

# Responses of *Xenopus laevis* Water Nose to Water-soluble and Volatile Odorants

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**abstract** Using the whole-cell mode of the patch-clamp technique, we recorded action potentials, voltage-activated cationic currents, and inward currents in response to water-soluble and volatile odorants from receptor neurons in the lateral diverticulum (water nose) of the olfactory sensory epithelium of *Xenopus laevis*. The resting membrane potential was  $-46.5 \pm 1.2$  mV (mean  $\pm$  SEM,  $n = 68$ ), and a current injection of 1–3 pA induced overshooting action potentials. Under voltage-clamp conditions, a voltage-dependent  $\text{Na}^+$  inward current, a sustained outward  $\text{K}^+$  current, and a  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  current were identified. Application of an amino acid cocktail induced inward currents in 32 of 238 olfactory neurons in the lateral diverticulum under voltage-clamp conditions. Application of volatile odorant cocktails also induced current responses in 23 of 238 olfactory neurons. These results suggest that the olfactory neurons respond to both water-soluble and volatile odorants. The application of alanine or arginine induced inward currents in a dose-dependent manner. More than 50% of the single olfactory neurons responded to multiple types of amino acids, including acidic, neutral, and basic amino acids applied at 100  $\mu\text{M}$  or 1 mM. These results suggest that olfactory neurons in the lateral diverticulum have receptors for amino acids and volatile odorants.

**key words:** olfactory receptor neuron • odor response • voltage-clamp • action potential • amino acid

## introduction

In *Xenopus laevis*, the olfactory organ is compartmentalized into two independent subregions, the medial and lateral diverticula. Anatomical observation has indicated that the olfactory sensory epithelium in the medial diverticulum comes into contact with air, while the epithelium in the lateral diverticulum is in contact with water (Altner, 1962). The latter, therefore, is called the water nose. The two olfactory subregions have a different affinity to lectins; the epithelium of the medial diverticulum was slightly labeled with soybean agglutinin-horseradish peroxidase, which has been shown to selectively label a portion of olfactory neurons, whereas the epithelium of the lateral diverticulum was not labeled (Hofmann and Meyer, 1991).

*Xenopus laevis* possesses a gene repertoire encoding two distinct classes of olfactory G-protein-coupled receptors; one class is related to the olfactory G-protein-coupled receptors of fish, and the other is related to the olfactory G-protein-coupled receptors of mammals (Freitag et al., 1995). An in situ hybridization study of the fish-like olfactory G-protein-coupled receptors resulted in labeling of the sensory epithelium in the lateral diverticulum, whereas the medial diverticulum was devoid of any hybridization signals. All the probes rep-

resenting mammalian-like receptor signals yielded results only in the medial diverticulum. On the assumption that these are odor receptors, the results suggest that olfactory neurons in the medial diverticulum are expected to respond to volatile odorants and neurons in the lateral diverticulum to water-soluble odorants (e.g., amino acids). Until recently, however, physiological functions including the odor selectivity of olfactory neurons in these two diverticula have remained unclear.

In the present study, we recorded the responses to water-soluble odorants as well as to volatile odorants from *Xenopus laevis* lateral olfactory receptor neurons under whole-cell voltage-clamp conditions. The results obtained indicated that the lateral olfactory neurons responded to both water-soluble and volatile odorants. Many single olfactory neurons responded to a variety of amino acids, including acidic, basic, and neutral amino acids.

## materials and methods

### *Slice Preparation of Xenopus laevis Olfactory Sensory Epithelium*

The method of preparation was essentially the same as that described in a previous paper (Taniguchi et al., 1996). Frogs, *Xenopus laevis*, were obtained from commercial suppliers and maintained at 15°C. Animals were fed porcine liver ad libitum. For the staining of olfactory epithelial slices with methylene blue (Merck), animals were bathed in 0.1% methylene blue solution for 6 h. For the preparation of olfactory epithelial slices, animals were cooled to 0°C to anesthetize completely, and then decapi-

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tated. Olfactory epithelium was quickly removed from the decapitated frogs. The epithelia were cut into slices  $\sim 120\text{-}\mu\text{m}$  thick with a vibrating slicer (DTK-1000; D.T.K.) in normal Ringer solution at  $0^\circ\text{C}$  and stored at  $4^\circ\text{C}$ . Epithelial slices were fixed on the glass at the bottom of a recording chamber, permitting access by patch pipette to neurons on the surface of the slice. The preparations were viewed under an upright microscope (OPTIPHOT; Nikon Inc.) using a  $40\times$  water immersion lens.

