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Dynamical behavior of lipid bilayer membranes for taste substances under random membrane-potential fluctuations

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Abstract

The dynamical behavior of the lipid bilayer membranes was experimentally studied under superposition of random or periodic membrane-potential fluctuations. The analysis of the mutual information has revealed that, in less than 10 Hz of random fluctuations, each of the time series of the mutual information of the transmembrane current for the five chemical substances (taste substances) has its inherent pattern, but not in a periodic fluctuation. On the other hand, the analysis of the power spectrum of the frequency could not distinguish those five basic taste substances in both random and periodic fluctuations. We provide the new detection idea of chemical substances by random fluctuations.

Keywords: Noise; Lipid bilayer membranes; Mutual information

1. Introductrion

Fluctuations, which are ubiquitous in real systems, have been the subject of various and extensive studies in nonlinear dynamical systems. In general, the effects of fluctuations can be quite difficult to predict and often yield counterintuitive behavior. The nonlinear dynamical systems exhibit a huge variety of noise-driven phenomena, ranging from a less ordered to a more ordered system dynamics. Prominent examples are the phenomena of noise-induced chaos [1,2], noise-induced order [3,4] or stochastic resonance [5]. Studies of fluctuations provide us the clue to the understanding of natural phenomena and help us to utilize the nonlinear dynamical systems [6–9].

A biological system is one of typical examples of nonlinear dynamical systems. Fluctuations have many roles in biological functions, including generation of errors in DNA replication leading to mutation and evolution, noise-driven divergence of cell fates and maintenance of the quantitative individuality of cells [10,11]. Especially, the recent studies suggest the possibility of fluctuation-supported signal transmission in neuronal tissue and other excitable biological media [9,12], i.e., stochastic resonance. This phenomenon is observed in the membrane potential of the biological membranes composed of proteins and lipid bilayer membranes. The biological membranes are the interface of cells for the outer circumstances and may have the functions that have still not been found.

It is very important for organisms to detect the chemical substances around themselves. In mammals, the detection of the chemical substances is performed in the biological membranes of taste and olfaction cells. Those studies have mainly been investigated from the point of view of protein receptors or ion channels [13–16], and have not much given attention to the lipid bilayer membranes. The lipid bilayer membranes are the skeletal structure of the biological membranes and have many functions. However, the effects of fluctuations for the lipid bilayer membranes have still not been revealed in detail. Therefore, in this paper, we investigate the new role of fluctuations in the lipid bilayer membranes. Especially, we focus on the dynamic behavior between the lipid bilayer membranes and the chemical

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substances under the membrane-potential fluctuations. Here, we used the five basic taste substances as the chemical ones.

2. Experimental section

In the present experiments, the planar lipid bilayer membranes [17,18] were used as a model of biological membranes, where proteins did not exist. The partially purified phosphatidylcholine (Type IV-S, Sigma) [19] and the buffer solution (50 mM Bis–Tris (Dojindo), 50 mM KCl (Wako), pH 7.3) or the 50 mM KCl solution were employed to form the planar lipid bilayer membranes. In order to investigate the dynamic behavior between the lipid bilayer membranes and the chemical substances, we used the five ones, acetic acid, D-glucose, L-glutamic acid, NaCl and quinine hydrochloride dihydrate (Wako, analytical grade), which were well-known as the five basic taste substances (acetic acid (sour), D-glucose (sweet), L-glutamic acid (umami), NaCl (salty) and quinine (bitter)) [13,14]. These taste substances were used without further purification.

The Gaussian distributed pulse noise (<ξ>(average of amplitude)=0 and σ (standard deviation of amplitude)=0.5 mV) and the sine wave (the amplitude was 5 mV) were applied to the planar lipid bilayer membranes. The Gaussian distributed pulse noise was added from the AD/DA converter (Contec AD16-16U(PCI)EH) and the sine wave was added from the function generator (Kenwood FG-273A). The frequencies were set at 100, 50, 20, 10, 5 and 1 Hz in the Gaussian distributed pulse noise and at 20, 10, 5 and 1 Hz in the sine wave. In the Gaussian distributed pulse noise, the frequency was fluctuated with σ of 20% of each frequency.

