Mechanisms Determining the Dynamic Range of the Bullfrog Olfactory Receptor Cell

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INTRODUCTION

Olfaction begins at the sensory cilia of the olfactory receptor cell (ORC) (Kurahashi 1989a; Kurahashi and Kaneko 1991; Lowe and Gold 1991). At the cilia, one type of odorant receptor protein is expressed exclusively in individual ORCs (Buck and Axel 1991; Chess et al. 1994; Ngai et al. 1993; Ressler et al. 1993; Serizawa et al. 2003; Vassar et al. 1993) and receives certain species of odorant molecules with relatively loose specificities (see e.g., Duchamp-Viret et al. 1999; Firestein et al. 1993; Touhara 2002). Binding of odorants to the receptor protein in turn activates adenylate cyclase via G protein. As a result, cytoplasmic cAMP concentration increases, which causes sequential openings of cyclic nucleotide-gated (CNG) cation channels (Kurahashi 1989a, 1990) and Ca²⁺-activated Cl⁻ channels (Kleene 1993; Kurahashi and Yau 1993; Lowe and Gold 1993; Reisert et al. 2003; Zhainazarov and Ache 1995). These two transduction channels are essential for olfactory perception in terms of two biological necessities; namely, stability of responsiveness under variable ionic conditions (Klee and Pun 1996; Kurahashi and Yau 1993) and making signal boosting expressing nonlinear amplification (e.g., Hill coefficient of 5) (Lowe and Gold 1993; Takeuchi and Kurahashi 2002, 2003; Takeuchi et al. 2003).

Openings of transduction channels cause a graded membrane depolarization that triggers self-regenerative action potentials (spikes) that transmit the odorant information to the olfactory bulb (Getchell 1977; Trotier and MacLeod 1983). It has been shown that the action potential is generated by openings of voltage-gated ion channels. Essentially, the rising phase is triggered by openings of Na⁺ channels, and the falling phase is shaped by the action of K⁺ channels (delayed rectifier and Ca²⁺-activated K⁺ channels: Firestein and Werblin 1987; Miyamoto et al. 1992; Schild 1989; Trotier 1986). Furthermore, it has been shown that T-type Ca⁺⁺ channels lower the threshold of spike generation (Kawai et al. 1996). After being coded into trains of action potentials, olfactory information regarding the strength of the stimuli is converted into the frequency of spike discharges (see e.g., Getchell and Shepherd 1978; Rospars et al. 2003; Trotier and MacLeod 1983). It has been reported that firing properties are influenced by passive membrane properties originally equipped in individual ORCs (Leinders-Zufall et al. 1995; Madrid et al. 2003; Trotier and Doving 1996).

Passing through these molecular and electrical chains, the odorant signal is processed to give necessary information selectively to the animal. When one considers about the odorant information regarding the strength of the stimuli, one of important factors is the dynamic range of individual ORCs; at oversaturating dose, cells cannot code the odorant intensity into biological information. Cells’ dynamic ranges are simply thought to be restricted by saturation of any steps of the signaling streams described above. There are several works that addressed to this question, but those experiments were performed with extracellular recordings (Rospars et al. 2003; Trotier 1994) or suction recordings (Reisert and Matthews 1999), which cannot monitor channel activities under the voltage-clamp condition. One aim of this study is to identify the limiting step determining the dynamic range throughout the stream of signal transmission. We applied the whole cell patch-clamp technique to ORCs in slice preparation and ex-
examined repetitive spike discharges that were caused by odorant stimuli. This allowed us to examine dose-response relations of both the transduction channel and spikes induced by the odorant in the same cells. As a result, saturating dose was found to be influenced largely by the transduction step, suggesting that signal saturation is essentially determined at the transduction system. However, the absolute dynamic range expressed slightly higher sensitivity in spike frequency when it was monitored under the current-clamp condition. In addition, a simple membrane model derived from the nonlinearity of the ORC membrane could reproduce the observed feature that the steepness of the depolarization-concentration curve became bigger as Hill coefficient was increased. It seems likely that a critical level of dynamic range is, at least in part, modified by the membrane nonlinearity.

