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# ACTIVATION BY ODORANTS OF CATION-SELECTIVE CONDUCTANCE IN THE OLFACTORY RECEPTOR CELL ISOLATED FROM THE NEWT

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SUMMARY

1. Ionic selectivity of the conductance activated by *n*-amyl acetate (odorant-activated conductance) was analysed in isolated olfactory receptor cells under the whole-cell voltage clamp condition.

2. Solitary receptor cells had a resting membrane potential of $-44{\cdot}7 \pm 7{\cdot}0$ mV (mean $\pm$ S.D.; $n = 70$). Application of 10 mM-*n*-amyl acetate caused a depolarizing response in about 30% of the cells. Sensitivity to the odorant was maximum at around the apical dendrite.

3. Odorant induced an inward current to cells voltage clamped at their resting potential and bathed in the standard medium. The response amplitude was voltage dependent, and the polarity reversed at $+2{\cdot}5 \pm 2{\cdot}2$ mV ($n = 6$). The *I–V* relation was almost linear at membrane potentials more positive than $-20$ mV, with an average slope of $3{\cdot}14 \pm 1{\cdot}59$ nS (measured at 0 mV), but showed a marked outward rectification at voltages more negative than $-30$ mV.

4. Removal of external $Ca^{2+}$ increased the amplitude of the odorant-induced current and prolonged response duration, but did not cause a significant change on the reversal potential. Thus, $Ca^{2+}$ affected the kinetics of the conductance, but did not seem to be a dominant charge carrier in the physiological condition.

5. Reduction of external $Na^+$ concentration ($[Na^+]_o$) (replaced with choline) shifted the reversal potential by about 57 mV per 10-fold change of $[Na^+]_o$. Removal of external $Cl^-$ (replaced with glutamate ions) did not affect the reversal potential.

6. The odorant-activated conducting channels were permeable to all alkali metal ions. The permeability ratios were; $P_{Li}:P_{Na}:P_K:P_{Rb}:P_{Cs} = 1{\cdot}25:1:0{\cdot}98:0{\cdot}84:0{\cdot}80$.

7. The present study strongly suggests that the olfactory receptor potential is generated by an increase in the membrane conductance to alkali metal ions.

## INTRODUCTION

Olfactory stimulation generates slow and graded depolarization in primary receptor cells. The depolarizing receptor potential in turn triggers action potentials which travel directly to the brain without being modified by synaptic interactions

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with neighbouring receptor cells (Getchell, 1977; Suzuki, 1977; Trotier & MacLeod, 1983; Masukawa, Hedlund & Shepherd, 1985).

It has been suggested that the receptor potential is produced by an increase in the membrane permeability to cations, since the polarity of the receptor potential is reversed by a strong depolarization of the membrane potential beyond 0 mV (Trotier & MacLeod, 1983). Further, indirect evidence has been presented from the studies of the effects of various ions on the electro-olfactogram (EOG), a mass response of olfactory cells in the mucosa (Tucker & Shibuya, 1965; Takagi, Wyse, Kitamura & Ito, 1968), or on evoked potentials at the olfactory bulb (Yosii & Kurihara, 1983), but the conclusion differs among reports. Diffusion barriers and other complications accompanying *in vitro* preparation might have been responsible for these disagreements.

Recently, enzymatic dissociation of the olfactory mucosa enabled us to obtain isolated olfactory receptor cells from amphibian olfactory epithelium. It has been shown that application of odorant stimuli depolarizes isolated olfactory receptor cells (Kurahashi & Shibuya, 1985, 1986*a*, 1989; Trotier, 1986; Anderson & Hamilton, 1987). The present study was carried out to further our understanding of the ionic mechanisms underlying the olfactory receptor potentials. Solitary receptor cells were voltage clamped and stimulated by odorant under various ionic environments. The present results show that the receptor potential is generated by an increase in the membrane conductance to alkali metal ions with low selectivity, but not to anions.

## METHODS

### *Materials*

Solitary receptor cells were dissociated enzymatically from the olfactory epithelium of the newt, *Cynops pyrrhogaster*. The newts were kept out of water for 2–3 days prior to the experiments, so that the olfactory cells of the animal become responsive to volatile substances (see Shibuya & Takagi, 1963). The animal was anaesthetized by cooling on ice, decapitated and pithed. Nasal cavities were opened and the olfactory mucosae were excised under a dissection microscope. The mucosae were cut into pieces of about 1 × 1 mm, and incubated for 5 min at 35 °C in a solution that contained 0·5–1·0% collagenase (Sigma, Type IA) and no added $Ca^{2+}$ or $Mg^{2+}$ (0 $Ca^{2+}$, 0 $Mg^{2+}$ solution, Table 1). The tissue was then rinsed twice with a standard solution (Table 1) and triturated (see Kurahashi & Shibuya, 1989). The cell suspension was dispensed into a plastic dish (35 mm, Falcon, no. 3001) coated with concanavalin A (Sigma, C2010; see Tachibana & Kaneko, 1984). After cells were anchored to the bottom of the dish, 2 ml of the standard solution (Table 1) was added. Cells were maintained at 4 °C (up to 10 h) before use.

Solitary receptor cells were identified unequivocally by their characteristic morphology which was maintained after dissociation (Fig. 1). Cells which had intact cilia were selected for experiments, because cilia are believed to be the site of the transduction (see below; also see Getchell, 1986; Lancet, 1986). In these cells, cilia moved randomly with a rate 1–3 beats/s.

### *Recording*

A dish containing isolated receptor cells was mounted on the stage of an inverted microscope with phase-contrast optics (Olympus, IMT-2). The dish was surrounded by a water jacket, where cool water was continuously supplied, to maintain the temperature of the dish at 15 °C. A stainless-steel ring was put into the dish to reduce the volume of the chamber (to approximately 0·8 ml) and to facilitate heat exchange. Cells were superfused continuously (at 1 ml/min) with one of the solutions listed in Table 1. When a solution was switched from one to the other, the flow rate was doubled so that the solution change was completed within 3 min.

Cells were voltage clamped by a patch pipette in the whole-cell recording configuration (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). The tip diameter of the pipette was 1–2 $\mu$m and

resistance was 10–20 MΩ when measured in a bath solution. In most experiments the recording pipette was filled with a $Cs^+$-containing solution (Table 2, 120 mM-$Cs^+$ solution) to minimize $K^+$ currents (a mixture of $I_{K(V)}$ and $I_{K(Ca)}$; Kurahashi & Shibuya, 1986*b*; T. Kurahashi, unpublished observation; Maue & Dionne, 1987; Firestein & Werblin, 1987). Recordings were started after the cell interior was dialysed with the pipette solution (a few minutes after the rupture of the patch membrane).

