Odorant Receptors Activated by Amino Acids in Sensory Neurons of the Channel Catfish *Ictalurus punctatus*

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**Abstract** Odorant receptors activated by amino acids were investigated with patch-clamp techniques in olfactory receptor neurons of the channel catfish, *Ictalurus punctatus*. The L-isomers of alanine, norvaline, arginine, and glutamate, known to act predominantly on different olfactory receptor sites, activated non-sensitizing inward currents with amplitudes of −2.5 to −280 pA in olfactory neurons voltage-clamped at membrane potentials of −72 or −82 mV. Different amino acids were shown to induce responses in the same sensory neurons; however, the amplitude and the kinetics of the observed whole cell currents differed among the stimuli and may therefore reflect activation of different amino acid receptor types or combinations of receptor types in these cells. Amino acid-induced currents appeared to have diverse voltage dependence and could also be classified according to the amplitude of the spontaneous channel fluctuations underlying the macroscopic currents. A mean single-channel conductance (γ) of 360 fS was estimated from small noise whole-cell currents evoked by arginine within the same olfactory neuron in which a mean γ value of 23.6 pS was estimated from 'large noise' response to norvaline. Quiescent olfactory neurons fired bursts of action potentials in response to either amino acid stimulation or application of 8-Br-cyclic GMP (100 μM), and voltage-gated channels underlying generation of action potentials were similar in these neurons. However, in whole-cell voltage-clamp, 8-Br-cyclic GMP evoked large rectangular current pulses, and single-channel conductances of 275, 220, and 110 pS were obtained from the discrete current levels. These results suggest that in addition to the cyclic nucleotide-gated transduction channels, olfactory neurons of the channel catfish possess a variety of odor receptors coupled to different types of transduction channels.

**Introduction**

Since 1971, amino acids have been recognized as potent olfactory stimuli in different species of fish (Sutterlin and Sutterlin, 1971; Suzuki and Tucker, 1971). Evidence...
obtained from electrophysiological recordings from the entire olfactory organ suggested that olfactory receptor thresholds to amino acids generally ranged from $10^{-8}$ M to $10^{-6}$ M, and that not all amino acids were equally effective stimuli. For the majority of fishes studied, neutral amino acids with long (>2 carbons) side-chains were generally more stimulatory than neutral amino acids with short side-chains and either acidic or basic amino acids (Caprio and Byrd, 1984; Caprio, 1988 for reviews); however, studies in carp (Goh and Tamura, 1978), lamprey (Li and Sorensen, 1992), and goldfish (Zippel, Lago-Schaaf, and Caprio, 1994) indicated that L-arginine, a basic amino acid, was the most potent amino acid stimulus. The different amino acid specificities in different species, along with evidence from cross-adaptation (Caprio and Byrd, 1984), receptor binding (Bruch and Rulli, 1988; Rehnberg and Schreck, 1986; Cagan and Zeiger, 1978), lectin inhibition (Kalinoski, Bruch, and Brand, 1987) and mixture (Caprio, Dudek, and Robinson, 1989; Kang and Caprio, 1991) studies clearly showed that different receptor sites existed for different categories (neutral, acidic, and basic) of amino acids, and possibly also for different amino acids within each category. The distribution of these amino acid receptor sites across individual olfactory neurons, however, is currently unknown. Apparently, because of the high density and small size of the olfactory receptor neurons located within the olfactory lamellae along with the general instability of a lamellar organization, only one in vivo study of single olfactory receptor units in fishes have been reported (Suzuki, 1978). Direct recordings of the odorant-activated membrane current in solitary olfactory receptor neurons (Kurahashi, 1989; Firestein, Shepherd, and Werblin, 1990) provided a different approach to the characterization of odorant receptors present in sensory neurons. Because olfactory receptor neurons of the channel catfish readily form a high resistance seal with the clean tip of a glass pipette, this preparation is especially suitable for patch-clamp recordings. Further, these neurons can be obtained in relatively large numbers in an isolation procedure which does not require enzyme treatment, and therefore the cells are likely to maintain their odorant sensitivity. Finally, despite the small size (soma, 3-5 μm diam), dissociated olfactory receptor cells can be reliably identified by the presence of an apical dendrite and olfactory knob. The voltage-activated currents of these neurons have been previously characterized, and features of the whole-cell currents activated by a complex mixture of amino acids have been described (Miyamoto, Restrepo, and Teeter, 1992b). Given the diversification of receptor currents activated by this mixture, Miyamoto and colleagues suggested that multiple mechanisms contribute to the signal processing. Two of these mechanisms might involve G-protein-coupled adenyl cyclase and phospholipase C pathways because both cytoplasmic second messengers, cyclic-AMP and inositol-1,4,5-trisphosphate (IP₃) have the ability to activate depolarizing responses in olfactory receptor cells of the channel catfish (Miyamoto, Restrepo, Cragoe, and Teeter, 1992a; Goulding, Ngai, Kramer, Colicos, Axel, Siegelbaum, and Chess, 1992). We used patch-clamp techniques to address questions concerning odorant receptor distribution among individual olfactory receptor cells and to characterize amino acid receptors present in these cells. Our observations linked the excitatory responses to odorants in these neurons to the activation of more than one type of transduction channels.
MATERIALS AND METHODS

