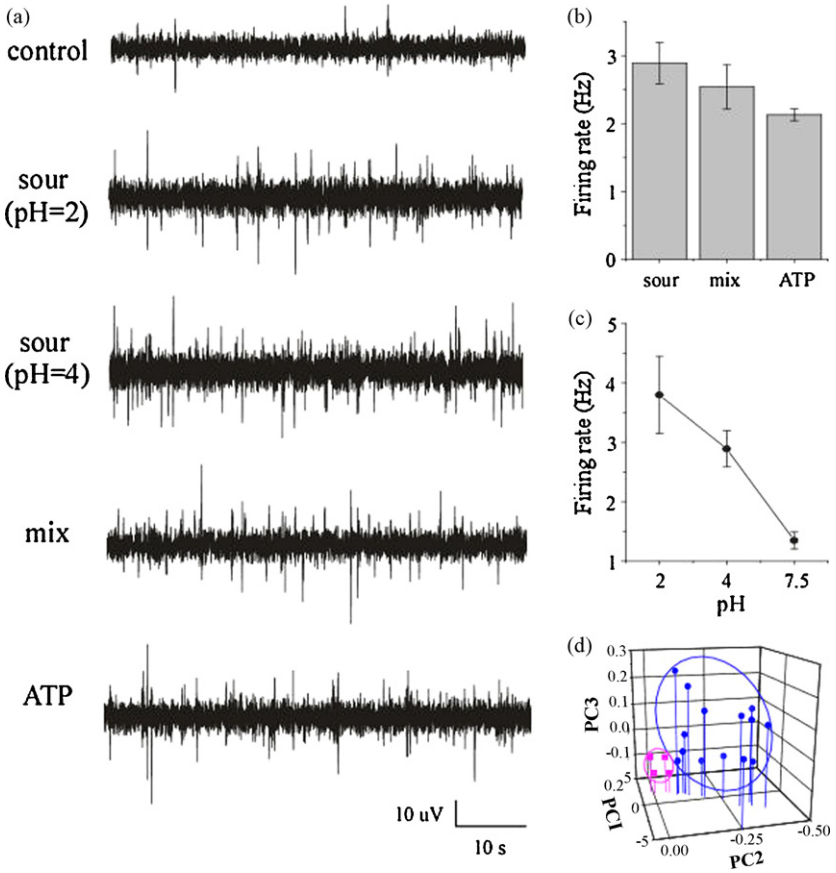

Fig. 2. The firing responses of taste cells to different stimuli. (a) The firings without stimulus, upon sour (HCl), mix (bitter ($MgSO_4$), sweet (sucrose), umami (monosodium glutamate)) tastants and exogenous ATP were obtained. Scale bar: ordinate, 10 μV, abscissa, 10 s. (b) The statistics of firing rate with different stimuli. (c) The pH-dependency of firing rate upon sour tastants. (d) PCA analysis of the temporal firing responses to sour (blue) and ATP (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