Methods

Preparation

Olfactory epithelia were obtained from the bullfrog (*Rana catesbeiana*). The experiments were performed under the latest ethical guidelines for animal experimentation at Osaka University, based on international experimental animal regulations. In this study, we used slice preparation, because spike discharges were more frequently observed in slice preparation than in solitary cells (preliminary observation in our laboratory). Protocols obtaining slice preparation were similar to those reported previously by Imanaka and Takeuchi (2001). The animals were anesthetized by cooling on ice (0°C, 20 min) and pithed, both rostrally and caudally. Nasal cavities were opened and the olfactory epithelia were removed and washed with normal Ringer solution (in mM) 110 NaCl, 3.7 KCl, 1 CaCl2, 1 MgCl2, 10 HEPES, 15 glucose, and 1 Na-pyruvate. All solutions were adjusted to pH 7.2 with NaOH. The epithelia were kept in chilled normal Ringer solution until use (4°C). A small piece of the epithelium having no mechanical damages was selected and was cut into a rectangle shape (3 mm wide and 5 mm long). This piece of the olfactory epithelium was plated on a circular filter paper (Nitrocelulose, pore size 3.0 μm, 13 mm diam; Advantec Toyo, Tokyo, Japan). The filter paper with the epithelium was placed on a syringe filter holder (SX0001300, Millipore) with the surface side up. Suction was applied to the backward of the filter paper so that the epithelium was fixed to the chamber with a small amount of silicone grease (High Vacuum Silicone Grease, Dow Corning) at both ends of the paper was indispensable for making rigid preparations which were needed for identification of cell positions.

Whole cell recordings

Electrophysiological recordings were applied to cells in fresh slices. Patch pipettes were made of borosilicate tubing with filament (1.2 mm OD; 0.68 mm ID; World Precision Instrument) by using a two-stage vertical patch electrode puller (PP-830, Narishige Japan, Tokyo, Japan). The recording pipette was filled with K+ solution containing (in mM) 119 KCl, 1 CaCl2, 5 EGTA, and 10 HEPES (pH adjusted to 7.2 with KOH) or Cs+ solution containing (in mM) 119 CsCl, 1 CaCl2, 5 EGTA, and 10 HEPES (pH adjusted to 7.2 with CsOH). Lucifer yellow CH (0.2%) was dissolved in the pipette solution for identification of ORCs.

Prior to recordings, the debris on the surface of the slices was removed with a jet of normal Ringer solution ejected from a glass capillary placed near the slice. After cleaning, the whole cell recording configuration was established. For recordings, the tip of the whole cell pipette was always placed on the cell body. The pipette resistance was 15–20 MΩ.

The recording pipette was connected to a patch-clamp amplifier (Axopatch 200B, Axon Instruments, CA). The signal was low-pass filtered at 5 kHz, digitized by an A/D converter (sampling frequency, 10 kHz), and connected to a computer (Express 5800, NEC, Tokyo, Japan). Simultaneously, signals were monitored on an oscilloscope. Generation of the command voltages and data acquisition were controlled by pClamp software (version 8.0, Axon Instruments). The results were analyzed by an off-line computer and plotted using Microcal Origin 6.1 software (Origin Lab). Experiments were performed at room temperature (23–25°C).

Slice preparations were superfused with normal Ringer solution to remove stimulants and mucus supplied presumably from the supporting cells and the Bowman’s gland. The volume of the recording chamber was 2 ml. A reservoir (Falcon 352070, BD and Co.) containing the normal solution was placed in a higher position (300 mm height) than the recording chamber, and the fresh solution was continuously supplied from this tube by gravity. The solution in the chamber was flowed out positively by using the microtube pump (MP-3N, Tokyo Rikakikai Co., Tokyo, Japan). The external solution in the chamber was completely exchanged within 1 min.

Stimulation by the odorant

To stimulate ORCs, we used an independent manipulator (MO333, Narishige Japan) equipped with a puffer pipette having the same shape as the recording pipette. In a previous report, it has been shown that the dose threshold generating membrane responses in the ORC is about 1 μM (Firestein et al. 1993). With a higher concentration, it has also been shown that increasing the stimulus period (up to ~300 ms) is equivalent to the increase in the stimulus dose (Firestein et al. 1993; Takeuchi and Kurahashi 2002; Takeuchi et al. 2003). In this study, therefore the puffer pipette was filled with cineole dissolved in normal Ringers at 10 mM concentration, and the stimulus dose was regulated by changing the stimulus period with constant pressure (245 kPa).

Under the whole cell condition, the shape of the ORC under recording could be recognized with a fluorescent emission from Lucifer yellow CH that was diffused into the cell from the recording pipette. The tip of the puffer pipette was positioned near the knob estimated from Lucifer yellow visualization (see DISCUSSION). The time delay from the application of TTL command to the actual arrival of stimulant was measured with independent experiments by a change in the junction current. The delay, being 20 ms, was taken into consideration (see DISCUSSION).