Isolation of Cells

The olfactory organ was removed from the nasal cavity of immobilized (Flaxedil, 0.1 mg/100 g fish), anesthetized (Tetracaine, 4%) channel catfish. The tissue was maintained at 4°C in physiological solution and could be used for 8-10 h. The olfactory organ consists of numerous lamellae radiating outward from a central axis (Caprio and Raderman-Little, 1978). Only the lamellar tissue adjacent to the central axis containing the olfactory neurons was gently triturated in the experimental chamber. No enzymatic treatment occurred before trituration. After settlement of the isolated cells, the chamber was perfused with Ringer's solution to remove the damaged cells, and the remaining cells were examined with phase-contrast optics (total magnification ×400). Olfactory receptor neurons were identified by the presence of an elongated dendrite which formed an olfactory knob at its terminal (Fig. 1). The sensory cilia and the portion of the axon attached to the somata could not be differentiated with the optics used for viewing the electrophysiological experiments. The motile cilia, however, appeared to be present because slow motion of the solitary olfactory receptor neurons was often observed. Several cilia extending from the olfactory knob of receptor cells could be viewed with Nomarski optics (×60 oil immersion objective, total magnification ×900). In whole-cell voltage-clamp, the sensory neurons were further identified by the activation of a transient inward current followed by an outward current in response to step depolarization. Since with increasing time after isolation (more than 1 h) the inward voltage-activated currents were absent in some
neurons, all experiments were performed within 30–60 min after cell dissociation. Interpretation of voltage-clamp data obtained from an elongated structure like an olfactory receptor neuron would be complicated if the membrane potential varied from point to point along the cell. Although only receptor cells with elongated dendrites were selected for the experiments, the dendrite was absorbed by the somata within a few seconds of breakthrough in whole-cell configuration, and the cell became spherical. Further, the cilia could be considered as cables of finite length with one end sealed by the surface membrane. The deviation from the clamp potential in such a system can be ignored if the length of the finite cable is short compared to the length constant defined as \((r_m/r_i)^{1/2}\), where \(r_m\) and \(r_i\) represent the membrane resistance per unit length and the internal resistance of the cytoplasm, respectively (Armstrong and Gilly, 1992). Because the membrane resistance is considerably greater than the resistance of the cytoplasm and the cilia are short cables, a uniform voltage distribution along the cilia is expected under the experimental conditions.