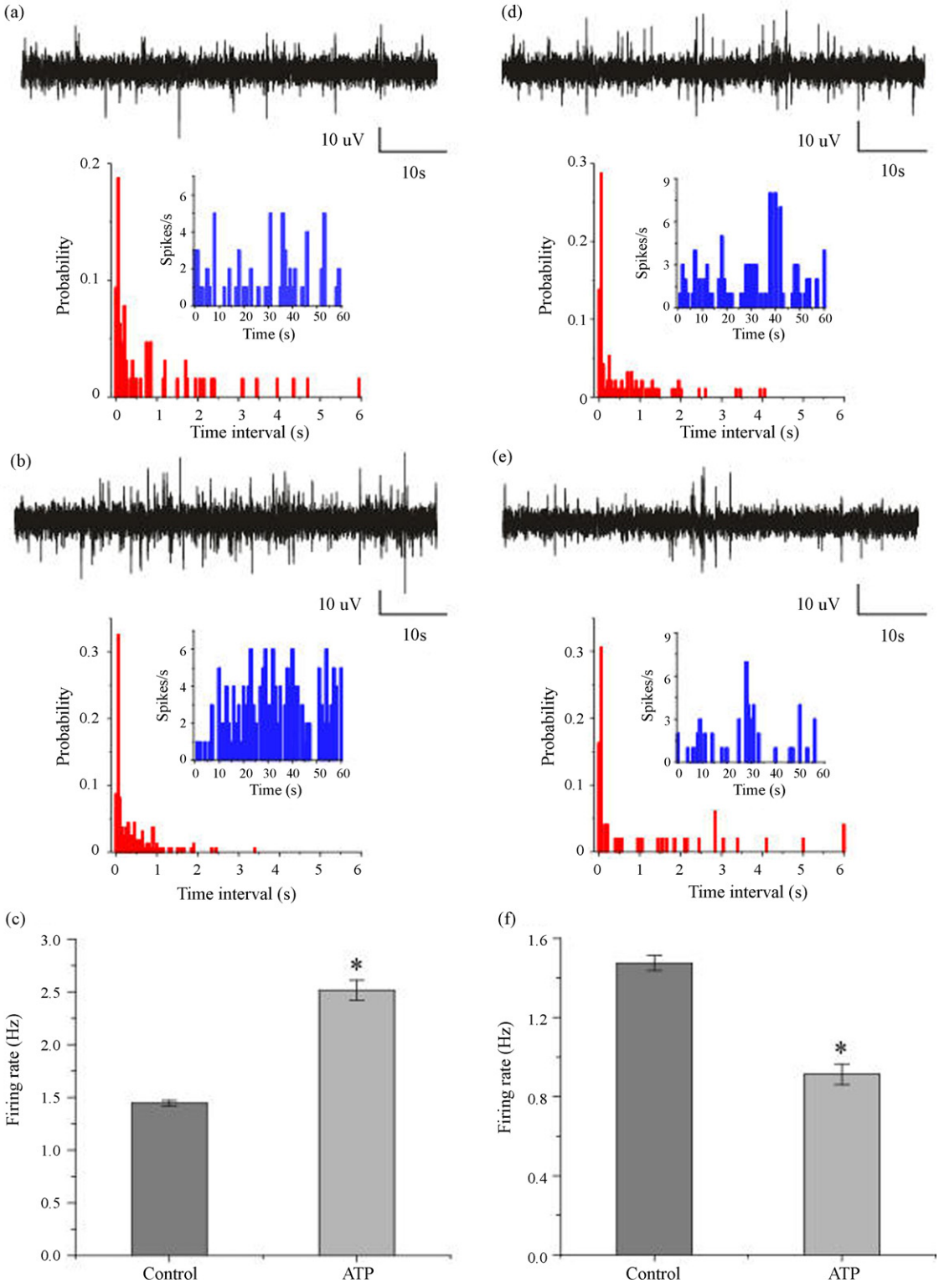

Fig. 3. The effects of 100 μM ATP on spontaneous firing of taste cells. PSTH (in blue) and ISIH (in red) were used. (a) Spontaneous firing from a taste cell. (b) The temporal firing after the application of 100 μM ATP on the taste cell in (a). (c) The firing rate was significantly increased upon ATP ($n = 5$). (d) The spontaneous firing pattern from another taste cell. In response to 100 μM ATP, the firing response was shown in (e). (f) The firing rate was significantly decreased in response to ATP ($n = 3$). Data are presented as mean ± SE. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the recursive least squares adaptive filter. The noise component obtained from above was regarded as the reference input of the filter. The spike timing of filtered signals was almost the same as the one before this process.

2.4. Analysis of temporal firing response

- *Mean firing rate*: The total number of spikes elicited by taste stimulus in a second was analyzed. Student's *t*-test was used to

calculate if there was a significant difference in the firing rate before and after the application of stimulus.

- *Peristimulus time histograms* (*PSTHs*) are the histogram of the times when cells fire. So we use PSTH to visualize the firing rate and timing of taste receptor cell firing spikes in response to a stimulus, like sour tastant, etc. We divide a period of observation time T into N bins with bin size $\Delta 1$ s. The amount of spikes n_i in each successive 1 s bin is extracted and the bar histogram with bar height of each bin given by the spikes per second was plotted.
- *Interspike interval histograms* (*ISIHs*) are the distribution of the intervals between spikes to unravel the patterns of spike activities. The interval is the abscissa and the spike amount in each intervals bin ($\Delta 50$ ms) is extracted. Then we normalized it by means of dividing by the overall amount of spikes in the observation time.

3. Results and discussion

Taste receptor cells fired in response to sour, mix (bitter, umami and sweet) tastants, and exogenous ATP. The temporal firing patterns in response to different stimuli were shown in Fig. 2(a). There are firing spikes upon stimuli. The noise level was about 15 μV. In control experiment, the recording from taste cells without stimuli was obtained (Fig. 2(a)). Few spikes could be observed.