### Data Recording and Analysis

The conventional whole-cell patch clamp method was used to measure transmembrane currents (Hamill et al., 1981). Patch pipettes with resistances of  $5\text{--}10\text{ M}\Omega$  were made from borosilicate glass capillaries with an inner filament (GD-1.5; Narishige Co.) using a two-stage electrode puller (PP853; Narishige Co.), and then heat-polished. Gigaohm seals were obtained by applying negative pressure ( $-30$  to  $-100\text{ cm H}_2\text{O}$ ). The whole-cell configuration was attained through the application of additional negative pressure. Membrane currents (holding potential,  $-70\text{ mV}$ ) and voltages were recorded in the whole-cell configuration. Data were recorded continuously using an EPC-7 patch clamp amplifier (List Electronic) and stored on video cassette via a digital audio processor (PCM-501; Sony Corp.). All recordings were carried out at room temperature. Membrane currents were low-pass filtered with two-poles, Bessel filter of EPC-7. The filter frequency was  $10\text{ kHz}$ . We estimated the magnitude of inward currents from just before the response to the peak. Analysis was carried out on a personal computer using pCLAMP software (Axon Instruments). All values are given as a mean  $\pm$  SEM. A liquid junction potential was measured as described by Neher (1992). The liquid junction potential never varied more than several mV in the altered solutions ( $-2.6 \sim 2.0\text{ mV}$ ). All data have been corrected for the liquid junction potential.

### Lucifer Yellow Dialysis

Lucifer yellow CH was dialyzed intracellularly by using patch pipettes filled with 1% Lucifer yellow solution as described previously (Taniguchi et al., 1996). After the measurement of voltage-activated currents, the pipettes were pulled back from the surface of the cells. The specimens were then transferred to the stage of a fluorescent microscope (OPTIPHOT) and observed.

### Stimulation

Olfactory neurons were stimulated with extracellular solutions by bath application from the outlet of the stimulating tube. Ringer solution, delivered by gravity, was alternated with odorant solutions by means of eight electrically actuated valves. The volume of the solutions in the chamber was  $\sim 90\text{--}350\ \mu\text{l}$ . A stimulating tube with a lumen  $160\text{--}200\ \mu\text{m}$  in diameter was placed under visual control  $\sim 3\text{ mm}$  from the cell to eliminate the mechanical effects of alternating among solutions. The flow rate, which was adjusted with needle valves, was  $12.3\text{--}23.3\ \mu\text{l/s}$ . The delay, due to dead space and to exchange solution in the chamber, was  $3\text{--}25\text{ s}$ , depending on the flow rate. Our attempt to eliminate deviations in the delay was unsuccessful because the precision of the needle valves was not sufficient to regulate the flow of small amounts of the solutions. To examine the extent of the dilution during stimulation,  $50\text{ mM}$  1-anilino-naphthalene-8-sulfonate (ANS) was applied from the stimulating tube to the recording chamber, and the fluorescence intensity of ANS at the point where the epithelium was usually placed was measured with an inverted microscope (Axiovert 135; Carl Zeiss, Inc.). The fluorescence intensity reached a saturated level within  $4\text{--}18\text{ s}$ . This saturated fluorescence intensity was similar to that of  $50\text{ mM}$  ANS.

### Solutions

The extracellular Ringer solution consisted of (mM):  $116\text{ NaCl}$ ,  $4\text{ KCl}$ ,  $2\text{ CaCl}_2$ ,  $1\text{ MgCl}_2$ ,  $10\text{ glucose}$ ,  $10\text{ HEPES-NaOH}$ , pH  $7.4$ . Patch pipettes were usually filled with a normal internal solution containing (mM):  $115\text{ KCl}$ ,  $2\text{ MgCl}_2$ ,  $2\text{ EGTA}$ ,  $10\text{ HEPES-KOH}$ , pH  $7.4$ . Further details on the solutions are shown in the individual figure legends. Lucifer yellow was dissolved in the internal solution at a concentration of 1%. The odorant cocktails were vigorously stirred with the magnetic stirrer for more than 30 min at room temperature. We confirmed that the solutions of odorant cocktails were completely clear. The final concentrations of each odorant in volatile odorant cocktail I, which induced inositol 1,4,5-trisphosphate accumulation in rat olfactory cilia, were  $40\ \mu\text{M}$  linal,  $40\ \mu\text{M}$  lylal, and  $20\ \mu\text{M}$  ethyl vanillin (Breer and Boekhoff, 1991). The final concentration of each odorant in volatile odorant cocktail II (citralva, hedione, eugenol, *l*-carvone, and cineole), which induced cAMP accumulation in the rat and frog olfactory cilia, was  $200\ \mu\text{M}$  (Sklar et al., 1986; Breer and Boekhoff, 1991). Stock solutions of amino acids were prepared at  $0.1\text{ M}$  and stored at  $4^\circ\text{C}$ . These solutions were added to normal Ringer solution to give the concentrations of odorants indicated. The final concentration of each amino acid in the amino acid cocktail (*l*-alanine, *l*-arginine, *l*-glutamic acid, and *l*-methionine) was  $1\text{ mM}$ . The deviations in pH in the stimulating solutions were within  $0.2$ .