**Recordings**

Whole-cell patch-clamp recordings were made as described by Hamill, Marty, Neher, Sakmann, and Sigworth (1981) and Marty and Neher (1983). The biphasic current transients driven by the action potentials were recorded with a gigaohm sealed pipette in cell-attached voltage clamp configuration (Frings & Lindemann, 1990). Patch pipettes were pulled from thick-walled filament-containing borosilicate glass and their tips were fire-polished. The resistance of pipettes was 5–10 M\(\Omega\) when filled with the internal solution used for recordings. The series resistance was usually 20 M\(\Omega\), and the input resistance exceeded 2 G\(\Omega\) in all experiments.

**Solutions**

The pipette solution contained (mM): KCl, 100; CaCl\(_2\), 0.2; EGTA, 1.0; ATP-Mg, 0.2; HEPES, 5.0; pH 7.2 and the extracellular solution contained: NaCl, 110; KCl, 3.5; CaCl\(_2\), 1.0; MgCl\(_2\), 1.6; HEPES, 5.0; pH 7.5. Amino acid odorants and membrane permeable analogs of cyclic AMP and cyclic GMP were dissolved in the external solution and were applied by perfusing the entire olfactory neuron through a miniature pressure puffing system (Picospritzer II, General Valve Corporation, Fairfield, NJ). Perfusion pipettes were fabricated from borosilicate glass with an initial diameter of 14–16 \(\mu\)M and the tips were fire-polished to uniform geometry and a final inner diameter of 10 \(\mu\)M. A plume (visualized with dye) ejected from such a pipette appeared linear and uniform in width downstream from the tip of the pipette. Because the high flow rate separated the cell from the recording electrode when the stimulus was applied near the electrode tip, the distance between the cell and the center of the perfusion pipette was 165 \(\mu\)M in all experiments and was continuously monitored to ensure a standardized delivery of the different odorants. All reported stimulus concentrations are the concentrations of the chemicals within the perfusion pipette.

**Analysis**

Membrane currents were recorded using a Dagan 3900 patch-clamp amplifier. Amino acid- and 8-Br-cyclic GMP-activated currents were low-pass filtered at 1 or 5 kHz and were stored on VHS tape for off-line analysis. Records were analyzed using the software package, Personal Scientific Workstation (Data Wave Technologies, Longmont, CO). For the analysis of whole-cell current fluctuations (Anderson and Stevens, 1973), the signals were high-pass filtered at 0.2 Hz and low-pass filtered at 800 Hz. Analog data were digitized in 0.5 ms intervals to form 10 blocks of 1,024 data points for each original record. The spectral densities for each block of data were calculated and averaged (10 blocks) to obtain a mean power spectrum. The spectral density of background (control) membrane noise was also calculated for 10 blocks of 1024 data points in
the presence of the extracellular solution alone before each amino acid stimulation. The differential spectral density, \( S(f) \), for the amino acid-induced current fluctuations was obtained by subtracting the mean control spectrum from the mean spectrum in the presence of the amino acid. The calculated differential spectra were fitted either with a single Lorentzian or the sum of two Lorentzian components:

\[
S(f) = S(0)/(1 + (f/f_c)^2),
\]

where \( S(f) \) is the spectral density at the frequency \( f \), and \( S(0) \) and \( f_c \) are the zero-frequency asymptope and half-power frequency of the Lorentzian, respectively. The total current variance was calculated from this fit as:

\[
\text{var}(I) = \frac{1}{2\pi} \sum S_i(0) f_i.
\]

The mean single-channel current (Neher and Stevens, 1977) was estimated as:

\[
i = \text{var}(I)/(\langle I \rangle),
\]

where \( \langle I \rangle \) is the mean whole-cell current. The half-power frequency of the Lorentzian corresponds to a time constant, \( \tau_{\text{noise}} \):

\[
\tau_{\text{noise}} = 1/(2\pi f_c).
\]

Voltage gated currents were stored on computer and analyzed using PCLAMP software (Axon Instruments, Foster City, CA).