TABLE 1. Ionic composition of the superfusates (mM)

| | $Li^+$ | $Na^+$ | $K^+$ | $Rb^+$ | $Cs^+$ | Choline | $Ca^{2+}$ | $Mg^{2+}$ | $Cl^-$ | Glutamate |
|---|---|---|---|---|---|---|---|---|---|---|
| Standard | – | 111·9 | 3·7 | – | – | – | 3 | 1 | 121·7 | – |
| 0 $Ca^{2+}$, 0 $Mg^{2+}$ | – | 117·9 | 3·7 | – | – | – | – | – | 119·7 | – |
| 0 $Mg^{2+}$ | – | 111·9 | 3·7 | – | – | – | 3 | – | 119·7 | – |
| 120 mM-$Li^+$ | 120 | 1·9 | – | – | – | – | – | – | 120 | – |
| 121·9 mM-$Na^+$ | – | 121·9 | – | – | – | – | – | – | 120 | – |
| 61·9 mM-$Na^+$ | – | 61·9 | – | – | – | 60 | – | – | 120 | – |
| 31·9 mM-$Na^+$ | – | 31·9 | – | – | – | 90 | – | – | 120 | – |
| 120 mM-$K^+$ | – | 1·9 | 120 | – | – | – | – | – | 120 | – |
| 120 mM-$Rb^+$ | – | 1·9 | – | 120 | – | – | – | – | 120 | – |
| 120 mM-$Cs^+$ | – | 1·9 | – | – | 120 | – | – | – | 120 | – |
| 0 $Cl^-$ | – | 121·9 | – | – | – | – | – | – | – | 120 |

All solutions contained 15 mM-glucose, 1 mM-pyruvate, 2 mM-HEPES and 0·001% (w/v) phenol red. The pH was adjusted to 7·4 with NaOH. Concentration of $Na^+$ used to adjust the pH has been included.

The patch pipette was connected to a current–voltage converter (Nihon Kohden, S-3666). Data were stored on FM magnetic tape (TEAC, R-100, bandwidth DC–1 kHz), and were sampled (at 0·2–1 kHz) by a 12-bit analog-to-digital converter connected to a microcomputer (NEC, PC9801VM). Command voltages were generated by a 12-bit digital-to-analog converter with a voltage and time resolution of 0·195 mV and 1 ms, respectively.

The liquid junction potentials at the pipette tip or at the indifferent electrode were measured by the method of Ohmori (1985). The data presented in this paper were corrected for these junction potentials.

*Application of odorant*

*n*-Amyl acetate (Wako Pure Chemical Industries, Osaka, Japan) was used as stimulant throughout the present experiments. In some experiments, D-limonene (D-*p*-mentha-1,8-diene; Tokyo Kasei Kougyo, Tokyo, Japan) was used for comparison, but no substantial difference was found. The odorant dissolved in the superfusate was filled in a pipette with a tip diameter of 1–2 μm and was ejected by pressure (0·16 kg/cm²) to the surface of the recording cells. Odorant of different concentrations was applied from a double-barrelled pipette (tip diameter, *ca* 5 μm).

## RESULTS

### *Response induced by odorants*

Isolated receptor cells had a resting membrane potential of $-44·7 \pm 7·0$ mV (mean ± S.D.; $n = 70$), when it was recorded by a pipette filled with a pseudo intracellular medium (Table 2, 130 mM- $K^+$ solution). Sensitivity to *n*-amyl acetate (10 mM, the concentration that evoked maximal responses in olfactory receptor cells *in situ*; see Kashiwayanagi & Kurihara, 1984; Arzt, Silver, Mason & Clark, 1986) was determined on sixty-three cells under the current clamp condition (130 mM-$K^+$ pipette). Of these, eighteen cells responded to the odorant with slow depolarization.

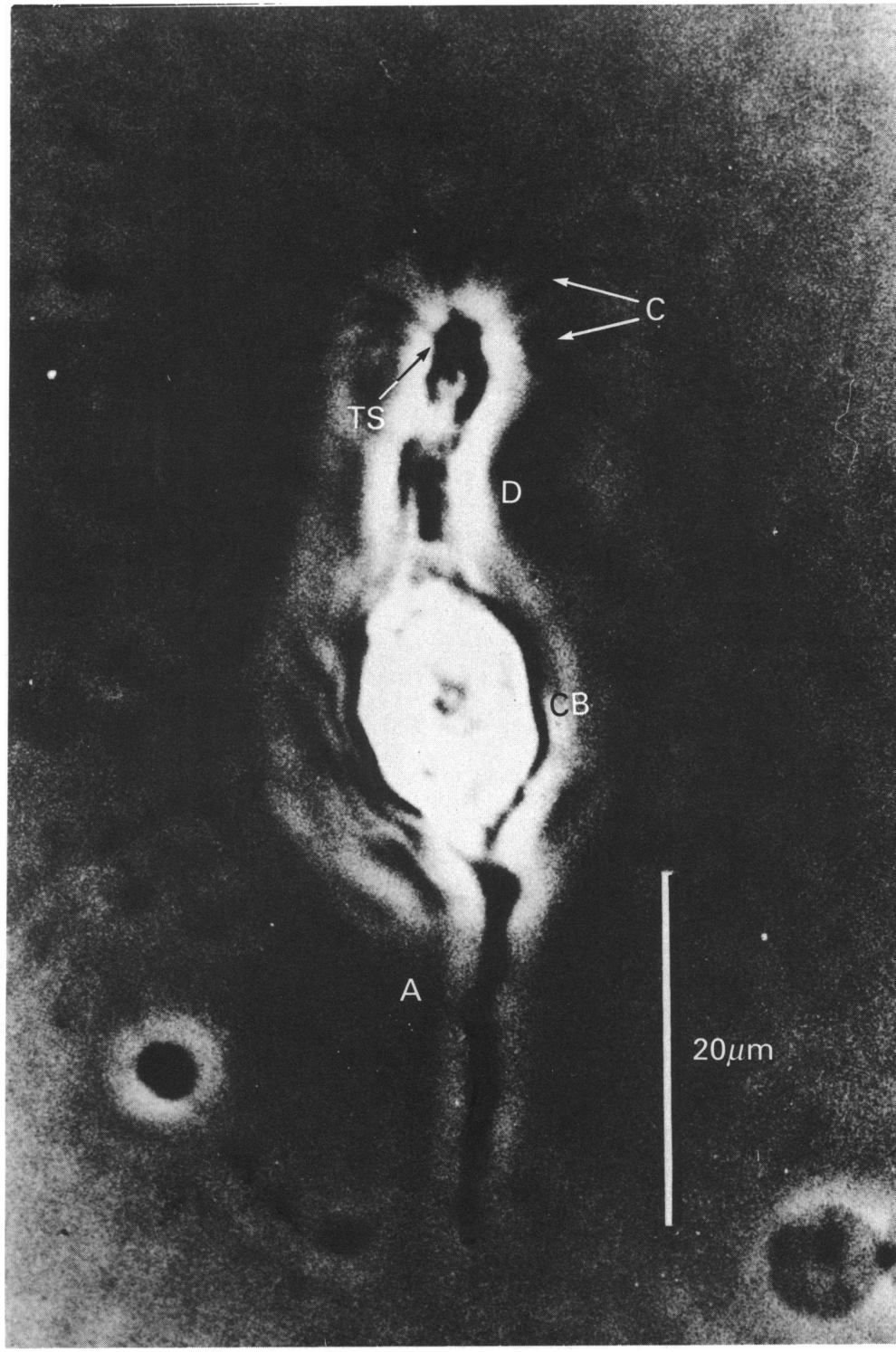


Fig. 1. Phase-contrast photomicrograph of an isolated olfactory receptor cell. C, cilia; TS, terminal swelling; D, dendrite; CB, cell body; A, axon.