### Chemicals

Tetrodotoxin (TTX)<sup>1</sup> and Lucifer yellow CH were obtained from Sankyo and the Aldrich Chemical Co., respectively. Amino acids (*l*-alanine, *l*-arginine, *l*-glutamic acid, and *l*-methionine) and *N*-methyl-*D*-glucamine were obtained from Wako. All volatile odorants were kindly supplied by Takasago International. All chemicals used were of the highest grade available.

## results

### Cell Morphology

Fig. 1, A and B, show micrographs of the olfactory epithelia of the lateral diverticulum and medial diverticulum of *Xenopus laevis* bathed in water containing 0.1% methylene blue. The surface of the lateral diverticulum was clearly stained with methylene blue, while that of the medial diverticulum was not stained at all. These results support a previous finding that the lateral and medial olfactory epithelia demonstrate differentiated contact with water-soluble odorants and volatile odorants, respectively (Altner, 1962). Fig. 1 D shows a fluorescent micrograph of an olfactory neuron dialyzed with Lucifer yellow in the olfactory epithelium of the lateral diverticulum. The cell bodies are elliptical with dimensions on the order of  $20 \times 12\ \mu\text{m}$ . The cells have dendritic processes that reach the surface of the epithelium.

### Current-Clamp Recordings

Initial studies of the whole-cell clamp technique were made using the current-clamp mode. With  $\text{K}^+$  and  $\text{Na}^+$

<sup>1</sup>Abbreviations used in this paper: TEA, tetraethylammonium; TTX, tetrodotoxin.

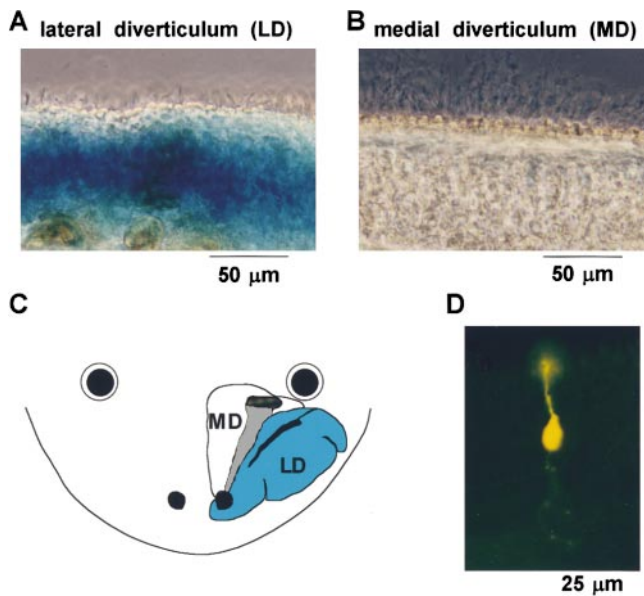


Figure 1. *Xenopus laevis* olfactory receptor neurons in the sensory epithelium. Light microscopic photomicrographs of slices of olfactory epithelia in the lateral diverticulum (A) and in the medial diverticulum (B) after the frog was cultured in 0.1% methylene blue water for 6 h. (C) A schema of the olfactory organ of *Xenopus laevis*. (D) An olfactory receptor neuron in the lateral diverticulum dialyzed with 1% Lucifer yellow. The knoblike structure, long dendrite, axon, and soma are visible.

as the main internal and external membrane-permeable cations, respectively, the mean resting membrane potential for these neurons was  $-46.5 \pm 1.2$  mV ( $n = 68$ ); a value similar to the value measured in the *Xenopus* olfactory receptor neuron (Schild, 1989). The injection of current (1–3 pA) elicited action potentials from a conditioning voltage at  $-60$  mV (Fig. 2 A). The threshold for action potential generation in these neurons was commonly between  $-35$  and  $-55$  mV. A large current injection generated brief trains of action potentials. The impulse trains typically consisted of up to 30 spikes. The firing frequency linearly increased with increasing current injections from 1 to 30 pA and reached a plateau at 30 pA (Fig. 2 B). The action potential latency was reduced with increases in the amplitude of the current injection. The spike induced by current (10 pA) was blocked by  $0.3 \mu\text{M}$  TTX (Fig. 2 C), suggesting that a voltage-sensitive  $\text{Na}^+$  current was involved in its generation.