**RESULTS**

**Whole-Cell Currents Induced by Amino Acids**

At negative holding potentials, applications of individual amino acids (100 \( \mu \)M) or their mixture (each component at 250 \( \mu \)M) caused inward currents in fourteen of 90 cells. Whole-cell currents of \(-2.5 \text{ to } -280 \text{ pA} \) were recorded at potentials of either \(-72 \text{ or } -82 \text{ mV} \). The currents developed 400–600 ms after the onset of stimulation and did not desensitize during application (3 to 10 s). Our attempt to eliminate the delay due to the 165 \( \mu \)M distance between the olfactory neuron and the perfusion pipette in order to estimate the latency in the activation of the receptors was unsuccessful because the pressure pulse from the perfusion pipette systematically disturbed the cells when the pipette was positioned closer.

The L-isomers of arginine, glutamate and either alanine or norvaline were applied to six of the responsive cells. Single olfactory neurons responded to all three amino acids; however, the amplitude of the whole-cell currents acquired at the same potential differed among the stimuli (Fig. 2, A–C). In some of the cells, activation of current during maintained stimulation followed a different time course for the different amino acids, suggesting that several mechanisms contributed to the activation of the odor response (Fig. 3). In addition, the most effective odorant varied among the cells, and the proportion of currents induced by neutral, basic and acidic amino acids was different for each receptor neuron examined (Fig. 4). Altogether, these results imply that the observed whole-cell currents in response to alanine, norvaline, arginine and glutamate represent activation of different amino acid receptor types or combination of receptor types in these cells.

Plots of whole-cell currents vs holding potential (\(-40 \text{ to } -100 \text{ mV} \)) revealed a
diversity in the voltage dependence of the receptors. In five cells, the current was a linear function of membrane potential (for example, Fig. 2D); however, the reversal potential could not always be determined by linear extrapolation because in the voltage range between -30 and +20 mV the current tended to be smaller than

![Image](A)

![Image](B)

![Image](C)

![Image](D)

**Figure 2.** Whole-cell currents evoked by 100 μM alanine (A), glutamate (B) and arginine (C) in a single olfactory neuron voltage-clamped at potentials indicated above each trace. The duration of each stimulus application is 3 s. Note the similarity in responses to glutamate and arginine at -60 mV and the differences in the amplitude and kinetics of currents induced by these amino acids at -100 mV. (D) Current-voltage relations of whole-cell currents induced in the same cell by alanine (filled squares), glutamate (filled triangles) and arginine (filled circles). The lines indicate the least-squares linear fit to the experimental points and give slope conductances of 0.3 nS (alanine, $R^2 = 0.9967$), 1.3 nS (arginine, $R^2 = 0.9999$) and 2.5 nS (glutamate, $R^2 = 0.9959$). The ratio of currents, $I_{\text{alanine}}/I_{\text{arginine}}/I_{\text{glutamate}}$, at -82 mV was 1:3:4.

Repeated application of each amino acid at the same membrane potential 10 min after the initial stimulation evoked increased (23–64%) currents (alanine [square], glutamate [triangle], arginine [circle]).

expected from a constant conductance corresponding to the linear segment of the $I-V$ curve in the more negative range (Fig. 5). The reversal potential, when we were able to measure it, was near 0 mV ($n = 4$ cells). The olfactory receptor neurons rarely tolerated holding potentials more positive than -40 mV. Driving the membrane
potential in this direction resulted in instant deterioration of the recording quality and was associated with both an increase in the volume of the cell and a change in its appearance. Generally, after breakthrough in whole-cell configuration, the healthy cells which were likely to provide stable recordings were compact and spherical with a contrast surface, while cells which began to swell and become transparent rarely survived the experiment. Thus, most of our experiments were restricted in the voltage range where olfactory receptor neurons were functional.