The remaining forty-five cells did not show a detectable response. Responsive cells and non-responsive cells had no difference either in morphology or in membrane current–voltage (*I–V*) relation measured under voltage clamp condition.

Figure 2*A* shows a typical depolarizing response to brief application of the

TABLE 2. Composition of the pipette solution (mM)

| | $K^+$ | $Cs^+$ | $Na^+$ | Choline | $Cl^-$ | EGTA | HEPES |
|---|---|---|---|---|---|---|---|
| 130 mM-$K^+$ | 130 | – | – | – | 120 | 5 | 2 |
| 120 mM-$Cs^+$ | – | 120 | 10 | – | 120 | 5 | 2 |
| 60 mM-$Cs^+$ | – | 60 | 10 | 60 | 120 | 5 | 2 |

All solutions were adjusted to pH 7·4 with KOH (130 mM-$K^+$) or NaOH (120 mM-$Cs^+$, 60 mM-$Cs^+$). The concentration of $Na^+$ and $K^+$ used to adjust the pH has been included. Indicator used was 0·001 % (w/v) phenol red.

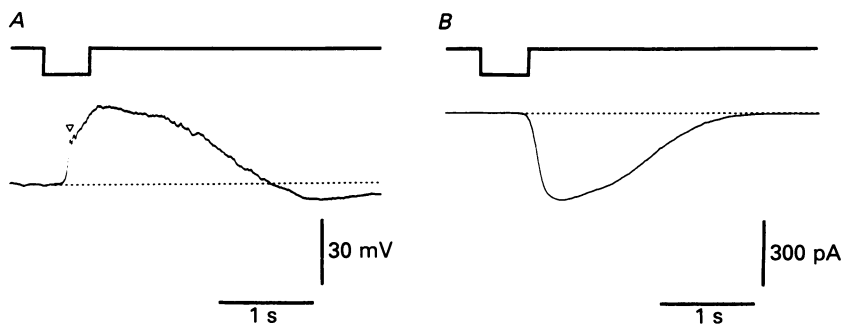


Fig. 2. Responses to odorant in isolated olfactory receptor cells bathed in the standard solution. Downward deflections of the upper traces show the timing of ejection of 10 mM-*n*-amyl acetate. *A*, voltage response evoked by odorant. Triangle indicates spike-like event. The recording pipette was filled with 130 mM-$K^+$ solution (Table 2). *B*, odorant-induced current recorded under voltage clamp (holding potential, −54·2 mV; different cell from *A*). A response delay longer than that in *A* is probably due to a longer diffusion time of the odorant to the receptive site of the cell in *B*. Recording pipette was filled with 120 mM-$Cs^+$ solution (Table 2).

odorant. The cell began to depolarize 230 ms after the onset of stimulus, reached a peak at about 620 ms, and then slowly repolarized. A small hyperpolarizing after-potential (7·5 mV) followed the depolarizing phase. The amplitude of depolarization varied largely from cell to cell (26·7 ± 8·2 mV; $n = 18$; 10 mM-*n*-amyl acetate). The minimum effective concentration of *n*-amyl acetate was 100 μM (also see Kurahashi & Shibuya, 1989). Pressure application of the superfusate alone caused no detectable response, thereby eliminating the possibility of mechanical artifacts. Similar depolarizing response was observed when cells were stimulated by D-limonene.

Although the modulation of the spike frequency by the level of odorant-evoked depolarization is the property commonly found in *in situ* olfactory receptor cells (Getchell, 1977; Suzuki, 1977; Trotier & MacLeod, 1983; Masukawa *et al.* 1985), depolarization by current injection generated repetitive spikes only in a few isolated

cells (three out of seventy). In these cells the spike frequency increased with an increase in the level of depolarization (Kurahashi & Shibuya, 1989). In the remaining cells, as exemplified by the cell in Fig. 2*A*, depolarization caused only small spike-like events at the rising phase, but no repetitive spike discharges. Neither were repetitive spikes evoked during depolarization evoked by current injection. Lack of repetitive spikes has also been reported in isolated olfactory receptor cells from the tiger salamander (Anderson & Hamilton, 1987; Firestein & Werblin, 1987). Amputation of axons by the dissociation procedure may be responsible for the lack of repetitive spikes in most of the preparations.

To determine whether the olfactory response desensitizes to odour, prolonged stimulation (up to 9 s) was applied. In fourteen out of seventeen cells, the amplitude of the odorant-evoked response declined significantly with time (to $29 \pm 10\%$ at 9 s, time to half-decay $1{\cdot}71 \pm 1{\cdot}10$ s, 10 mM-*n*-amyl acetate). The responses which had declined recovered within 15 s to more than 90% (five cells). Responses in three out of seventeen cells did not decline significantly during the stimulation.

The odorant induced an inward current in cells voltage clamped at their resting membrane potential (Fig. 2*B*). The inward current was seen only in cells which were depolarized by the odorant under the current clamp condition. The inward current developed 430 ms after the onset of stimulation (the longer delay compared with that of the voltage response in Fig. 2*A* was attributed to the larger distance between the receptive site and the stimulus pipette; see below), reached a peak at 830 ms and then slowly declined. No outward current was observed after the inward current, although the voltage response was followed by a small after-hyperpolarization. Thus, it seems likely that the after-hyperpolarization is caused by a voltage-dependent conductance. This was also supported by the findings that the maintained depolarization (*ca* 2 s) by extrinsic current injection was followed by a small hyperpolarization (not illustrated).

The amplitude of the inward current varied from cell to cell (compare Figs 2, 3 and 4 all obtained under the same condition), as did the voltage responses. Also the current decayed in amplitude during a prolonged stimulation (time to half-decay, $0{\cdot}82 \pm 0{\cdot}85$ s; $n = 7$; at $-54$ mV).

Responses evoked by odorants ran down slowly with time, due perhaps to the loss of some necessary cytoplasmic components by intracellular dialysis. The period for which response could be recorded varied among cells (ranging from a few minutes to 20 minutes). In this study, however, further analysis was not made on the response run-down.

### *Spatial distribution of the sensitivity to odorant*

The distribution of odorant sensitivity was mapped by giving a small amount of odorant locally to various regions of isolated receptor cells. Because of the movement of the cilia, mapping was made mainly on the dendrite and on the cell body. In the cell illustrated in Fig. 3, the maximum response (*ca* 400 pA) was evoked when the stimulus was given to the apical part of the dendrite (Fig. 3, location 1). Identical doses of the odorant given to the cell body (Fig. 3, location 2 and 3) induced much less current (72 pA). Longer response delay was seen when the stimulus was given to

the cell body (400 ms) than to the apical dendrite (140 ms). This difference may be attributed to the diffusion of the odorant to the receptive site. Similar results were obtained in four other cells. Only in one cell that had slow ciliary movement was sensitivity distribution examined also along the cilia. Within the cilium the apical part was more sensitive than the proximal part (not illustrated).