Results

Resting potential and membrane properties of ORCs in slice preparation

Whole cell recording configuration was established on the ORC in slice preparation. Under the current clamp, most of cells were silent at rest and the resting membrane potential was −56.6 ± 12.0 mV (mean ± SD, n = 286; measured at 30–60 s after the rupture of the patch membrane). Twenty cells
showed spontaneous spike discharges without receiving any external stimuli. The resting membrane potential of these cells was measured when the cell was occasionally silent and was $-44.8 \pm 9.5 \text{ mV}$ ($n = 18$). It thus seems likely that cells showing spontaneous spikes have higher resting membrane potentials (see Discussion).

After measuring the resting membrane potential (and observing spontaneous discharges), the recording mode was switched to the voltage-clamp with the holding potential of $-90 \text{ mV}$. Under this condition, application of depolarizing voltage steps (ranging from $-80$ to $+50 \text{ mV}$; duration, $100$ ms) induced time- and voltage-dependent current components (Fig. 1A). Mean input resistance was $2.4 \pm 1.9 \text{ G\Omega}$ ($n = 306$). A transient inward current (reaching to a peak within $\sim 20$ ms) was induced by depolarizing voltage steps to more positive than $-40 \text{ mV}$. The maximum amplitude of the inward current was $-629.6 \pm 256.0 \text{ pA}$ ($n = 306$, measured at $-30$ or $-40 \text{ mV}$). The transient inward current was observed only once at the beginning of the step pulse. It thus seems likely that ORCs were quite well space clamped (compare with data obtained by Ma et al. 1999). The inward current was followed by a slowly activated outward current that increased monotonically up to $+50 \text{ mV}$ (amplitude: $960.8 \pm 503.7 \text{ pA}$, at $+50 \text{ mV}$, $n = 306$). Current-voltage ($I-V$) relationship (Fig. 1B) showed nonlinear properties, and its shape was essentially the same as those obtained in previous works done on ORCs under dissociation (Firestein and Werblin 1987; Kawai et al. 1996; Kurahashi 1989b; Lynch and Barry 1991; Miyamoto et al. 1992) and on ORCs in slice preparation (Imanaka and Takeuchi 2001).

### Spiking properties by current injection under the current-clamp condition

Among 306 cells, 154 ORCs were subjected for current injection ($\leq 20 \text{ pA}$ in 66 cells, $\leq 100 \text{ pA}$ in 88 cells, 1-s duration) under the current-clamp condition. Ninety-five of 154 ORCs showed only one action potential. Fifteen of 154 cells showed only one action potential. Remaining 44 cells did not show any action potentials, even with a very strong stimulus. There was a possibility that spiking abilities may be correlated with cell damages during slicing protocols. In this study, however, we could not recognize the axon of cells under recordings, presumably because of the limited spatial resolution of the optical system.

To quantify repetitive firings induced by current injection, firing frequencies were analyzed with varied stimulus strengths. In this study, the firing frequency was defined as the averaged frequency during the firing period. Figure 2B shows the relation between the firing frequency and amount of injected current. As the current was increased, the firing frequency of the spikes increased. At strong intensities, however, the curve expressed nonlinearity, showing a saturating feature (see also Imanaka and Takeuchi 2001). The maximum frequency was $36.2 \pm 7.1 \text{ Hz}$ at $100 \text{ pA}$ ($n = 47$).

The observation that spiking system in the ORC expressed saturation brought up a possibility that such saturation at the spiking level might specify the dynamic range of the olfactory signaling process. This possibility will be investigated later in further detail.

### Voltage and current responses induced by the odorant

Odorant stimulation with cineole (10 mM in the puffer pipette) caused depolarization under the current-clamp and an inward current under the voltage-clamp (holding potential $=-60 \text{ mV}$, Fig. 3B). The amplitude of the inward current was $-132.5 \pm 100.7 \text{ pA}$ (at $-60 \text{ mV}$, $n = 54$; see Fig. 3B).

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**FIG. 1.** Voltage-induced currents of the olfactory receptor cell in slice preparation. **A:** whole cell membrane current recorded under the voltage-clamp condition. Holding potential was $-90 \text{ mV}$. Depolarizing voltage steps ($-80$ to $+50 \text{ mV}$) were applied with a constant duration (100 ms). Upward deflection of the top trace indicates the timing and duration of stimulation (step duration = 100 ms). **B:** current-voltage ($I-V$) relationship of the olfactory receptor cell (ORC) membrane. Each symbol represents the mean value obtained from 306 cells. Error bars show the SD. $\bullet$, outward current measured at 100 ms after the application of voltage steps; $\blacksquare$, peak of inward currents.
Response probability was 17.6% (54 of 306 cells). The response latency was 376.3 ± 33.8 ms (n = 54) and was relatively longer than that observed in experiments using isolated receptor cells (200–300 ms, Firestein and Werblin 1989; Kurahashi 1989a; Takeuchi et al. 2003).