A nonlinear current-voltage relation was found for norvaline, glutamate and arginine in a single neuron (Fig. 6) and for alanine and norvaline in two additional neurons. The I-V curves for these cells in the range of $-100$ mV to $-40$ mV were fitted by an exponential equation: $I = I(O) \exp\left(-V_m/H\right)$ with a constant of voltage-dependence, $H$, ranging from 41 to 90 mV (Fig. 7).
A transition in odor-activated channels from a low-conductance to a high-conductance state with membrane depolarization from -100 to -50 mV was previously described for receptor channels activated by an amino acid mixture in channel catfish olfactory neurons (Miyamoto et al., 1992b). This unusual voltage dependence for ligand-gated receptors was observed in our experiments for each of the neutral, basic and acidic receptor types. In these recordings, an amino acid applied at a holding potential of -50 mV produced inward current almost identical to that produced at -82 and -100 mV (Fig. 6 C). In other cells, the amplitude of amino acid-induced current even increased with depolarization of the cell from -100 to -50 or -40 mV.

FIGURE 4. Responses of six individual olfactory receptor neurons to glutamate, arginine, norvaline and alanine. The inward whole-cell currents activated by the different amino acids (100 µM) are plotted as vertical bars. Holding potential: -82 mV for cells 1, 3, 4, and 5; -72 mV for cell 2 and -100 mV for cell 6.

(Fig. 8). A closer study of these neurons showed that a second stimulation reduced rectification, and multiple applications of the same amino acid completely abolished it (Fig. 6 C and Fig. 8).

Inward currents evoked by amino acids in some olfactory neurons were accompanied by an obvious increase in current fluctuations (Fig. 9), whereas in other neurons, an increase in current noise was not detected despite the relatively large amplitudes of the induced whole-cell currents (Figs. 2, 3). These two types of responses closely resembled “large noise” and “small noise” responses to glutamate found in cerebellum granule cells (Cull-Candy, Howe, and Ogden, 1988). Both large noise and small
noise responses could be activated by different amino acids in the same olfactory neuron (Fig. 9). Power spectra resolved from whole-cell amino acid-induced current noise in such an olfactory neuron gave different estimates for the elementary characteristics of the channels underlying the macroscopic currents. The spectrum for alanine-evoked noise (Fig. 10 A) was fitted with a single Lorentzian with $f_c$ value of 75 Hz ($\tau_{\text{noise}} = 2.1$ ms) and a single-channel conductance of 3.5 pS was calculated, assuming a reversal potential of 0 mV. The $f_c$ (75 Hz) and $\gamma$ (3.6 pS) values obtained from a second single Lorentzian power spectrum resolved from current fluctuations during repeated application of alanine to the same cell (not shown) were consistent with $f_c$ and $\gamma$ estimates from the first alanine spectrum. Two Lorentzian components were required to fit both glutamate and norvaline noise spectra (Fig. 10, B and D). The half-power frequencies of the two components for glutamate were 10 and 75 Hz, which corresponded to $\tau_{\text{noise}}$ values of 15.9 and 2.1 ms; a single-channel conductance of 7.7 pS was estimated for this cell from the glutamate noise. The Lorentzian
components resolved in the norvaline noise spectrum (Fig. 10 D) had \( f_c \) values of 4.5 and 120 Hz, and these values gave \( \tau_{\text{noise}} \) estimates of 35.4 and 1.3 ms. The single-channel conductance estimated from the ratio of current variance to the mean norvaline-induced whole-cell current was 23.6 pS for an extrapolated reversal potential of 0 mV. Because of the small increase in the current variance during the arginine-evoked currents, the power spectra were not well-defined at -72 mV; however, a single Lorentzian spectrum was resolved at a clamp potential of -90 mV.