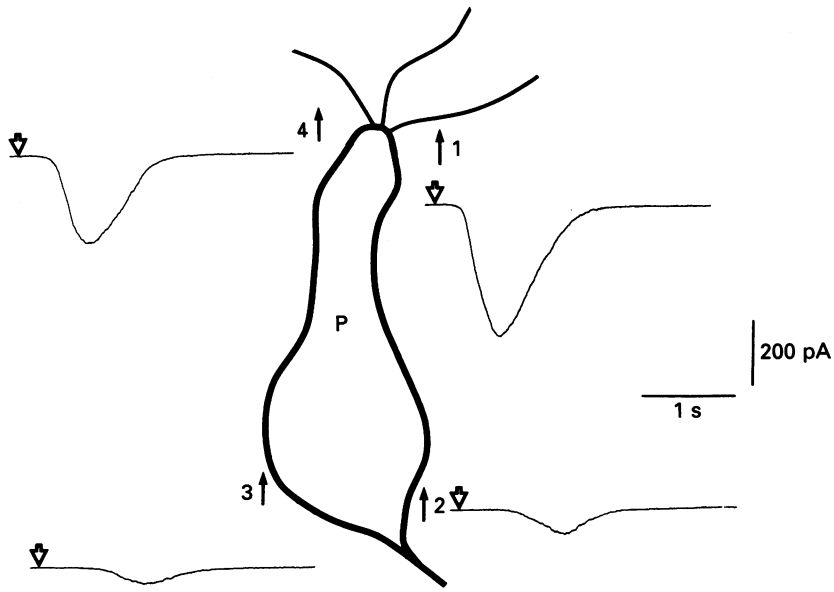


Fig. 3. Spatial distribution of sensitivity to the odorant. The cell was bathed in the standard solution and voltage clamped (holding voltage, −43·7 mV). Each trace shows the current response when a small amount of *n*-amyl acetate (10 mM) was ejected by a brief pulse (50 ms duration indicated by open arrows) from a glass pipette (tip diameter, *ca* 1 μm) placed at numbered arrow-heads (1–4; the number also indicates the sequence of odorant application). The shape of the cell (diameter at the cell body, *ca* 15 μm) was traced from a photomicrograph. This cell had more than ten cilia, but only three are illustrated. The recording pipette was filled with 120 mM-$Cs^+$ solution (Table 2), and attached to the cell at the symbol P.

*Voltage dependence and reversal potential of the odorant-induced current*

The amplitude and polarity of the odorant-induced current were voltage dependent (Fig. 4). At the resting potential (approximately −43·7 mV), *n*-amyl acetate induced an inward current. The response decreased in amplitude as the holding potential was shifted toward 0 mV, and at membrane potentials more positive than 6 mV the current polarity reversed to outward. The relation between the odorant-induced current and the membrane potential is shown in Fig. 4*B*. The average reversal potential was 2·5 ± 2·2 mV (10 mM-*n*-amyl acetate, $K^+$ pipette; $n = 6$). The average conductance was 3·14 ± 1·59 nS (measured at 0 mV). Response to D-limonene had a similar reversal potential.

The *I–V* curve was almost linear in the voltage range more positive than −20 mV, but was markedly non-linear in negative voltage regions (more negative than

−30 mV). It might be argued that such a strong non-linearity could be caused by a suppression by the odorant of an underlying membrane current that is activated at the negative voltage region. Blockage of voltage-activated channels by chemicals has been reported in solitary retinal horizontal cells in which glutamate blocked the

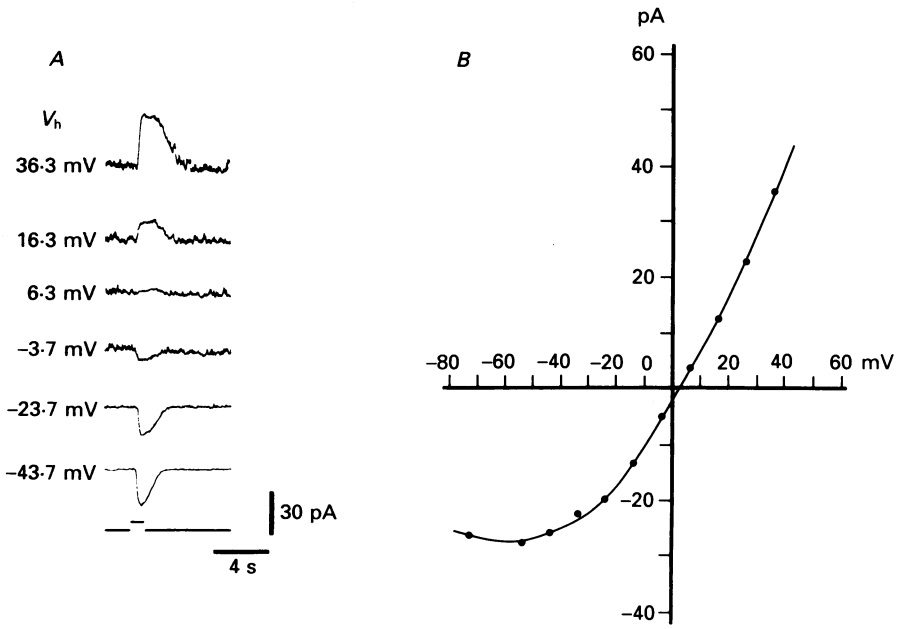


Fig. 4. *A*, odorant-induced currents (downward deflection, inward) recorded under various holding voltages (indicated on the left of each current trace) from a cell bathed in the standard solution. The current traces were arbitrarily shifted. The bottom trace indicates the timing of 10 mM-*n*-amyl acetate application from a glass pipette positioned less than 20 $\mu$m away from the apical dendrite. The recording pipette contained 130 mM-$K^+$ solution (Table 2). *B*, voltage dependence of odorant-induced current. Replotted from *A* and additional records from the same cell.

anomalous rectifier $K^+$ channels (Kaneko & Tachibana, 1985). However, this possibility does not seem likely in the olfactory receptors, because a voltage-dependent conductance activated at the negative region has not been detected (Trotier, 1986; Firestein & Werblin, 1987; Kurahashi & Shibuya, 1989). It is inferred, therefore, that the odorant-activated conductance itself has a non-linear property. The transition voltage at which the slope of the *I–V* curve changes differed from cell to cell (ranging from −20 to −50 mV).

*Effects of external $Ca^{2+}$ on the odorant-activated conductance*

Removal of external $Ca^{2+}$ dramatically enhanced the response amplitude and prolonged the time course of odorant-induced current. Figure 5 shows a typical result. The maximal amplitude of the current induced by a short odour pulse (at −24 mV) increased 4·6 times, and the duration was prolonged to more than 10 s (Fig. 5*A*).