#### Voltage Response to Injected Current

Voltage-clamp recordings confirm that the current responsible for the rising phase of the current-induced action potential is carried by  $\text{Na}^+$ . Fig. 3 A shows the two major currents elicited by the depolarizing steps of voltage from a holding potential of  $-70$  mV. A transient inward current appeared at greater than  $-40$  mV, and an outward current was observed after the rapid in-

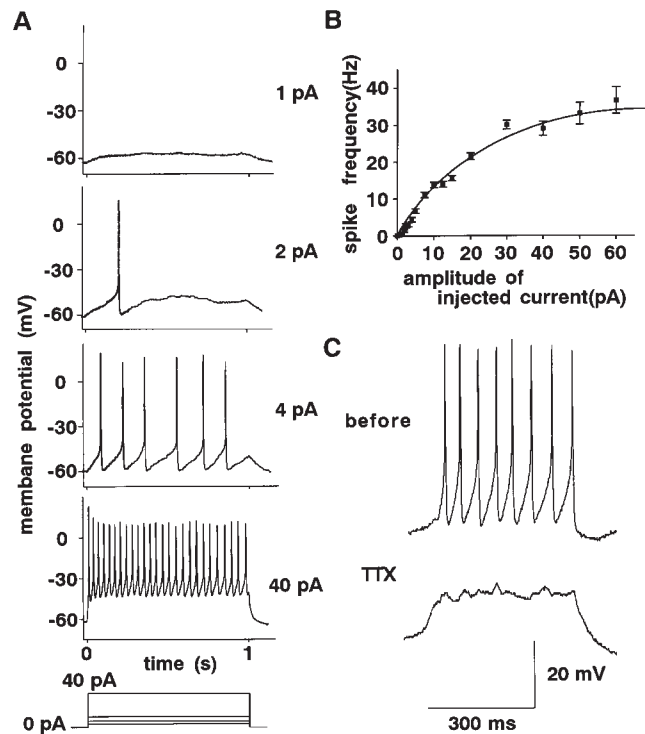


Figure 2. Current-clamp recordings from olfactory receptor neurons in the lateral diverticulum of *Xenopus laevis*. Current-clamp recordings showing the voltage responses to positive current injections from a conditioning potential of  $-60$  mV (A). Above a certain threshold, repetitive action potential spikes were observed. The number next to the action potentials indicates the amplitude of currents injected. These traces were recorded from the same cell. (B) Spike frequency of olfactory receptor neurons as a function of current injection for six neurons that fired repeatedly. (C) An action potential recorded in response to a positive current pulse (40 pA) from the conditioning potential ( $-60$  mV). This action potential response was reversibly blocked by  $1 \mu\text{M}$  TTX.

ward current. The current–voltage curves taken at the peak of the inward and outward currents are shown in Fig. 3 B.

To examine the transient inward currents, internal KCl was substituted with CsCl. Replacing  $\text{Na}^+$  with choline in the external solution reversibly and completely abolished the transient inward current in all five neurons (Fig. 3 C). The addition of  $1 \mu\text{M}$  TTX to the external solution also completely inhibited this current in all four neurons (Fig. 3 D). These results suggest that the current is probably carried by  $\text{Na}^+$ .

To study the outward  $\text{K}^+$  currents, 25 mM tetraethylammonium (TEA) was substituted with an equivalent amount of NaCl, and  $1 \mu\text{M}$  TTX was added to the external medium. The outward current was partially blocked by the addition of 25 mM TEA to the external solution (Fig. 3 E) in all six neurons. The magnitude of the outward currents was reduced to  $32.3 \pm 4.4\%$  ( $n = 6$ ) by 25 mM TEA. The presence of a  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  current in the neuron was studied by measuring the