![Image](image.png)

**Figure 6.** Whole-cell currents evoked by 100 \( \mu \)M norvaline (A), arginine (B), alanine (C), and glutamate (D) in the same olfactory receptor cell voltage-clamped at -50, -82, and -100 mV. The minor decline of the norvaline-induced current at -100 mV (A) is the only example of desensitization observed in our experiments. Initially, alanine evoked currents with similar amplitudes at each membrane potential; however, repeated application of alanine at -82 and -100 mV induced larger inward currents which increased linearly with hyperpolarization. (E) norvaline re-applied 50 min after its first presentation to the same cell produced currents similar to those recorded initially. The duration of each stimulus application was 10 s. (F) plots of the current-voltage relations of whole-cell currents induced by norvaline, shown in A (filled squares), arginine (filled circles), and glutamate (filled triangles). Points represent the absolute value of the mean inward whole-cell currents. The lines are the exponential fits to these points \( (R^2 = 0.9962 \text{ for norvaline, } R^2 = 0.9985 \text{ for glutamate, } R^2 = 0.9969 \text{ for arginine}). \)

An \( f_c \) value of 55 Hz was obtained from this spectrum \( (\tau_{\text{noise}} = 2.9 \text{ ms}) \), and a single-channel conductance of 360 fS was estimated from noise analysis.

**Firing Properties of Olfactory Receptor Neurons**

The currents flowing through the amino acid-activated channels depolarized the membrane and resulted in the production of action potentials by olfactory receptor neurons which could be detected in the cell-attached configuration, but not under...
whole-cell current-clamp. Therefore, the biphasic current transients driven by the action potentials (Frings and Lindemann, 1990) were used to survey cell excitability. Several patterns of excitatory responses to amino acid stimulation were distinguished in olfactory receptor cells of channel catfish. In sensory neurons exhibiting complex spontaneous activity, excitation was typified by an increase in the firing rate (Fig. 11 A). Other cells which were quiescent or had low, irregular spontaneous activity elicited a train of action potentials in response to amino acid stimulation (Fig. 11 B). There was an appreciable variability in the time scale of evoked neural activity. The onset of the evoked activity followed the onset of stimulation with a delay ranging from 400 ms to 20–30 s (Fig. 11 C). Such a remote generation of action potentials in some of the sensory neurons suggests that amino acids act on G-protein-coupled receptors, initiating openings of transduction channels different from the relatively fast receptor channels underlying the inward currents previously described. Recent patch-clamp studies indicated that both cyclic nucleotides (Goulding et al., 1992) and IP3 (Miyamoto, Restrepo, Cragoe, Jr. and Teeter, 1992a) directly activate depolarizing conductances in channel catfish olfactory neurons. Since only membrane permeable analogs of cyclic AMP and cyclic GMP were available, we examined the effect of 8-Br-cyclic AMP (100 μM) and 8-Br-cyclic GMP (100 μM) on quiescent olfactory neurons. Both membrane-permeable cyclic nucleotide analogs were applied in identical conditions to eight sensory cells. In four neurons, 8-Br-cyclic GMP, but not 8-Br-cyclic AMP, evoked a burst of action potentials (Fig. 11 D), analogous to the neural activity observed in different cells in response to odor stimulation. The initial onset of activity was followed by subsequent bursts of spikes.

Six voltage-activated currents underlying excitability of olfactory receptor neurons of the channel catfish were differentiated and previously described: a transient Na+ inward current, a small sustained inward current classified as t-type Ca2+ current, a
Ca\textsuperscript{2+}-activated K\textsuperscript{+} current, a transient K\textsuperscript{+} current, a delayed rectifier K\textsuperscript{+} current, and a tiny inward rectifier K\textsuperscript{+} current (Miyamoto et al., 1992b). In our experiments, step depolarization of the cell membrane under voltage clamp from a holding potential of \(-82\) mV to various test potentials elicited similar voltage-gated currents in the cells responsive to amino acids as in the cells responsive to 8-Br-cyclic GMP (Fig. 12). Thus, the background complex of voltage-activated conductances which contribute to the generation of action potentials was possibly the same in these neurons. However, in two of the neurons in which whole-cell configuration was successfully obtained,