Of these 54 cells, 20 cells showed repetitive spikes in response to odorant stimulation under the current-clamp condition (Fig. 3A). Seven cells showed a single spike. The remaining 27 cells did not show any spikes during the graded receptor potential. All cells expressing repetitive firings in response to odorant stimulation also induced repetitive spikes with current injection (examined in 11 cells). Vice versa, odor-sensitive cells that failed to initiate spikes with odor stimuli did not show any spikes with current injection (n = 5).

The I-V relation of the odorant-induced conductance was almost linear when measured with ramp clamp (Fig. 3C). The reversal potential and slope conductance were -1.39 ± 0.71 mV (n = 3) and 1.9 ± 0.7 nS (measured at 0 mV; n = 3), respectively. The results were consistent with previous data obtained from isolated ORCs (e.g., Kurahashi 1989a).

Relation between stimulus intensity and firing frequency

It is generally known that ORCs encode the intensity of odorant stimuli into the firing frequency and transmit this information to the olfactory bulb. In this study, we examined the relation between the odorant dose and firing frequency using slice preparation. For regulating odorant dose in this study, we changed the duration of the stimulus pulse applied to the solenoid valve. It has been known that increasing the stimulus period is equivalent to the increase in the dose (Firestein et al. 1993; Kurahashi and Menini 1997; Takeuchi et al. 2003).

![Image](http://jn.physiology.org/)

**FIG. 2.** Spiking activities induced by current injection. A: spike activities induced by current steps (0–100 pA, 1-ms duration). Note that the frequency of the repetitive discharges increases as the size of injected current was increased. Even at 80 or 100 pA, there were 2 spikes that allowed us to obtain the firing frequency. B: relationship between the firing frequency and the amount of the injected current. Each symbol represents the mean value obtained from 44 (●), 91 (●), and 47 cells (●), respectively. Error bars show the SD. Average of the inverse of the interspike intervals during the current step was measured as the frequency (F). Smooth line was drawn by least square fitting of the data points by the Michaelis-Menten equation, 
\[ F = \frac{F_{max}I_{inject}}{I_{inject} + I_{1/2}} \]
where \( I_{inject} \) is the intensity of injection and \( I_{1/2} \) is the half-maximum intensity. \( F_{max} \) = 49.5 Hz, \( I_{1/2} \) = 33.1 pA.

**FIG. 3.** Voltage and current responses induced by odorant stimulation. A: voltage response induced by cineole (10 mM in the puffer pipette) under the current-clamp condition. Odorant stimuli induced a slow depolarization and in turn initiated repetitive spikes. Downward deflection of the top trace indicates the timing and duration of the odorant stimulation (stimulus period = 50 ms). K⁺-pipette solution. B: current response induced under the voltage-clamp condition. Same cell as in A. Holding potential was -60 mV. Downward deflection of the top trace indicates the timing and duration of the odorant stimulation (stimulus period = 200 ms). C: voltage dependence of the odorant-induced current of the ORC in slice. Ramp clamp (ramp rate = 400 mV/s) was applied to the ORC responding to cineole. Voltage-dependent current observed in the absence of the odorant was subtracted. Recording pipette was filled with Cs⁺-pipette solution.
and Kurahashi 2002; Takeuchi et al. 2003). In this method, critically, the stimulus dose does not represent the linear function with pulse duration. However, in the following experiments, such nonlinearity does not influence the interpretation of data, except for obtained biophysical parameters (e.g., Hill coefficient, see Fig. 4B).

In a cell shown in Fig. 4, 8 ms (pressure, 245 kPa) of stimulus was sufficient to induce a depolarization accompanied by a spike discharge. As the stimulus period was increased (≥80 ms), the time latency for triggering the first spike became shorter and the following spikes were discharged more frequently. In Fig. 4B, the firing frequency was plotted against the stimulus period. Relation between the firing frequency and the stimulus intensity showed a sigmoidal feature and could be fitted by the Hill equation. The same result was obtained from nine other cells that showed repetitive firings (11 cells that showed response run-down were omitted from the analysis). The average of maximum frequency observed in individual ORCs was 24.8 ± 7.8 (SD) Hz (n = 9). The shortest stimulus period causing spikes differed between cells widely, and it ranged from 8 to 100 ms, reflecting the heterogeneous responsiveness of cells to cineole. It is also possible to think that variations observed in sensitivities are due to irregular efficiency of odorant arrival to the cilia in the experiment on slice preparation (see DISCUSSION).