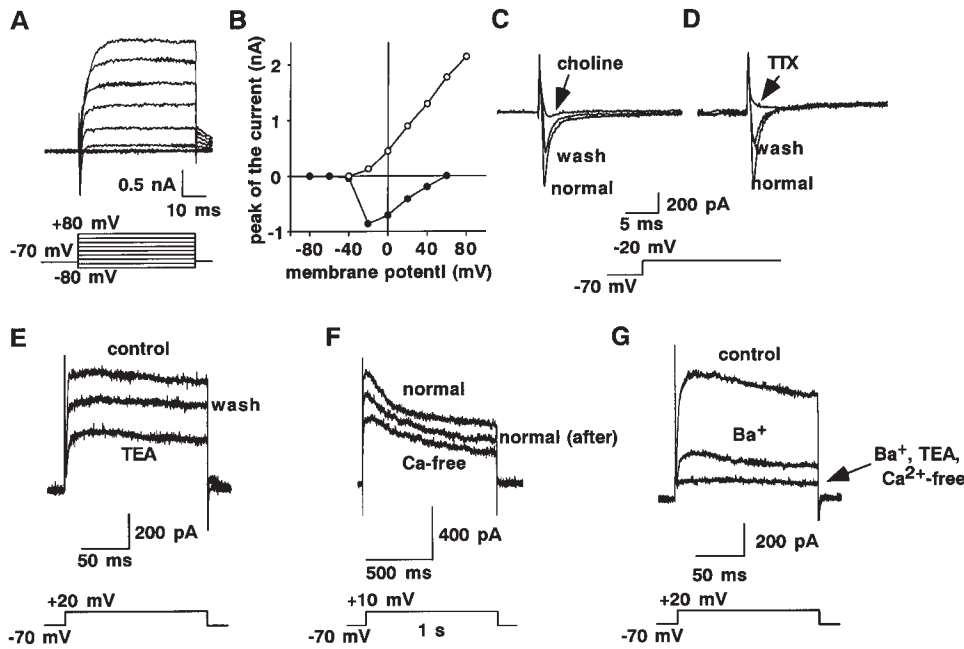


Figure 3. (A) The currents seen under voltage-clamp conditions in response to negative and positive voltage pulses from  $-70$  mV. (B) Current-voltage relations. The peak of the transient inward current (●) and the outward current (○). Isolated  $\text{Na}^+$  currents from olfactory neurons in the lateral diverticulum. The voltage-activated  $\text{Na}^+$  current is blocked after the substitution of  $\text{Na}^+$  with choline in the external solution (C) or the addition of  $1 \mu\text{M}$  TTX to the external solution (D). The internal solution contained (mM): 115 CsCl, 2  $\text{MgCl}_2$ , 2 EGTA, 10 HEPES-NaOH, pH 7.4. The external EGTA solution contained (mM): 116 NaCl, 4 KCl, 1  $\text{MgCl}_2$ , 1 EGTA, 10 glucose, 10 HEPES-NaOH, pH 7.4. Isolated  $\text{K}^+$  currents in olfactory receptor neurons in the lateral diverticulum of *Xenopus laevis*,

and the effect of the application of TEA and the elimination of  $\text{Ca}^{2+}$  (E–H). (E) The  $\text{K}^+$  current responses to positive voltage pulses from a holding potential of  $-70$  mV. This current was reversibly blocked by 25 mM TEA (F and G). The external solution contained (mM): 116 *N*-methyl-*D*-glucamine, 1 EGTA, 2  $\text{CoCl}_2$ , 10 glucose, 10 HEPES-KOH, pH 7.4. (H) The outward  $\text{K}^+$  current is partially attenuated by the elimination of  $\text{Ca}^{2+}$  from the external solution. The  $\text{Ca}^{2+}$ -free solution contained (mM): 116 NaCl, 4 KCl, 1  $\text{MgCl}_2$ , 1 EGTA, 10 glucose, 10 HEPES-KOH, pH 7.4.

outward current in an external solution with and without  $\text{Ca}^{2+}$ , respectively. The outward current was partially attenuated by the elimination of  $\text{Ca}^{2+}$  into the external solution (Fig. 3 F), suggesting the presence of a  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channel. Recently,  $\text{K}^+$  currents mediated with RELK1 channels insensitive to 100 mM TEA have been shown to be blocked by  $\text{Ba}^{2+}$  (Engeland et al., 1998). As shown in Fig. 3 G, TEA-insensitive outward currents in *Xenopus* water nose olfactory receptor neurons were also blocked by  $\text{Ba}^{2+}$ .

#### Current Responses to Water-soluble and Volatile Odorants

To evaluate the odor selectivity of the olfactory neuron of the lateral diverticulum, an amino acid cocktail and two volatile odorant cocktails were separately applied to neurons. The application of these three odorant cocktails induced inward currents in 46 of 238 neurons (Fig. 4). In 7 of 32 neurons responding to the amino acid cocktail, the magnitude of the inward current was reduced rapidly but not completely during continuous stimulation (Fig. 4 A). The remaining responses to the amino acid cocktail did not show sharp peaks (Fig. 4 B). The peak amplitude of inward currents in response to the amino acid cocktail ranged, typically, from 0 to 126 pA. The mean amplitude of inward currents in response to odorant cocktails was calculated using neurons that responded to any one of the three odorant cocktails.

The volatile odorant cocktails I and II induced inward current responses in 17 (37%) and 14 (30%) neurons, respectively (Fig. 4, C and D). The peak amplitudes of inward currents induced by the odorant cocktails I and II ranged, typically, from 0 to 100 and 0 to 164 pA, respectively. There are single neurons that responded to both cocktails. Some single neurons responded to all three cocktails (i.e., the amino acid cocktail as well as the two volatile odorant cocktails). One example of plural responses is shown in Fig. 4 E. In this neuron, the response to the amino acid cocktail is larger than that to the volatile odorant cocktail I or the volatile odorant cocktail II.

Fig. 5 shows the response profiles of individual olfactory neurons to the amino acid cocktail, the volatile odorant cocktail I, and the volatile odorant cocktail II. The population of neurons responding to the amino acid cocktail was larger than that responding to either of the two volatile odorant cocktails. 5 of 46 single olfactory neurons responded to all three odorant cocktails. Seven neurons responded to two odorant cocktails. Thus,  $\sim 26\%$  of the single olfactory neurons responded to plural odorant cocktails. More than 19% of the neurons responded to both the amino acid cocktail and the volatile odorant cocktail.