![Diagram of whole-cell inward currents evoked by norvaline (100 \mu M) in an olfactory receptor neuron.](image)

**Figure 8.** (A) Whole-cell inward currents evoked by norvaline (100 \mu M) in an olfactory receptor neuron. The initial two applications of norvaline at \(-82\) and \(-60\) mV activated small currents of similar amplitude. Norvaline applied at \(-40\) mV caused a larger inward current compared to those initially recorded at \(-60\) and \(-82\) mV. Subsequent applications of norvaline at \(-60\) and \(-82\) mV (not shown) activated larger currents than those recorded initially. Currents were recorded consecutively, with 2-min intervals between stimulations. The extracellular solution contained physiological concentrations of Ca\textsuperscript{2+} and Mg\textsuperscript{2+}. (B) Plot of current-voltage relation for norvaline-induced whole-cell currents shown in A. Numbers indicate the order of the applications of norvaline.

8-Br-cyclic GMP (100 \mu M) activated large unitary inward current pulses (Fig. 13) rather than a macroscopic current seen in response to amino acids. The maximal unitary current amplitude of 22.5 pA at \(-82\) mV appeared the same for both olfactory neurons and was larger than the amplitude of the mean whole-cell currents induced in several cells by amino acids (Fig. 4). Assuming a reversal potential value of 0 mV for the ionic current flowing through the nonselective cyclic-nucleotide activated channels, a single-channel conductance of \(\sim 275\) pS was obtained from the maximal current level. Direct transitions between the maximal conductance level and
FIGURE 9. 'Large noise' and 'small noise' inward currents recorded in whole-cell voltage clamp from the same sensory neuron. At a membrane potential of -72 mV, arginine induced the largest whole-cell current (mean = -159 pA) and the smallest increase in current noise, whereas the macroscopic currents evoked by alanine (mean = -113 pA), glutamate (mean = -46.5 pA) and norvaline (-21 pA at -90 mV) were accompanied by large increases in current fluctuations.

the closed state and from the closed state to the subconductance levels of approximately 110 and 220 pS were also observed (Fig. 13).

DISCUSSION

Binding of odor molecules to receptors located in the fine sensory cilia of olfactory receptor cells initiate a sequence of events which controls the firing rate of the axon
Figure 10. Power spectra of whole-cell current noise evoked by application of alanine, glutamate, arginine and norvaline to the cell in Fig. 9. Presented are the net spectra obtained by subtracting the spectral density of the basic membrane noise from the spectral density of the current noise in the presence of an amino acid. (A) Power spectrum of the current noise evoked by 100 μM alanine at -72 mV. The spectrum was fitted as a single Lorentzian with a half-power frequency, $f_0$, of 75 Hz. The increase in current variance, $\text{var}(I)$, was calculated from the fit to the net spectrum. The mean inward current, $\langle I \rangle$, was 123 pA. The single-channel current, $i$, estimated from the ratio $\text{var}(I)/\langle I \rangle$ was 0.25 pA. (B) Power spectrum of current noise evoked by 100 μM-glutamate at -72 mV (mean inward current 46.5 pA). The spectrum was fitted as the sum of two Lorentzians with $f_0$ values of 10 and 75 Hz. The single-channel current, $i$, estimated from this spectrum was 0.56 pA. (C) Power spectrum of current noise evoked by 100 μM-arginine applied at -90 mV (mean inward current: 164 pA). The spectrum was fitted as a single Lorentzian with a half-power frequency of 55 Hz. The estimated single-channel current was 32.6 fA. (D) Power spectrum of noise evoked by 100 μM-norvaline was fitted with the sum of two Lorentzians with half-power frequencies of 4.5 and 120 Hz (holding potential: -90 mV; mean inward current: 21 pA). The estimated single-channel current was 2.12 pA.