Figure 5*B* shows the *I–V* relations of the odorant-activated conductance in the absence and presence of 3 mM-$Ca^{2+}$. Removal of $Ca^{2+}$ increased the slope of the *I–V*

curve, but did not affect the reversal potential. These results suggest that $Ca^{2+}$, at least at a physiological concentration, does not carry significant current through the odorant-activated conducting channels, but affects the kinetics of the conductance. The present findings agree well with the report by Nakamura & Gold (1987) who

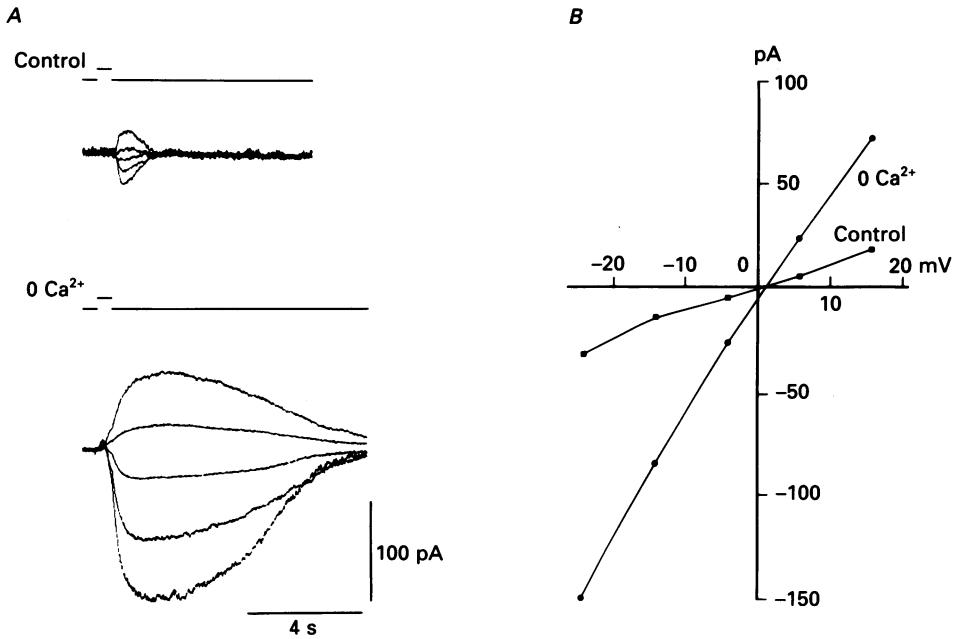


Fig. 5. Effects of removal of external $Ca^{2+}$ on odorant-induced current. *A*, currents induced by 10 mM-*n*-amyl acetate (applied for 0·5 s) in a cell bathed in a solution containing 3 mM-$Ca^{2+}$ (upper group of current traces; 0 $Mg^{2+}$ solution in Table 1) or in a $Ca^{2+}$- and $Mg^{2+}$-free solution (lower group of current traces; 0 $Ca^{2+}$, 0 $Mg^{2+}$ solution in Table 1). Holding potentials were −24, −14, −4, 6 and 16 mV in both records. Current traces were superimposed by shifting by an arbitrary amount. The pipette contained 120 mM-$Cs^{+}$ solution (Table 2). *B*, the *I–V* relation of the odorant-induced current obtained with 3 mM (control) and 0 $Ca^{2+}$ solution. Replotted from *A*.

showed that the cyclic nucleotide-gated conductance on the membrane patch excised from the olfactory cilia was enhanced in low-$Ca^{2+}$ medium. The non-linearity of the odorant-activated conductance was reduced when external $Ca^{2+}$ was removed (compare Figs 4*B* & 6*A*, but note that the data of Fig. 5 cover a narrower voltage range). A similar observation has been reported for the cyclic nuleotide-gated conductance (Nakamura & Gold, 1987). In the present study, however, this matter was not further analysed.

Since it was easier to analyse a large response, subsequent experiments were made on cells superfused with solutions to which no $Ca^{2+}$ was added.

### *Measurement of the reversal potential by ramp clamp*

As described in the previous section, the odorant-evoked response ran down with time. To overcome this complication, the *I–V* relation was measured more efficiently by using a ramp clamp. The *I–V* relation of the odorant-induced response was

obtained by subtracting the *I–V* curve measured in the absence of odorant from the curve obtained in its presence. The reversal potential value obtained by the ramp clamp was almost identical to that obtained by voltage clamp with step pulses (compared in seven cells).

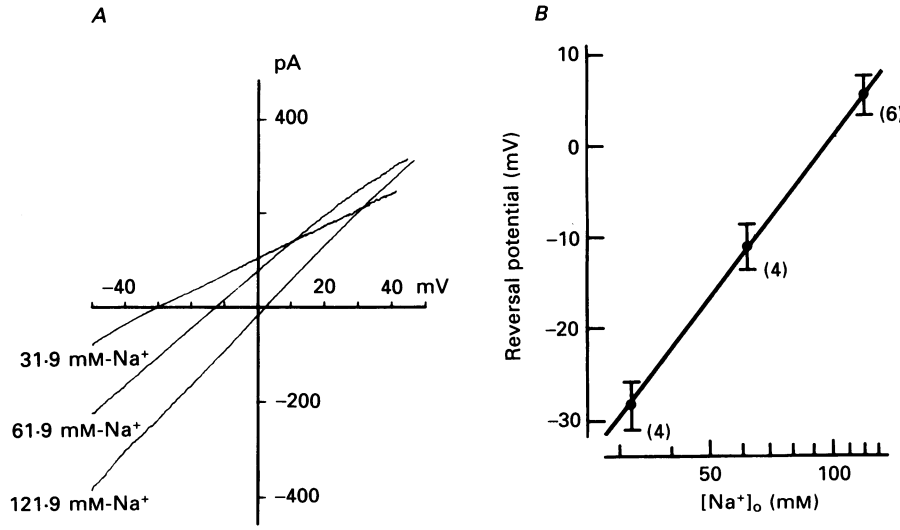


Fig. 6. Effects of $[Na^+]_o$ on the conductance activated by 10 mM-*n*-amyl acetate. *A*, the *I–V* relations of the odorant-activated conductance obtained by ramp clamp in 121·9, 61·9 and 31·9 mM-$Na^+$ (Table 1). Each curve represents the average of the data for the two currents in response to rising and falling voltage ramps (ramp rate = 195 mV/s). The pipette contained 120 mM-$Cs^+$ solution (Table 2). *B*, dependence of the reversal potential on $[Na^+]_o$ (plotted on a logarithmic scale). Means (●) and standard deviation (vertical line) are shown with the number of cells examined (in parentheses). The diagonal line has a slope of 57 mV per 10-fold change of $[Na^+]_o$. The position of the line was determined by calculating a Goldman–Hodgkin–Katz equation, with $P_{Na} = 1$, $P_{Cs} = 0{\cdot}72$ and $P_{choline} = P_{Cl} = 0$.