**Molecular site determining the system saturation**

Through the aforementioned experiments, we recognized that both the transduction site and the spiking system express saturation. Now, the question is what step(s) actually limits the dynamic range of final output of the ORC.

To identify the molecular step(s) determining the dynamic range, we measured dose dependences of both the transduction current and spikes induced by the odorant in the same cells. Figure 5 shows dose dependences of the transduction current and spike frequency obtained from two different preparations. Both cells expressed different sensitivities to applied cineole, displaying parallel shifts of the dose dependence. It is noticeable to see that the dose dependences of spike discharges are shifted essentially depending on the change in the sensitivity of cells. The width of dynamic range for the spikes is essentially the same as that of transduction current. These results suggest that the saturation of the signaling process is essentially determined by the transduction current. In other words, it is likely that the ORC uses the linear region of the spiking system. To support this idea further, we observed that the maximum frequency of the spikes induced by the odorant stimulation (25 Hz) was lower than the saturating frequency of spikes induced by current injection (36 Hz).

However, there were slight deviations in the absolute position of the dose dependence between the transduction current

![Fig. 4](http://jn.physiology.org/)

**FIG. 4. Relation between firing frequency and odorant dose. A**: cineole stimuli were applied to ORC while membrane potentials were recorded. Dose of the odorant stimulation was controlled by manipulating duration of the stimuli (8–80 ms). **B**: smooth line was drawn by least square fitting of the data points by the Hill equation, \( F = F_{\text{max}} P^n/(P^n + K_{1/2}^n) \), where \( F \) is the firing frequency, \( P \) is the stimulus period, \( K_{1/2} \) is the half-maximum period, and \( n \) is the Hill coefficient. \( F_{\text{max}} = 22.8 \text{ Hz}, K_{1/2} = 11.5 \text{ ms}, n = 4.9 \). Since in our puff protocols, the actual dose of stimulant is assumed to be a function of stimulus period superlinearly, this \( n \) value could be overestimation. **Inset**: expanded data displaying spiking activities. Data from A. Input resistance was 6.7 GΩ.

![Fig. 5](http://jn.physiology.org/)

**FIG. 5. Dose dependence of the transduction current and the spikes induced by the odorant in the same cells. Normalized transduction current (○) and firing frequency (●) obtained from 2 different cells. Each relation was fitted by the Hill equation. Filled and empty symbols show data from different cells. Fitting values for each curve were \( K_{1/2} = 12.0 \text{ ms}, n = 8.3 \text{ (●),} K_{1/2} = 13.7 \text{ ms,} n = 9.7 \text{ (●),} K_{1/2} = 28.4 \text{ ms,} n = 4.1 \text{ (●), and} K_{1/2} = 48.5 \text{ ms,} n = 3.8 \text{ (●). Note that the high-sensitive cell displayed with ○ or ● shows saturation at lower pressure level than the low-sensitive cell (□ or □). This indicates that response saturation is independent from the saturation in the puffer system.**
and spikes. The dose dependence of the spike frequency actually showed slightly higher sensitivity than that of the transduction current in all cells examined \((n = 4)\). This slight difference could be explained by two possibilities. First, the adaptation state of the transduction channels could be different between two experimental conditions; \(\text{Ca}^{2+}\) influx through the CNG channels would be higher under the voltage-clamp condition than under the current-clamp condition. Second, membrane nonlinearity could attribute to the shift of the dose-dependence (see following section). Similar ideas have been also proposed by previous works (loose patch, Trotier 1994; suction, Reisert and Matthews 1999; simulation model, Rospars et al. 2003). This possibility is examined later.

**Odorant-induced depolarization predicted from nonlinear membrane model**

One may still feel it very puzzling that the receptor potential is only \(<40\, \text{mV} \) depolarization, whereas the odorant-induced current exceeds 100 pA. Since the input resistance of the ORC is about 5 G\(\Omega\), simple calculation estimated from Ohm’s law would give much bigger voltage changes. In fact, the experiments of Fig. 2 showed that <100 pA of current injection caused subsaturation in the spiking frequency (see also, Imanaka and Takeuchi 2001).

This question could be simply explained by the fact that the odorant-activated conductance has a reversal potential near 0 mV (Fig. 3C) and that olfactory receptor cell membrane shows nonlinear \(I-V\) relations (Fig. 1B, see APPENDIX). It is therefore reasonably considered that membrane depolarization caused by the transduction channel never exceeds 0 mV, even with a conductance increase to the infinity (see Fig. 6). In contrast, current injection just simply shifts the membrane \(I-V\) relation to downward (see Fig. 7). Therefore membrane depolarization induced by current injection becomes much bigger than that induced by the odorant stimulation (Fig. 7B).