The planar lipid bilayer membranes were formed by the folding method across a 0.2 mm circular hole in a septum (thickness 25 μm) separating two compartments of a Teflon chamber (2 ml), where the 1.5 ml solution was added into each of the two compartments. The Ag–AgCl electrodes placed in the agar bridges (2% agarose (Wako), 3M KCl) was employed for the stimuli of the voltage and the measurement of the current. The temperature was kept constant at 25±1 °C.

In 30 min after the formation of the planar lipid bilayer membranes (0.6~0.8 μF/cm^2), the solutions of the taste substances were added into the one side of the two compartments of the Teflon chamber (10 μl in acetic acid, D-glucose and NaCl, and 100 μl in L-glutamic acid and quinine). The final concentrations of the taste substances in the compartment were all set at 10^{-2} M. Moreover, the same volume of the buffer solution was added into the other side to cancel the effects of the water pressure. Next, using the patch clump amplifier (Nihon Kohden CEZ-2400), we added the voltage fluctuations to the planar lipid bilayer membranes and measured the transmembrane current through the 500 Hz low pass filter. The data of the transmembrane current were recorded on PC. The sampling times of 100, 50, 20, 10, 5 and 1 Hz were set at 0.04, 0.04, 0.4, 0.4, 0.8 and 1 ms, respectively.

In the present study, we employed the mutual information for the dynamical analysis of the experimental data. In the next session, we describe the concept of the mutual information.

3. Mutual information

The mutual information, I, is usually discussed in terms of a signalling system. Consider a process in which messages are sent to an experimenter across the channel of his instruments. Let S denote the whole system which consists of a set of possible messages $s_1, s_2, s_3, \ldots, s_n$ and the associated probabilities $P_s(s_1), P_s(s_2), \ldots, P_s(s_n)$. If the possible messages are continuous, S denotes a possible message, and $P_s(s)$ is the probability density at s. And $P_{sq}(s_i, q_i)$ is the joint probability distribution of S and Q.

The mutual information $I(S, Q)$ measures the general independence of S and Q. The following definition holds

$$I(S,Q) = H(S) + H(Q) - H(S,Q), \tag{1}$$

where

$$H(S) = -\sum_i P_s(s_i)\log P_s(s_i), \tag{2}$$

$$H(S) = -\sum_{i,j} P_{sq}(s_i,q_i)\log\left[P_{sq}(s_i,q_j)\right]. \tag{3}$$

Thus, $I(S, Q)$ is the information from the individual $H(S)$ and $H(Q)$, reduced by $H(S, Q)$ obtained from both of them. When S and Q is completely independent, $I(S, Q)$ is zero.

In this paper, we calculated the dependence of $I(S, Q)$ on time using the theory and the algorithm described by Fraser and Swinney [20]. The time series of the mutual information, $I(t)$, means the information transmission from time t to $t+\Delta t$. Namely, when the values of the mutual information are small, the time series of the transmembrane current are random. As a result, we can investigate the dynamic behavior of the time series using $I(t)$.

4. Results and discussion

First, the Gaussian distributed pulse noise of 1 Hz was applied to the planar lipid bilayer membranes as fluctuations. The buffer solution (50 mM Bis–Tris and 50 mM KCl, pH 7.3) was used. Fig. 1 shows the time series of the Gaussian distributed pulse noise of 1 Hz and the transmembrane current of acetic acid. The tendencies of the time series of the transmembrane current for the other four taste substances were also similar to that of acetic acid. To investigate the interactions between the five basic taste substances and the planar lipid bilayer membranes under membrane-potential fluctuations, we calculated the

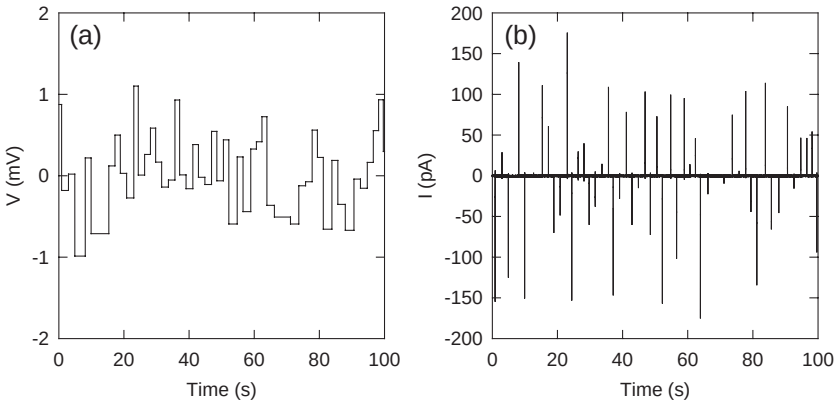

Fig. 1. Time series of the Gaussian distributed pulse noise and the transmembrane current. The buffer solution was used. (a) The Gaussian distributed pulse noise of 1 Hz and $\sigma = 0.5$ mV (standard deviation of the amplitude) with frequency fluctuation of $\sigma = 0.2$ Hz (standard deviation). (b) The transmembrane current of acetic acid for (a).