*Effects of ions on odorant-activated conductance*

To identify the species of ions carrying the odorant-induced current, a series of *I–V* relations were examined in various ionic environments. In most experiments bi-ionic conditions were established to simplify the analyses.

*External $Na^+$*

Since $Na^+$ is the richest ion in the extracellular environment, the effect of $Na^+$ on the reversal potential was first analysed. Figure 6*A* shows the *I–V* relations of the odorant-activated conductance under three different concentrations of external $Na^+$ ($[Na^+]_o$). The reversal potential was +3 mV in the cell bathed with a solution containing 121·9 mM-$Na^+$. The reversal potential was shifted to −13 mV and −30 mV when $[Na^+]_o$ was reduced to 61·9 mM and 31·9 mM, respectively. The slope conductance decreased with the reduction of $[Na^+]_o$.

Figure 6*B* illustrates the relation between $[Na^+]_o$ and the reversal potential. The

data points (mean values obtained from cells whose number is shown in parentheses) were very close to the line that has a slope of 57 mV per 10-fold change of $[Na^+]_o$. This agreement indicates that the odorant-activated conducting channels are highly permeable to $Na^+$.

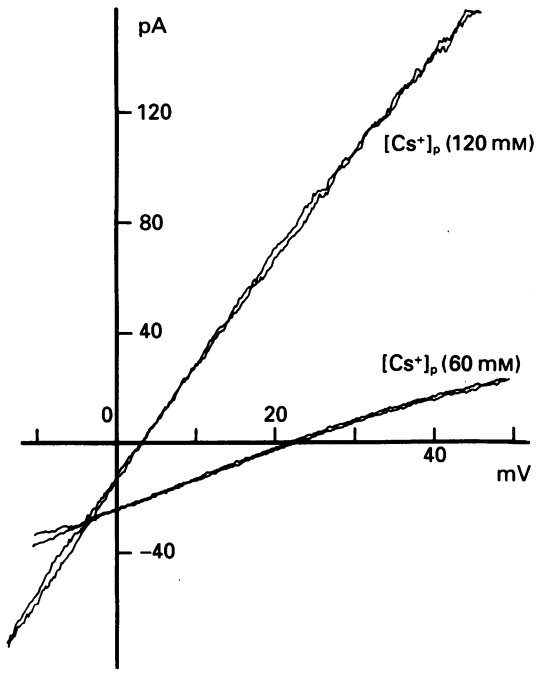


Fig. 7. Effect of $Cs^+$ concentration in the pipette solution ($[Cs^+]_p$; 60 and 120 mM) on the conductance activated by 10 mM-*n*-amyl acetate. Data obtained from two different cells which were superfused with 121·9 mM-$Na^+$ solution.

*Internal $Cs^+$*

In the aforementioned experiment, the cytoplasm was dialysed with a pipette solution consisting of 120 mM-$Cs^+$ and 10 mM-$Na^+$. Since the reversal potential was much more negative than the equilibrium potential for $Na^+$ (+62 mV), it is reasonable to assume that $Cs^+$ permeates through the odorant-activated conducting channels.

The experiment illustrated in Fig. 7 was carried out to test this possibility. The figure shows *I–V* curves obtained from two different cells recorded with a pipette filled with either 120 mM- or 60 mM-$Cs^+$ (replaced with 60 mM-choline$^+$ which is assumed not to contribute to the conductance, see Fig. 6). The reversal potential was 21·7 ± 4·6 mV ($n = 4$) in cells dialysed with 60 mM-$Cs^+$, approximately 16 mV more positive than the reversal potential obtained with 120 mM-$Cs^+$ (+5·3 mV). This observation suggests that $Cs^+$ also passes through the odorant-activated conducting channels.

The establishment of bi-ionic conditions was verified by the finding that $Cl^-$ did not pass through the odorant-activated conducting channels. The reversal potential of the odorant-induced current measured in 120 mM-$Cl^-$ (121·9 mM-$Na^+$ solution;

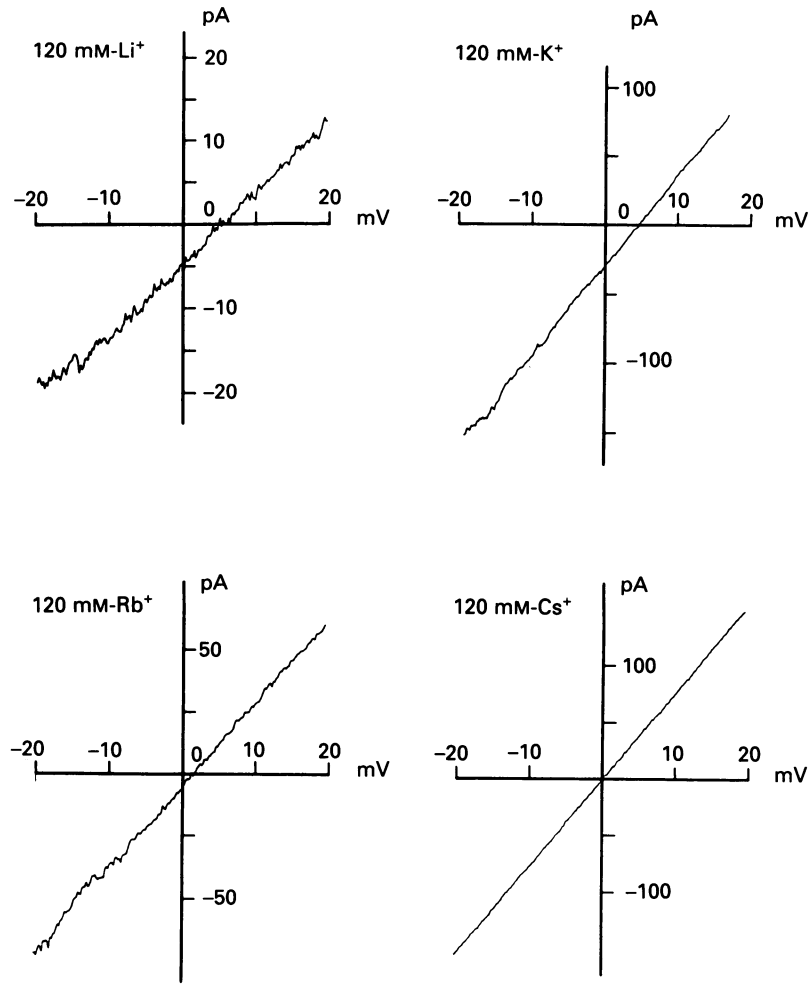


Fig. 8. The *I–V* relations of the conductance activated by 10 mM-*n*-amyl acetate recorded in cells bathed in solutions containing various alkali metal ions. The ionic composition of the solutions is listed in Table 1 (120 mM-$Li^+$, 120 mM-$K^+$, 120 mM-$Rb^+$, 120 mM-$Cs^+$). Each record was obtained from a different cell. The pipette contained 120 mM-$Cs^+$ solution (Table 2).