The mean firing rates upon stimuli were shown in Fig. 2(b). Upon sour stimulus (HCl), the firing rate was around 2.89 ± 0.3 Hz ($n = 14$), when pH equaled to 4. With the application of mixed tastants (sweet, bitter and umami), the firing rate was 2.54 ± 0.32 Hz ($n = 10$). Meanwhile, to mimic and test the effects of neural transmitter ATP released from type II taste cells, exogenous ATP was applied. The mean firing rate was around 2.13 ± 0.09 Hz ($n = 4$). Upon the above three tested stimuli, the firing rate was significantly increased compared with the Tyrode solution condition (pH 7.5). According to the firing rate, we found it was hard to discriminate the responses from different tastants. However, the firing rate was proportional to the concentration of the same stimulus (HCl). The mean firing rate was 3.8 ± 0.65 Hz, when pH equaled to 2 ($n = 12$). As shown in Fig. 2(c), with pH going more acidic, the mean firing rate notably increased which was consistent with the patch-clamp result from hamster taste cells (Gilbertson et al., 1992). The pH-dependency trend indicated that information about the intensity of taste stimuli could be represented and transmitted by the firing rate code, as the same in the other sensory systems. In order to confirm that there were no effects of stimuli on LAPS chip, recordings were also obtained without taste cells and with HEK 293 cells cultured on LAPS. No firing spikes could be observed (data not shown here).

Molecular biological and genetic studies demonstrated that three taste modalities including sweet, bitter and umami were encoded by type II cells. Sour sensation was coded by type III cells. Taste information from type II cells was transmitted to type III by releasing ATP. However, whether different taste modalities could be distinguished by type III cells was not well known. In addition, evidence from behavior study indicated that taste quality can be transmitted by temporal firing patterns and temporal codes (Di Lorenzo and Victor, 2003). Therefore, we performed principle component analysis (PCA) of the temporal firing responses upon exogenous ATP and sour tastants. The responses from ATP and sour stimuli formed distinct clusters, as shown in Fig. 2(d), which indicated that the taste information from different types of taste cells (II and III) could be discriminated.

In recordings, we found another subset of taste cells, which fired spontaneously, could also respond to 100 μM ATP. The recordings before and after the application of ATP were shown in Fig. 3. Five cells increased their firing rate in response to ATP (Fig. 3(a and b)). The firing rate after applying ATP (2.52 ± 0.096 Hz) was significant higher than their spontaneous firing (1.44 ± 0.027 Hz), as shown in Fig. 3(c), whereas the firing rate of another three cells notably decreased (0.91 ± 0.052 Hz) compared with spontaneous firing (1.475 ± 0.038 Hz) (Fig. 3(d–f)).

Temporal encoding analysis methods, such as peristimulus-time histograms (PSTHs) and interspike interval histograms (ISIH) were utilized. In the subset of taste cells with increasing firing rate, the time interval is comparatively smaller and more centralized in ISIH after the application of 100 μM ATP (Fig. 3(b)). The firing spikes were notably increased in PSTH. On the contrary, another three taste cells with decreased firing rate, had smaller time interval, mostly distributed before 2 s in ISIH (Fig. 3(d)). Upon ATP, the time interval scattered in the range of 0–6 s (Fig. 3(d)). The spikes in PSTH were less compared with its spontaneous firing. The results showed that there were dual effects of ATP on the spontaneous firing, both enhancement and inhibition, which were consistent with the results obtained from patch clamp recordings (Hayato et al., 2007).

The results from our recordings indicate that taste density can be coded by firing rate. Different types of cells probably carried specific taste information, which can be discriminated and then transmitted to nerves. Furthermore, this taste receptor cell-based biosensor is demonstrated to provide a promising platform for the investigation of extracelluar electrophysiology, taste sensation mechanism and taste encoding in a non-invasion manner. In this work, due to the low density of taste cell culture, the recordings were just from a single cell. A more direct way to study taste cell-to-cell communication was to culture a taste cell network on biosensor. Therefore, a method to obtain taste cell clusters is to be exploited.

4. Conclusion

In this work, a hybrid biosensor based on taste receptor cell was developed for the purpose of specific taste sensation based on taste firing encoding. Upon stimuli, extracellular temporal firing responses correlated with taste cells were recorded by this hybrid biosensor. The firing rate and temporal firing were analyzed. It showed firing rate was dose-dependent upon sour tastant. PCA analysis of the temporal firing upon exogenous ATP and sour stimuli demonstrated that information from different cell types could be distinguished. In addition, ATP had both enhanced and inhibitory effects on spontaneous firing, which was consistent with the recordings from patch clamp. This biomimetic taste receptor cell-based hybrid biosensor was potential and suitable for specific taste sensation and transduction mechanism, which could be realized in a non-invasive and convenient operation way.

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