power spectrum of the frequency (FFT), $F(\omega)$, from the time series of the transmembrane current. Fig. 2 indicates the tendencies of $F(\omega)$ of the five basic taste substances and the blank solution (the buffer solution without the taste substances). All of the patterns of $F(\omega)$ are almost equal to that of the blank solution. Namely, the five basic taste qualities cannot be distinguished by $F(\omega)$.

The analysis of $F(\omega)$ does not take account of the time variation of the time series of the transmembrane current, i.e. dynamic behavior. Therefore, in the capacity of a means to investigate the dynamic behavior of the transmembrane current, we examined the time dependence of the mutual information of the time series of the transmembrane current [20]. Fig. 3 shows that the tendencies of $I(t)$ of acetic acid,

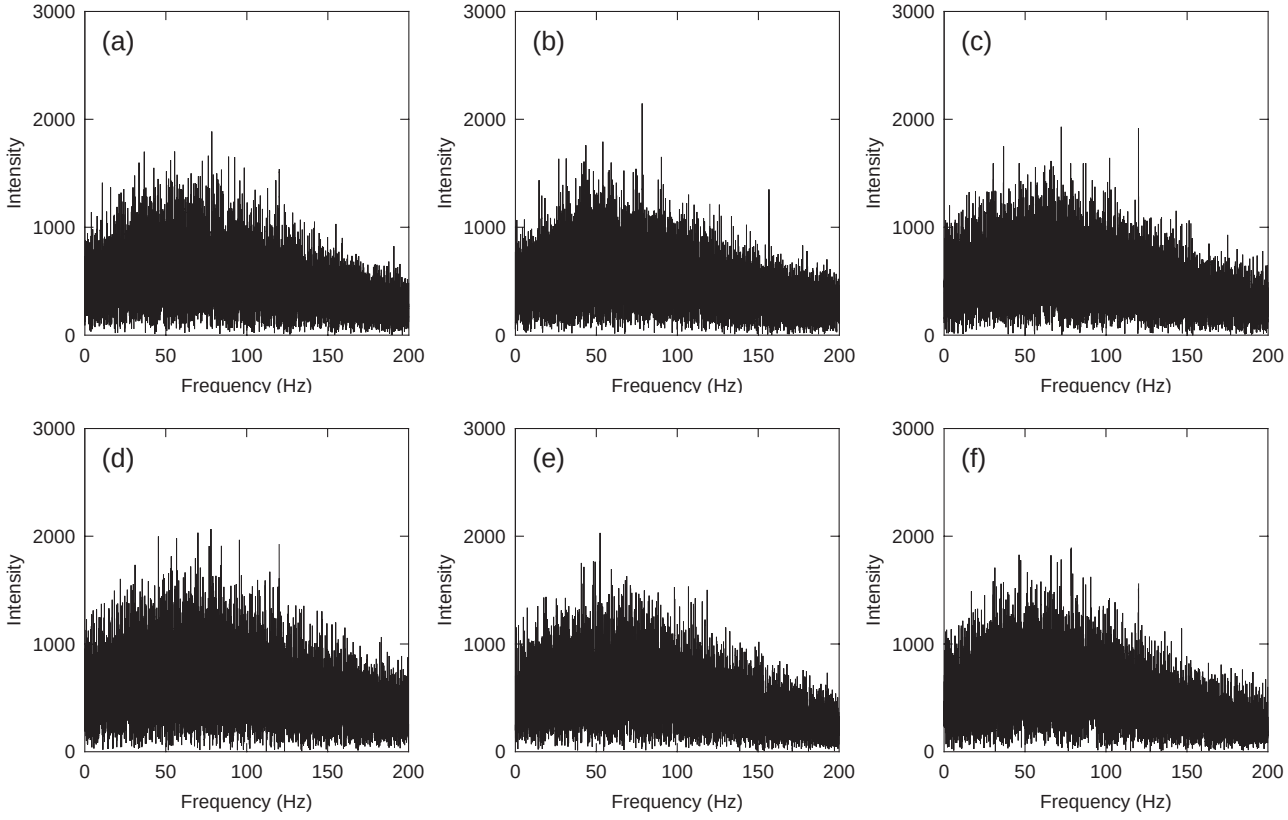