$5{\cdot}3 \pm 2{\cdot}0$ mV, $n = 6$) and in $Cl^-$-free medium (replaced with glutamate ions; $2{\cdot}5 \pm 2{\cdot}3$ mV, $n = 3$) was nearly identical.

*Comparison of permeabilities of various alkali metal ions*

To examine the ionic selectivity of the odorant-activated conductance the *I–V* relations of the response were measured in cells bathed in an external medium that contained one of the alkali metal ions, $Li^+$, $K^+$, $Rb^+$ or $Cs^+$ (each at 120 mM, Fig. 8). The pipette contained 120 mM-$Cs^+$ and 10 mM-$Na^+$. The reversal potential obtained under each of these bi-ionic conditions was $10{\cdot}7 \pm 3{\cdot}3$ mV for $Li^+$ ($n = 5$), $5{\cdot}3 \pm 2{\cdot}0$ mV for $Na^+$ ($n = 6$), $4{\cdot}8 \pm 0{\cdot}4$ mV for $K^+$ ($n = 3$), $1{\cdot}5 \pm 1{\cdot}0$ mV for $Rb^+$ ($n = 4$) and

$-0{\cdot}4 \pm 0{\cdot}4$ mV for $Cs^+$ ($n = 3$). These results suggest that the odorant-activated conducting channels do not strongly discriminate between these cations.

The relative permeability was derived from the equation

$$E_{rev} = \frac{RT}{F} \ln \frac{\Sigma P_X [X]_o}{\Sigma P_X [X]_i}$$

where $E_{rev}$ represents the reversal potential, X the ionic species, $P_X$, relative permeability of the membrane to ion X, $T$ is temperature, and $R$ and $T$ are physical constants (Hille, 1984). The relative permeability of various cations through the odorant-activated conductance (in relation to permeability to $Na^+$) was

$$P_{Li} : P_{Na} : P_K : P_{Rb} : P_{Cs} = 1{\cdot}25 : 1 : 0{\cdot}98 : 0{\cdot}84 : 0{\cdot}80.$$

## DISCUSSION

The present study has demonstrated that odour stimulants, *n*-amyl acetate and D-limonene, depolarize isolated olfactory receptor cells by increasing their membrane conductance to cations. The sensitivity to the odorant was confined to the apical portion of receptor cells. The present finding supports the notion that the cilia and/or the terminal swelling is the site of olfactory transduction (see Getchell, 1986; Lancet, 1986). Here, an odorant-binding protein (Rhein & Cagan, 1980) and odorant-activated adenylate cyclase (Pace, Hanski, Salomon & Lancet, 1985) have been found. Furthermore, the length of the olfactory cilia and the amplitude of the EOG response have a direct relationship (Adamek, Gesteland, Mair & Oakley, 1984).

### *Transduction current and depolarizing receptor potential*

The membrane of the olfactory cells have a high input resistance around the resting potential (−45 mV), exceeding a few gigaohms, when measured in the isolated preparation with a patch pipette (2·1 GΩ, Kurahashi & Shibuya, 1985; 2·2 GΩ, Trotier, 1986; 5·2 GΩ, Firestein & Werblin, 1987), with almost linear *I–V* relations at membrane potentials more negative than −20 mV. Thus the current required to depolarize the membrane by 10 mV from the resting potential was estimated to be as small as 5 pA (with 2 GΩ input resistance). Thus, the odorant-induced current recorded in the present study in standard solution (30–400 pA) was large enough to depolarize the olfactory receptor cells.

### *Ionic dependence of the olfactory response*

The odorant-activated conducting channels observed in the isolated olfactory receptor cells were permeable to all alkali metal ions with low selectivity. But choline or anions did not permeate. Previous studies on the ionic dependence of olfactory responses have been made by using indirect indices such as EOG (Tucker & Shibuya, 1965; Takagi *et al.* 1968) or evoked potentials recorded at the olfactory bulb (Yosii & Kurihara, 1983). Some of these results are very puzzling in the light of present study. It is surprising that Tucker & Shibuya (1965) observed a large EOG when they superfused the turtle olfactory epithelium with a $Na^+$-free sucrose solution. Yosii &

Kurihara (1983) showed that the replacement of $Na^+$ with $Tris^+$ or $choline^+$ did not affect the response amplitude of evoked potentials at the olfactory bulb. These puzzling results could be understood if one assumes that the ionic composition of the medium in the immediate surrounding of olfactory cells was not much influenced by the experimental condition due to a diffusion barrier or by a strong buffering system of the preparation *in situ*.

*Comparison with ion channels found in the receptive membrane*

The odorant-activated conductance observed in the present study did not show a strong discrimination between the species of various alkali metal ions. Similar low cationic selectivity has been reported in various preparations from different sources. Nakamura & Gold (1987) showed that the cyclic nucleotide-gated conductance has the same permeability values either to $Na^+$ or $K^+$ in membrane patches excised from olfactory cilia. Suzuki (1988) has reported that intracellular injection of cyclic GMP into isolated olfactory cells increases the cationic permeability with a ratio of $P_{Li}:P_{Na}:P_K:P_{Rb}:P_{Cs} = 1{\cdot}03:1:0{\cdot}95:0{\cdot}78:0{\cdot}75$. Ionic selectivity was similar even to that of the cyclic GMP-gated channels in the rod outer segment (Fesenko, Kolesnikov & Lyubarsky, 1985).

Cyclic nucleotides are thought to be the second messenger in the olfactory transduction (Pace *et al.* 1985; Suzuki, 1986; Nakamura & Gold, 1987), but there are also several reports that odorant can directly gate ionic channels (Vodyanoy & Murphy, 1983; Labarca, Simon & Anholt, 1988). The preparation by Labarca *et al.* (1988) (the odorant-gated channels reconstructed in the lipid bilayers fused with the ciliary membrane) has a cationic selectivity similar to that of the present study ($P_K/P_{Na} = 1$). However, the preparation by Vodyanoy & Murphy (1983) (diethylsulphide-gated channel reconstructed in the lipid bilayer) has selective permeability to $K^+$.

External $Ca^{2+}$ reduced the slope of the odorant-activated conductance. In addition, the $I$–$V$ relation of the conductance showed non-linearity in the presence of external $Ca^{2+}$. Similar properties have been found in the cyclic nucleotide-gated conductance (Nakamura & Gold, 1987) of the olfactory cilia and in the cyclic GMP-gated conductance of the rod photoreceptor cell (Haynes, Kay & Yau, 1986; Matthews, 1986; Zimmerman & Baylor, 1986).

The odour response declined with internal dialysis (see also Trotier, 1986). Although the present study was not directed to answer the underlying mechanism of intracellular signal transmission, these findings favour the notion that cyclic nucleotide and/or some other intracellular substances play an important role in olfactory transduction.

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## REFERENCES

ADAMEK, G. D., GESTELAND, R. C., MAIR, R. G. & OAKLEY, B. (1984). Transduction physiology of olfactory receptor cilia. *Brain Research* **310**, 87–97.