Using this simple model, we compared dynamic ranges of the transduction current obtained under the voltage-clamp

![FIG. 6. Degree of odorant-induced depolarization obtained from the nonlinear membrane model. A: change in membrane \(I-V\) curve when the transduction channels are open. The Hill coefficient of \(I_{\text{odor}}\) was set to 4. Odorant concentration \(S\) was changed from 0 to 61.3. Stimulation was normalized to the \(K_{1/2}\) of odorant-induced current (see APPENDIX). The membrane property of the ORC was expressed as a mixture of the steady-state currents and leak currents. The steady-state current \((I_{\text{steady}})\) was expressed by the Boltzman function as \(I_{\text{steady}} = A_2 + (A_1 - A_2)[1 + \exp(VV_2 - V)(V_2)]\), where \(A_1, A_2, V_1,\) and \(V_2\) are the constants obtained by fitting to the data of Fig. 1B and \(V\) is the holding potential. \(A_1 = -45.0, A_2 = 1,200.3, V_1 = 21.0,\) and \(V_2 = 21.1.\) (For detail, see APPENDIX). B: depolarization-stimulation curve. Estimated depolarization was plotted against the stimulation normalized to the \(K_{1/2}\) of odorant-induced current (Eq. 1, see APPENDIX). Smooth line was drawn by the least square fitting of data point by the Hill equation, \(D = D_{\text{max}}S^{n}_{\text{odor}}(S^{n}_{\text{odor}} + K_{1/2}),\) where \(D\) is the depolarization, \(S_{\text{odor}}\) is the normalized intensity of odorant stimulation, and \(K_{1/2}\) is the half-maximum stimulation. \(D_{\text{max}} = 19.4\, \text{mV}, K_{1/2} = 0.80,\) and \(n = 3.83.\) C: normalized transduction current curve and depolarization curve. Both curves were fitted by the Hill equation. Fitting values for each curve were \(K_{1/2} = 0.80, n = 3.83 (\bullet)\) and \(K_{1/2} = 1.0, n = 4.0 (\bullet).\)

![FIG. 7. Difference in the size of depolarization between current injection and odorant-induced conductance. A: calculated change of the membrane \(I-V\) relation induced by current injection \((0-100\, \text{pA}, \text{every} 10\, \text{pA})\). The membrane \(I-V\) relation was described as in Fig. 6. Current injection was expressed as a parallel shift of the \(I-V\) relation. B: comparison of the depolarization induced by current injection and odorant stimulation. Note that depolarization induced by current injection becomes bigger than that induced by odorant stimulation.](http://jn.physiology.org/)

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condition and that of the membrane depolarization. Based on the model, the relation between the membrane depolarization and the odorant dose could be expressed as a sigmoidal function, and was fitted by the Hill equation (Fig. 6B). The dose dependence of the depolarization was shifted to a higher sensitivity than that of the transduction current as predicted before (Fig. 6C).

In the model, we could also examine the effect of changed Hill coefficients (from 2 to 6) on the depolarization-stimulation curve (Fig. 8). It is noticeable to see that the steepness of the depolarization-stimulation curve becomes bigger as Hill coefficient is increased. These results indicate that the dynamic range of ORCs is set to a narrow region by the nonlinear amplification equipped in the olfactory transduction machinery.

We also tried to obtain the relation between the spike frequency and the increase of the membrane conductance, referring to the Hodgkin-Huxley model (Hodgkin and Huxley 1952). However, we could not obtain repetitive spikes in the model when we used parameters that express membrane currents measured under the voltage-clamp condition. At this point, unfortunately, it is not understood whether this failure is due to inappropriate choice of parameters or due to other unknown reasons.

Discussion

In this study, we recorded the train of action potentials that were induced by odorant stimulation to ORCs in the frog slice. Using slice preparation, the input-output relation of the ORC was investigated by checking at intermediate points through the olfactory signal processing. By examining dose-response relations of both the transduction current and spikes induced by the odorant in the same cells, it was suggested that the final output was fundamentally determined by the transduction step. It is likely, however, that the crucial level of the dynamic range is influenced by membrane nonlinearity. In addition, it was confirmed that the steepness of the depolarization-concentration curve became bigger depending on Hill coefficient of the transduction system. It is therefore likely that the dynamic range of ORCs is set to a narrow region by the nonlinear amplification equipped in the olfactory transduction machinery.