Fig. 2. Power spectra of the frequency for the five basic taste substances in the Gaussian distributed pulse noise of 1 Hz. The buffer solution was used. (a) Blank solution. (b) Acetic acid. (c) D-glucose. (d) L-glutamic acid. (e) NaCl. (f) Quinine. The power spectra were calculated from the data points of 32,768. The measurements were repeated three times.

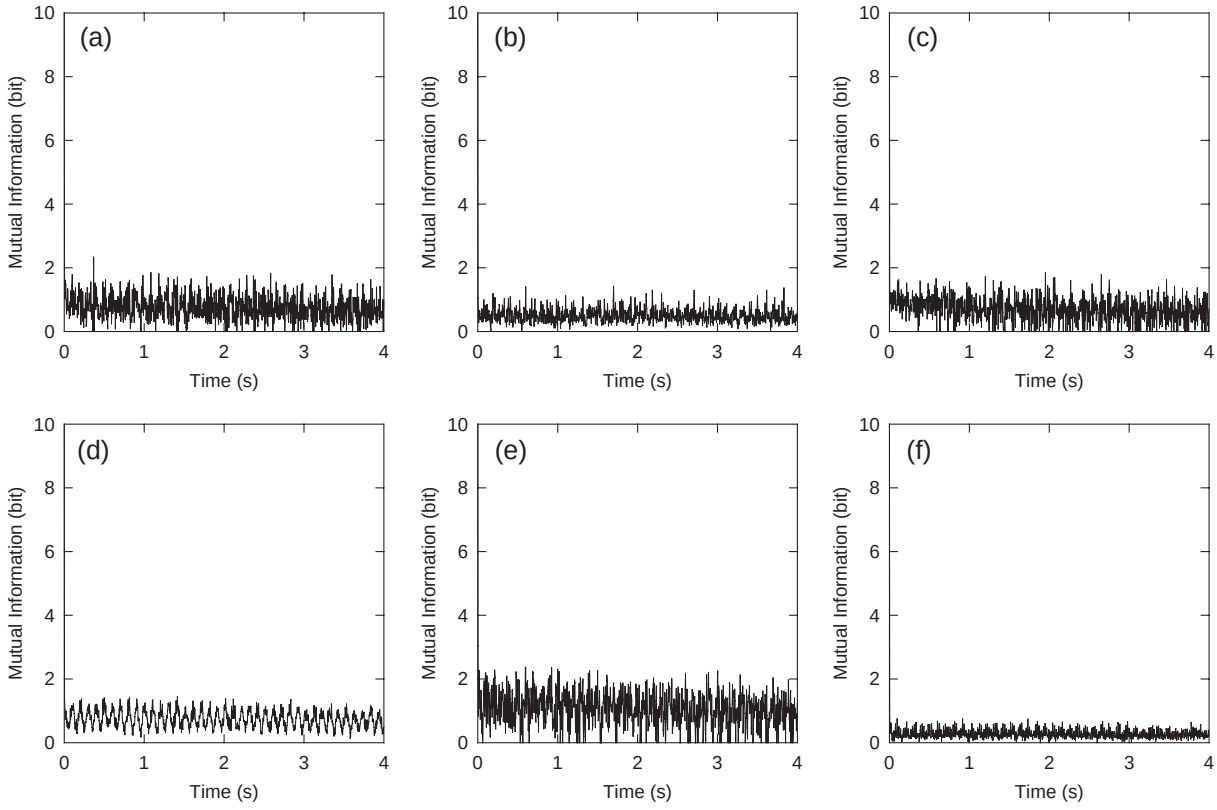

Fig. 3. Time series of the mutual information of the transmembrane current for the five basic taste substances in the Gaussian distributed pulse noise of 1 Hz. The buffer solution was used. (a) Blank solution. (b) Acetic acid. (c) D-glucose. (d) L-glutamic acid. (e) NaCl. (f) Quinine. The time series of the mutual information, $I(t)$, were calculated under the following conditions [20]: the number of data was 65,536, the confidence level for χ-square test was 20% and the maximum resolution was set at 20 bits. The average values of $I(t)$ for the taste substances are as follows: (a) 0.702. (b) 0.489. (c) 0.627. (d) 0.716. (e) 0.988. (f) 0.268. The measurements were repeated three times.