ANDERSON, P. A. V. & HAMILTON, K. A. (1987). Intracellular recordings from isolated salamander olfactory receptor neurons. *Neuroscience* **21**, 167–173.

ARZT, A. H., SILVER, W. L., MASON, J. R. & CLARK, L. (1986). Olfactory responses of aquatic and terrestrial tiger salamanders to airborne and waterborne stimuli. *Journal of Comparative Physiology A* **158**, 479–487.

FESENKO, E. E., KOLESNIKOV, S. S. & LYUBARSKY, A. L. (1985). Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature* **313**, 310–313.

FIRESTEIN, S. & WERBLIN, F. S. (1987). Gated currents in isolated olfactory receptor neurons of the larval tiger salamander. *Proceedings of the National Academy of Sciences of the USA* **84**, 6292–6296.

GETCHELL, T. V. (1977). Analysis of intracellular recordings from salamander olfactory epithelium. *Brain Research* **123**, 275–286.

GETCHELL, T. V. (1986). Functional properties of vertebrate olfactory receptor neurons. *Physiological Reviews* **66**, 772–818.

HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

HAYNES, L. W., KAY, A. R. & YAU, K.-W. (1986). Single cyclic GMP-activated channel activity in excised patches of rod outer segment membrane. *Nature* **321**, 66–70.

HILLE, B. (1984). *Ionic Channels of Excitable Membranes*. Sunderland, Massachusetts: Sinauer Associates Inc.

KANEKO, A. & TACHIBANA, M. (1985). Effects of L-glutamate on the anomalous rectifier potassium current in horizontal cells of *Carassius auratus* retina. *Journal of Physiology* **358**, 169–182.

KASHIWAYANAGI, M. & KURIHARA, K. (1984). Neuroblastoma cell as model for olfactory cell: mechanism of depolarization in response to various odorants. *Brain Research* **293**, 251–258.

KURAHASHI, T. & SHIBUYA, T. (1985). Membrane electrical properties and responses to odor in the isolated olfactory receptor cells of newt. *Dobutu seiri* **2**, 167.

KURAHASHI, T. & SHIBUYA, T. (1986*a*). The odor responses and odor-induced current in the solitary olfactory receptor cells isolated from newts. *Proceedings of the 20th Japanese Symposium on Taste and Smell*, pp. 33–36. Asahi University, Gifu, Japan: Japanese Association for the Study of Taste and Smell.

KURAHASHI, T. & SHIBUYA, T. (1986*b*). Membrane ionic currents in the solitary olfactory receptor cells of newt. *Zoological Science* **3**, 982.

KURAHASHI, T. & SHIBUYA, T. (1989). Membrane responses and permeability changes to odorants in the solitary olfactory receptor cells of newt. *Zoological Science* **6**, 19–30.

LABARCA, P., SIMON, S. A. & ANHOLT, R. R. H. (1988). Activation by odorants of a multistate cation channel from olfactory cilia. *Proceedings of the National Academy of Sciences of the USA* **85**, 944–947.

LANCET, D. (1986). Vertebrate olfactory reception. *Annual Review of Neuroscience* **9**, 329–355.

MASUKAWA, L. M., HEDLUND, B. & SHEPHERD, G. M. (1985). Electrophysiological properties of identified cells in the *in vitro* olfactory epithelium of the tiger salamander. *Journal of Neuroscience* **5**, 128–135.

MATTHEWS, G. (1986). Comparison of the light-sensitive and cyclic GMP-sensitive conductances of the rod photoreceptor: noise characteristics. *Journal of Neuroscience* **6**, 2521–2526.

MAUE, R. A. & DIONNE, V. E. (1987). Patch-clamp studies of isolated mouse olfactory receptor neurons. *Journal of General Physiology* **90**, 95–125.

NAKAMURA, T. & GOLD, G. H. (1987). A cyclic nucleotide-gated conductance in olfactory cilia. *Nature* **325**, 442–444.

OHMORI, H. (1985). Mechano-electrical transduction currents in isolated vestibular hair cells of the chick. *Journal of Physiology* **359**, 189–217.

PACE, U., HANSKI, E., SALOMON, Y. & LANCET, D. (1985). Odorant-sensitive adenylate cyclase may mediate olfactory reception. *Nature* **316**, 255–258.

RHEIN, L. D. & CAGAN, R. H. (1980). Biochemical studies of olfaction: isolation, characterization, and odorant binding activity of cilia from rainbow trout olfactory rosettes. *Proceedings of the National Academy of Sciences of the USA* **77**, 4412–4416.

SHIBUYA, T. & TAKAGI, S. F. (1963). Electrical response and growth of olfactory cilia of the olfactory epithelium of the newt in water and on land. *Journal of General Physiology* **47**, 71–82.

SUZUKI, N. (1977). Intracellular responses of lamprey olfactory receptors to current and chemical stimulation. In *Food Intake and Chemical Senses*, ed. KATSUKI, Y., SATO, M., TAKAGI, S. F. & OOMURA, Y., pp. 13–22. Tokyo: University of Tokyo Press.

SUZUKI, N. (1986). Cyclic nucleotide-induced conductance increase in solitary olfactory receptor cells. *Proceedings of the 20th Japanese Symposium on Taste and Smell*, pp. 37–40. Asahi University, Gifu, Japan: Japanese Association for the Study of Taste and Smell.

SUZUKI, N. (1988). Cation selectivity of cyclic nucleotide-gated conductance in isolated olfactory receptor cells. *Zoological Science* **5**, 1194.

TACHIBANA, M. & KANEKO, A. (1984). γ-Aminobutyric acid acts at axon terminals of turtle photoreceptors: difference in sensitivity among cell types. *Proceedings of the National Academy of Sciences of the USA* **81**, 7961–7964.

TAKAGI, S. F., WYSE, G. A., KITAMURA, H. & ITO, K. (1968). The roles of sodium and potassium ions in the generation of the electro-olfactogram. *Journal of General Physiology* **51**, 552–578.

TROTIER, D. (1986). A patch-clamp analysis of membrane currents in salamander olfactory receptor cells. *Pflügers Archiv* **407**, 589–595.

TROTIER, D. & MACLEOD, P. (1983). Intracellular recordings from salamander olfactory receptor cells. *Brain Research* **268**, 225–237.

TUCKER, D. & SHIBUYA, T. (1965). A physiologic and pharmacologic study of olfactory receptors. In *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 30, pp. 207–215. New York.

VODYANOY, V. & MURPHY, R. B. (1983). Single-channel fluctuations in bimolecular lipid membranes induced by rat olfactory epithelial homogenates. *Science* **220**, 717–719.

YOSII, K. & KURIHARA, K. (1983). Role of cations in olfactory reception. *Brain Research* **274**, 239–248.

ZIMMERMAN, A. L. & BAYLOR, D. A. (1986). Cyclic GMP-sensitive conductance of retinal rods consists of aqueous pores. *Nature* **321**, 70–72.