Action potential generated by current injection

In this study, we could observe repetitive action potentials in ORCs embedded in olfactory epithelium. The firing frequency increased as injected current (or odorant dose) was increased, as has been shown in previous works (Firestein and Werblin 1987; Imanaka and Takeuchi 2001; Kurahashi 1989b; Masukawa et al. 1985a,b; Trottier 1994). However, it is also reported that repetitive firings frequently abolishes in ORCs after dissociation (Kurahashi 1989a). The reason why most of solitary receptor cells do not generate repetitive action potentials is still a matter for speculation. The most likely possibility is the injury of the axon. Many of solitary receptor cells just retain very short axon (±10–20 μm). Even with slice preparation, 29% of cells examined current injection did not show repetitive firings. It is possible that the axon of these cells has been injured during the slicing processes. Unfortunately, however, we could not recognize the entire length of the axon in cells under recordings with our optical system. Further systematic study which employs visualization of axons would be necessary to examine this question.

In a previous work by Imanaka and Takeuchi (2001), it was concluded that the relation between the injected current and the spike frequency showed a linear property. We confirmed, however, that this interpretation needs to be modified. The resting potential of the ORC is present around −70 to −80 mV (after compensation of the seal resistance during recording; Kawai et al. 1996). However, the threshold of voltage-gated Na+ channels and T-type Ca2+ channels that trigger action potential generation is around −50 mV (Fig. 1B). Therefore it is reasonably assumed that the ORC generates the action potential only when the membrane potential is depolarized by 20 to 30 mV. Because of this, the input-output relation must express a nonlinear region between the resting state and the threshold. Furthermore, this study showed that injection of large current caused almost complete saturation in the spike frequency (Fig. 2B). Coupling both the input-output relation at the spiking step is thought to express a sigmoidal function.

However, the nonlinear region around the resting state may be negligible in terms of the total signal processing, because only a very small current (a couple of picoamperes) is sufficient to induce the action potential. The current induced by odorant frequently exceeds 100 pA. In addition, high cooperativity equipped in the transduction system provides a dramatic increase in the transduction current by a very small change in the odorant concentration. This property would simply cancel the nonlinearity of the spiking system around the resting membrane potential.

Odorant application to the ORC in slice preparation

In this study, odorant stimulation was applied to the ORC in slice preparation. The size of the inward current response under the voltage clamp was almost the same as that reported for the ORC under dissociation (Firestein et al. 1993; Kurahashi 1989a). However, response latency (376 ms) was obviously longer in this study than that observed in previous studies that used solitary cells (200–300 ms, Firestein and Werblin 1989; Kurahashi 1989a; Takeuchi et al. 2003).

These discrepancies are not due to the difference in the pressure ejection system. The time delay needed for drug
application after the opening of the electromagnetic valve has been measured to be 20 ms (monitored by the junction current). Actually, this value could be derived from simple calculation using Bernoulli’s principle when it was applied to the geometry of our pressure ejection system. The obtained 20 ms delay cannot explain the difference in the response latency between our experiments and previous data.

It thus seems likely that the longer latency observed in these experiments attributes to the use of slice preparation. In case of slice preparation, there is a possibility that the stimulant does not arrive directly to the site of the olfactory transduction (olfactory cilia). For instance, olfactory mucus may be the cause of this delay. Other debris in slice preparation may also be a reason for this response delay. Also, it is possible that the position of the pipette tip has been simply far from the knob (and therefore far from the cilia) that was unclear in our optical views.

Spontaneous spiking of the ORC

In this study, we saw that 6.5% of cells (20 of 306 cells) showed spontaneous spiking under the current-clamp condition. These cells showed relatively positive resting membrane potential than that obtained from silent cells. It is highly likely that the positive resting potential triggers spike discharges with a very slight potential drift. In a previous study using intracellular recordings, it was reported that 67% of ORCs showed spontaneous spiking (Trotier and MacLeod 1983). Our result may suggest that in slice preparation the spiking ability of the ORC is smaller than that observed in the in vivo preparation; cells may have been, in part, affected by the slicing procedure. On the other hand, however, it is possible that in intracellular recordings cells are depolarized by the leakage current that is caused by injection of the microelectrode. This will simply cause the increase in the number of cells showing spontaneous discharges.