Alanine of varying concentrations was applied to the receptor neurons (Fig. 6 A). The magnitude of the inward current induced by alanine increased with increases in alanine concentrations. Fig. 6 B shows the

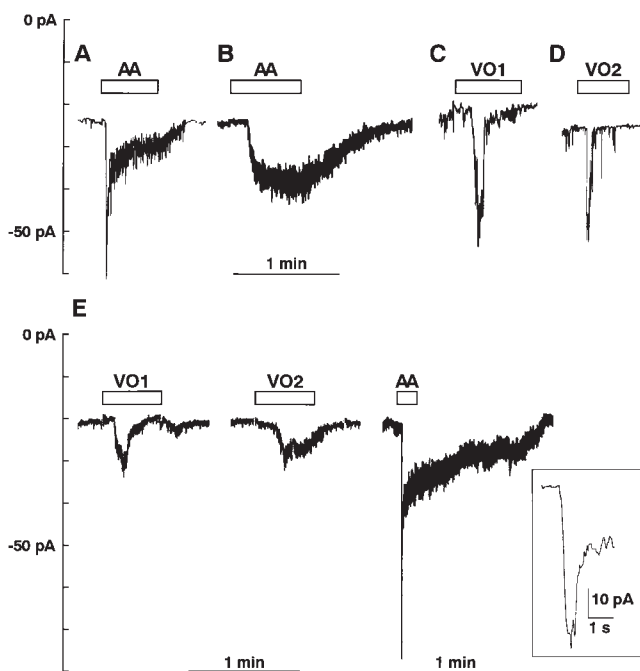


Figure 4. Inward currents in response to the amino acid cocktail (A and B). Inward currents in response to volatile odorant cocktail I (C) and cocktail II (D). All responses were recorded from different neurons (A~D). (E) Inward currents in response to volatile odorant cocktail I, volatile odorant cocktail II, and the amino acid cocktail, respectively. The same inward current in response to the amino acid cocktail was shown with a short time scale in inset. All responses were recorded from the same neuron (E). The holding potential was  $-70$  mV.

magnitudes of the responses to alanine of various concentrations. The currents appeared at  $100 \mu\text{M}$  and increased with increases in concentration. The application of arginine also induced inward current responses in a dose-dependent manner (Fig. 6 C).

To explore the selectivity of single olfactory neurons with neutral, basic, and acidic amino acids, alanine, arginine, and glutamic acid were applied to the olfactory neuron of the lateral diverticulum (Fig. 7). The application of  $100 \mu\text{M}$  of individual amino acids induced inward current responses in the single sensory neuron, as shown in Fig. 7 A. The magnitude of the response to arginine was larger than that to alanine or glutamic acid. The neurons shown in Fig. 7 B responded to either  $1$  mM alanine or  $1$  mM glutamic acid, but not to  $1$  mM arginine. Thus, although single olfactory neurons responded to plural amino acids, the response selectivity was differentiated among neurons. Fig. 7 C shows the response profiles of individual olfactory neurons to  $100 \mu\text{M}$  or  $1$  mM alanine, arginine, and glutamic acid, respectively. These amino acids induced responses in 13 of 152 neurons. More than 50% of the sensory neurons responded to plural amino acids. In particular, four neurons (31% of neurons stimulated by three odorants) responded to all three amino acids.

Cell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
AA	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
VO1	○	○	○	○	○	○	○	○	○	×	×	×	×	×	×	×
VO2	○	○	○	○	○	×	×	×	×	×	×	×	×	×	×	×
Cell	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
AA	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
VO1	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
VO2	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Cell	33	34	35	36	37	38	39	40	41	42	43	44	45	46		
AA	×	×	×	×	×	×	×	×	×	×	×	×	×	×		
VO1	○	○	○	○	○	○	○	○	×	×	×	×	×	×		
VO2	○	○	○	×	×	×	×	×	○	○	○	○	○	○		

Figure 5. Response profile of single olfactory neurons to various odorants as obtained by the whole-cell clamp technique. The ○ and × indicate an inward current and no current, respectively, upon the application of odorants.

Lilial and citralva at different concentrations were applied to single olfactory neurons. The neuron shown in Fig. 8 A responded to  $1$  and  $10 \mu\text{M}$ , and to  $1$  mM alanine. Six of nine cells responded to alanine, and to lilial, and/or citralva. Fig. 8, B and C, shows the magnitudes of response to lilial and citralva of varying concentrations. Distinct responses appeared at  $1 \mu\text{M}$  for the odorants, indicating that the olfactory receptor neuron of the water nose responds sensitively to volatile odorants.

## discussion

In this study, we examined the electrophysiological properties of receptor neurons in the lateral diverticulum of the olfactory sensory epithelium of *Xenopus laevis* using the whole-cell voltage-clamp technique, and showed that olfactory receptor neurons in the water nose respond to both amino acids and volatile odorants.