L-glutamic acid, NaCl and quinine are clearly different from that of the blank solution. To obtain further insights into those differences, the trajectories were constructed from the time-delay vectors of the time series of the mutual information, $(I(t), I(t+\Delta t))$ [21,22]. Each of the trajectories of acetic acid, glutamic acid and quinine is converged into its inherent attractor (Fig. 4). The trajectory of NaCl is more scattered than that of the blank solution. On the other hand, the trajectory of D-glucose is similar to that of the blank solution. However, the average value of $I(t)$ of the D-glucose is 0.627 bits (Fig. 3c) and that of the blank solution is 0.702 bits (Fig. 3a). The analysis of $I(t)$ suggests that the lipid bilayer membranes can sense the five basic taste qualities under the Gaussian distributed pulse noise.

Furthermore, to investigate the effects of the fluctuation frequency, 100, 50, 20, 10 and 5 Hz of the Gaussian distributed pulse noise were applied to the planar lipid bilayer membranes. In 10 and 5 Hz, we could sense the five basic taste qualities by $I(t)$, where the tendencies of $F(\omega)$ and $I(t)$ were the same as those of 1 Hz. On the other hand, in 100, 50 and 20 Hz, the patterns of $F(\omega)$ and $I(t)$ were all equal to those of the blank solution. In addition, the average values of $I(t)$ of the five basic taste substances were all equal approximately to that of the blank solution (1.5 bits in 100 Hz, 0.83 bits in 50 Hz and 0.35 bits in 20 Hz). These results suggest that the lipid bilayer membranes can distinguish the five basic taste qualities by $I(t)$ only in the frequency less than 10 Hz of the Gaussian distributed pulse noise.

We also investigated the behavior of the lipid bilayer membranes for periodic fluctuations. In the present study, the sine wave of 20, 10, 5 and 1 Hz were used. In all frequencies, each of power spectra of the transmembrane current of the five basic taste substances had only one peak corresponding to the applied voltage frequency, where the intensities of the one peak were all equal to that of the blank solution. Moreover, the tendencies of the trajectories and the average values of $I(t)$ were equal to those of the blank solution. Namely, the lipid bilayer membranes cannot sense the five basic taste qualities by a periodic fluctuation, i.e., sin wave.

In acetic acid, the behavior of the transmembrane current was examined in the 50 mM KCl solution under 1 Hz of the Gaussian distributed pulse noise. This attempt can reveal the pH effect. As a result, we have found that the pattern of $F(\omega)$ was the same as that of the buffer solution and that the tendencies of the time series and the trajectory of the mutual information were almost equal to those of the buffer solution, except that those were slightly scattered in comparison to those of the buffer solution. On the other hand, the average