Input-output relation of the ORC derived from both physiology and the nonlinear membrane model

The ORC model that was constructed based on experimental data allowed us to estimate the degree of odorant-induced depolarization. According to calculation, relation between the degree of depolarization and odorant concentration shows saturation (and therefore fitted by the Hill equation). This tendency was essentially the same as data obtained with physiological experiments. The model also allowed us to examine the role of high cooperativity that is equipped in the transduction system. It has been believed that the high cooperativity of the olfactory transduction system is actually achieved by the sequential openings of CNG channels and Ca^{2+}-activated Cl^- channels (Lowe and Gold 1993; Takeuchi and Kurahashi 2002). With a nonlinear membrane model, it was confirmed that the rising phase of the depolarization-stimulation curve became steeper as Hill coefficient was increased. Consequently, it is supposed that the dynamic range of ORCs is set to a narrow region by a nonlinear amplification equipped in the transduction system.

Appendix

In this section, we describe a simple mathematical model for explaining the differences between membrane depolarizations induced by conductance increase and by extrinsic current injection. The degree of depolarization can be obtained by the shift of the I-V curve. The membrane properties were obtained by the experiment of Fig. 1B. To calculate this V-intercept, we used the following concepts.

As has been shown by experiments with the voltage clamp, \( I_{\text{odor}} \) is fitted by the Hill equation (for example, see Firestein et al. 1993; Lowe and Gold 1993; Takeuchi and Kurahashi 2002)

\[
I_{\text{odor}} = I_{\text{max}} \frac{S^n}{S^n + K_{\text{H}}^{n/2}}
\]

where \( I_{\text{max}} \) is the maximal current response, \( S \) is the odorant stimulation, \( K_{\text{H}} \) is the odorant stimulation necessary to activate one-half of the maximal current, and \( n \) is Hill coefficient. To express the current size in our calculation, parameters were obtained from physiological data (\( K_{1/2} \) = 16.3 ms and \( I_{\text{max}} = -132.5 \) pA).

For describing the steady-state I-V relation of the ORC membrane, we used \( I_{\text{steady}} \) as a mixture of \( I_{\text{Kv}} \) and leak current. \( I_{\text{steady}} \) was obtained by fitting the result of Fig. 1B with the Boltzmann function

\[
I_{\text{steady}} = \frac{A_1 - A_2}{1 + \exp \left( \frac{V - V_1}{V_2} \right)} + A_2
\]

where \( A_1, A_2, V_1, \) and \( V_2 \) are the constants obtained by fitting to the data of Fig. 1B, and \( V \) is the membrane potential. We adopted \( A_1 = -45.0, A_2 = 1.200,3, V_1 = 21.0, \) and \( V_2 = 21.1, \) respectively.

Since odorant-activated conductance has a reversal potential near 0 mV (Fig. 3C), the final current (\( I_{\text{final}} \)) and voltage relation is represented as the equation as follows

\[
I_{\text{final}} = I_{\text{steady}} + I_{\text{inj}} \frac{V}{V_h} = \left[ \frac{A_1 - A_2}{1 + \exp \left( \frac{V - V_1}{V_2} \right)} + A_2 \right] + \left( \frac{I_{\text{max}} S^n}{S^n + K_{\text{H}}^{n/2}} \right) \frac{V}{V_h}
\]

where \( V_h = -60 \) mV. This parameter was also obtained from the physiological experiment.

In comparison with odorant-induced depolarization, depolarization induced by current injection is described as follows

\[
I_{\text{final}}' = I_{\text{steady}} + I_{\text{inj}} \left( \frac{A_1 - A_2}{1 + \exp \left( \frac{V - V_1}{V_2} \right)} + A_2 \right) + I_{\text{inj}}
\]

where \( A_1, A_2, V_1, \) and \( V_2 \) are the constants obtained by fitting to the data of Fig. 1B, \( V \) is the membrane potential, and \( I_{\text{inj}} \) is the injected current. For parameters, we adopted the same numbers described above. Thus depolarization induced by current injection is determined by the parallel shift of I-V relation to downward direction (Fig. 7A). Therefore current-induced depolarization becomes infinite when the amount of injected current becomes infinity. In this aspect, both depolarizing phenomena must be considered in different ways in terms of the amount of the current. In fact, the depolarization is much smaller when it is caused by the odorant-induced conductance increase (Fig. 7B). This sometimes makes qualitative confusion when one simply considers about the dose-response relation caused by current injection and odorant-induced depolarization.

Finally, odorant-induced depolarization (\( \Delta V \)) can be described with the following formula with \( V_{\text{shift}} \) that is a V-intercept derived from the Eq. 3

\[
\Delta V = V_{\text{shift}} - V_0
\]

where \( V_0 \) is a V-intercept of the I-V relation when \( I_{\text{odor}} = 0 \).

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REFERENCES