Odorants are considered to be received by olfactory G-protein-coupled receptors, which are linked to adenylyl cyclase or phospholipase C via the G-proteins (Buck and Axel, 1991; Breer et al., 1994). In situ hybridization experiments to identify olfactory G-protein-coupled receptors in the rat, mouse, and catfish have suggested that a single olfactory G-protein-coupled receptor was expressed in only 0.1–2% of olfactory neurons, and that each olfactory neuron might have only a single particular type of olfactory G-protein-coupled receptor (Axel, 1995; Kishimoto et al., 1994; Ngai et al., 1993; Ressler et al., 1993, 1994; Sullivan et al., 1995; Vassar et al., 1993). In the *Xenopus laevis* olfactory system, olfactory G-protein-coupled receptors have been cloned by Freitag et al. (1995). Probing for individual receptor mRNA suggests that only a small per-



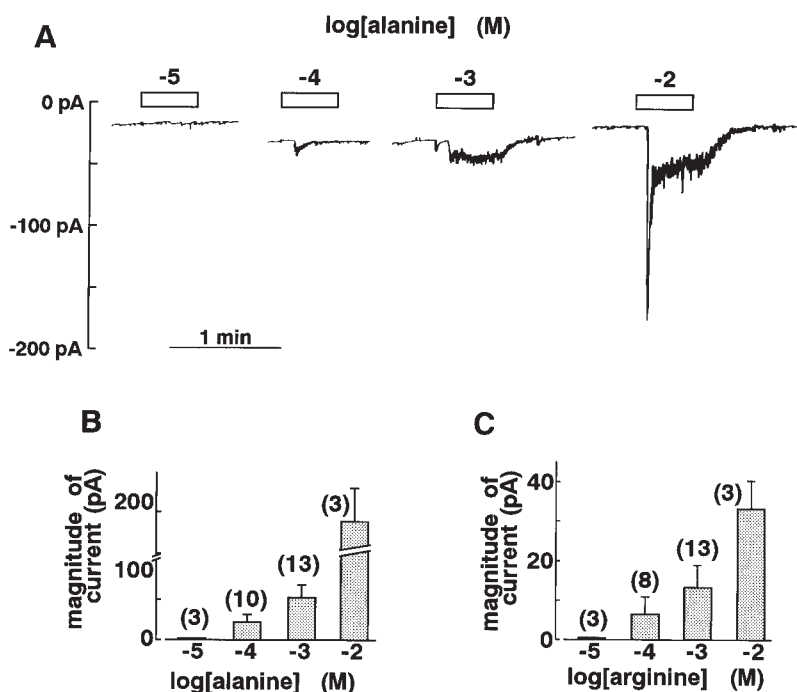


Figure 6. Inward currents in response to alanine of varying concentrations (A). Responses from different neurons were recorded. Mean magnitude of inward currents in response to alanine (B) and arginine (C) of varying concentrations. Each point is the mean  $\pm$  SEM of data obtained from  $n$  preparations that responded to any of the amino acids applied. The holding potential was  $-70$  mV.

centage of the *Xenopus laevis* olfactory neurons ( $<1\%$ ) express any particular olfactory receptor protein.

In the present study, we showed that  $>50\%$  of single olfactory neurons of *Xenopus laevis* respond to a variety of amino acids, including acidic, neutral, and basic

amino acids. Single olfactory neurons in catfish also responded to plural amino acids (Ivanova and Caprio, 1993; Kang and Caprio, 1995). Single olfactory neurons in other animals in the rat, frog, and turtle respond to many species of odorants with diverse struc-

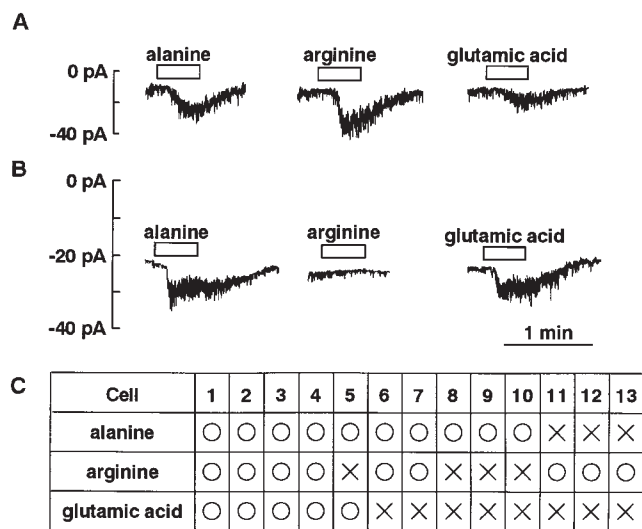


Figure 7. Inward currents in response to  $100 \mu\text{M}$  alanine,  $100 \mu\text{M}$  arginine, and  $100 \mu\text{M}$  glutamic acid (A), and those to  $1 \text{ mM}$  alanine,  $1 \text{ mM}$  arginine, and  $1 \text{ mM}$  glutamic acid (B). All responses to each of the three traces were recorded from the same neurons. The holding potential was  $-70$  mV. (C) Response profile of single olfactory neurons to various odorants of  $1 \text{ mM}$  as obtained by the whole-cell clamp technique. The  $\circ$  and  $\times$  indicate an inward current and no current, respectively, upon the application of odorants.