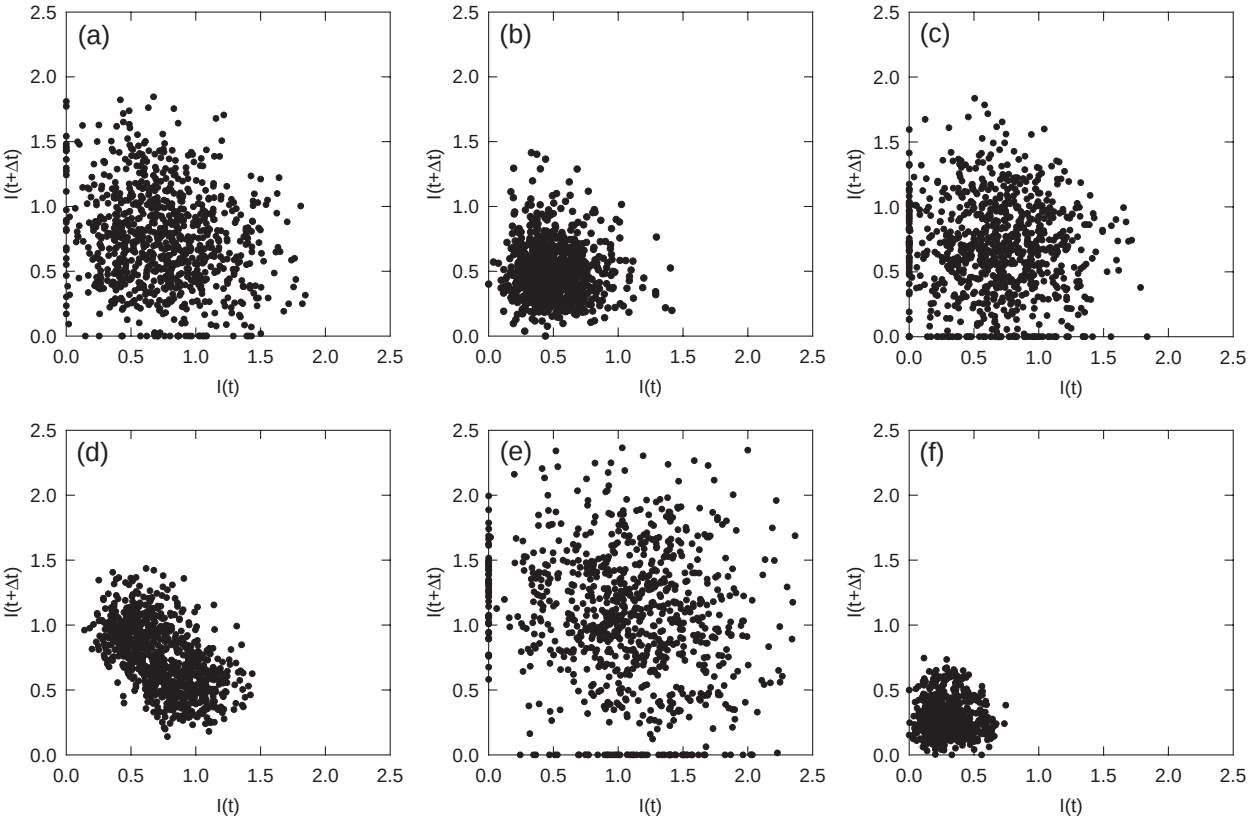

Fig. 4. Trajectories constructed from the time-delay vector of the time series of Fig. 3, ($I(t)$, $I(t+\Delta t)$). The value of Δt was set at 40 ms, which was appropriately chosen as the most distinguishable one [21]. (a) Blank solution. (b) Acetic acid. (c) D-glucose. (d) L-glutamic acid. (e) NaCl. (f) Quinine.

value of $I(t)$ was 0.593, which was larger than that of the buffer solution. That is, the decrease of pH generates the increase of the periodicity of the transmembrane current and leads to the scatter of $I(t)$.

5. Conclusion

In this paper, it has become clear that the lipid bilayer membranes can sense the five basic qualities by the Gaussian distributed pulse noise less than 10 Hz. Each of acetic acid, glutamic acid and quinine has its inherent attractor of the mutual information dissimilar to the blank solution. In NaCl, the scatter of $I(t)$ increases in comparison to that of the blank solution. In D-glucose, the average value of $I(t)$ decreases. It is obvious that these results are based on the distinction of the interactions between the lipid bilayer membranes and the taste substances. In acetic acid, glutamic acid and quinine, the analysis of $I(t)$ may reveal the distinction of interactions between the inner region of the lipid bilayer membranes and the three taste substances, and, in NaCl, may indicate the change of the electric charge of the interface between the lipid bilayer membranes and the solutions. In D-glucose, the decrease of the mutual information may result from the characteristics of the non-electrolytic substance, where the trajectory of the mutual information of D-glucose is similar to that of the blank solution. We are now investigating the detection mechanism of the five basic taste substances by the Gaussian distributed pulse noise in more detail. However, it is obvious that the detection of the five basic taste qualities is possible by the Gaussian distributed pulse noise, but not by the sine wave, and that $F(\omega)$ cannot distinguish the five basic taste substances. These results suggest that the randomness of membrane-potential fluctuations and the dynamic behavior of the transmembrane current are useful for the recognition of the five basic taste qualities by the lipid bilayer membranes.

There are inevitably fluctuations less than 10 Hz in biological systems. The analysis of $I(t)$ may reveal a part of the mechanism of the information transmission of the taste qualities in the taste system. The olfaction system may utilize the membrane-potential fluctuations also. The present results propose that the lipid bilayer membranes may enable us to develop an artificial taste sensor. Furthermore, the present method may be useful for the quality detection of the chemical substances other than the tastes ones.

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