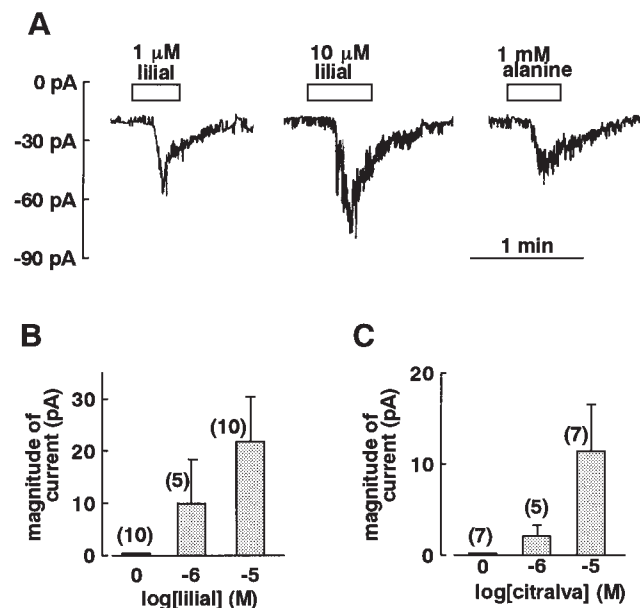


Figure 8. Inward currents in response to  $1$  and  $10 \mu\text{M}$  lilial, and  $1 \text{ mM}$  alanine (A). All responses to each of the four traces were recorded from the same neurons. The holding potential was  $-70$  mV. Mean magnitude of inward currents to lilial (B) and citralva (C) of varying concentrations. Each point is the mean  $\pm$  SEM of data obtained from  $n$  preparations that responded to the odorants applied. The holding potential was  $-70$  mV.

tures (Gesteland et al., 1982; Sicard and Holley, 1984; Kashiwayanagi and Kurihara, 1994; Kashiwayanagi et al., 1996). An olfactory G-protein-coupled receptor (OR5) has a broad specificity for volatile odorants (Raming et al., 1993), hence each olfactory neuron carrying a single type of olfactory G-protein-coupled receptor may respond to many odorants. It is also possible that single olfactory neurons have multiple receptors for various odorants. A cross-adaptation experiment of single olfactory receptor neurons in the turtle and the bullfrog has directly indicated that multiple odorant receptors exist in single olfactory neurons (Kashiwayanagi and Kurihara, 1994; Kashiwayanagi et al., 1996). For example, the magnitude of inward currents induced by the application of hedione to a single neuron after desensitization of the current in response to lylal or citralva is similar to that induced by hedione applied alone, and vice versa.

An *in situ* hybridization study of the olfactory G-protein-coupled receptors of *Xenopus laevis* has shown that G-protein-coupled receptors similar to those in fish exist not in the epithelium of the medial diverticulum, but in the epithelium of the lateral diverticulum, while G-protein-coupled receptors similar to those in mammals exist in the epithelium of the medial diverticulum, but not in the epithelium of the lateral diverticulum (Freitag et al., 1995). These results suggest that olfactory neurons in the medial and lateral diverticula dem-

onstrate differentiated responses to volatile odorants and water-soluble odorants such as amino acids, respectively. Volatile odorants induce inward current responses in the olfactory neuron in the medial diverticulum of the *Xenopus laevis* (Lischka and Schild, 1993; Schild and Lischka, 1994), supporting this idea. In the present study, we showed that olfactory neurons in the lateral diverticulum respond to acidic, neutral, and basic amino acids. Therefore, it is likely that olfactory neurons having G-protein-coupled receptors in the lateral diverticulum similar to those in fish will respond to amino acids.

It should be noted, however, that many olfactory neurons in the lateral diverticulum respond not only to amino acids, but also to volatile odorants. The sensitivity of the neurons to volatile odorants is similar to the olfactory neurons of salamanders (Firestein and Werblin, 1989). It is possible that olfactory G-protein-coupled receptors similar to those in fish receive both water-soluble odorants and volatile odorants, or that unknown olfactory G-protein-coupled receptors similar to those in mammals, which were not observed by Freitag et al. (1995), exist in the lateral diverticulum olfactory neurons. In addition, it is also possible that volatile odorants induce responses in the lateral diverticulum olfactory neurons in an olfactory G-protein-coupled receptor-independent manner.

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