of these receptor neurons. The experiments described here provide information on the ion channels specialized for mediating odorant transduction in olfactory receptor cells of channel catfish and allow comparison with transduction channels found in olfactory neurons of other species. Our observations indicate that olfactory receptor neurons possess a variety of channels which open in response to alanine, norvaline, arginine and glutamate. Currents carried through these channels depolarize the cell
membrane, and this depolarization is sufficient to open voltage-gated Na channels, initiating an increase in spike frequency. The present experiments are consistent with evidence from a previous study showing that a complex mixture of amino acids can activate heterogeneous currents in olfactory receptor neurons of the channel catfish, implicating the involvement of multiple pathways in olfactory signal transduction in this species (Miyamoto et al., 1992b). The percentage of responsive cells (20% in Miyamoto et al., 1992b vs 16% in the present report) is similar in both studies. Although the procedures for isolation of sensory neurons were different in the two studies, a consistent percentage of responsive cells indicates that the enzyme (papain) treatment (Miyamoto et al., 1992b) apparently did not affect odorant sensitivity.

![Figure 11](image)

**Figure 11.** Excitatory responses of olfactory receptor neurons. Biphasic current transients related to action potentials were recorded in cell-attached voltage clamp configuration in different cells. (A) the olfactory neuron fired with a complex pattern. Bursts of spontaneous action potentials alternated with quiet intervals. A 4-s superfusion with alanine (100 μM) increased the firing rate in the burst which was followed by a long silent period. (B) glutamate initiated a train of action potentials in another olfactory neuron showing low, irregular spontaneous activity. (C) Remote generation of action potentials in response to a 4-s application of arginine in a different quiescent olfactory neuron. A stimulation of increased duration (10 s) did not eliminate the delay in spike generation. (D) a 20-s application of 8-Br-cyclic GMP (100 μM) initiated bursts of action potentials in another sensory neuron. Responses, shown in B, C, and D were reproducible upon recurrent stimulation.

Because the responses evoked by alanine, norvaline, arginine, and glutamate were notably diverse, three aspects of the odorant-activated membrane currents will be discussed successively: the time course, the voltage dependence and the spontaneous fluctuations of the ionic channels underlying these currents.

**Time Course of the Odorant Responses**

Alanine, norvaline, arginine and glutamate activated inward currents which did not desensitize in the continued presence of the stimulus. In contrast, previous studies using olfactory receptor cells of newt (Kurahashi and Shibuya, 1990) and salamander (Firestein, et al., 1990) showed that maintained odor stimulus evoked a transient
FIGURE 12. Families of voltage-gated currents recorded under voltage clamp in two different olfactory neurons. (A1) The membrane potential of an olfactory neuron exhibiting inward currents in response to amino acid stimulation was depolarized from a holding potential of -82 mV to potentials increasing in 20-mV steps from -62 to +58 mV (the duration of depolarizing steps: 50 ms; interval between the steps: 2 s). Leakage and capacity currents were subtracted by PCLAMP software. Only current traces at membrane potentials of -42, -22, -2, +18, and +38 mV are presented. (A2) Current-voltage relations of currents in A1. Peak transient inward current (circles), peak outward current (filled squares) and outward current at the end of the 50-ms depolarization pulse (squares) from seven traces were plotted against the test potential. Data from two trials with the same cell were remarkably reproducible and were superimposed in the I-V relations. (B1) The membrane potential of the olfactory neuron in Fig. 11 D under voltage clamp was held at -82 mV and depolarized for 50 ms to various test potentials ranging from -67 to +68 mV in 15-mV steps (interval between steps: 2 s). Current traces at test potential of -52, -22, +8, +38, and +68 mV are presented. (B2) Current-voltage relations of voltage-gated currents in the receptor neuron in B1. Peak inward current (circles), peak outward current (filled squares) and outward current at the end of each 50-ms voltage pulse (squares) from ten traces are plotted vs membrane potential. Despite differences in amplitudes of the voltage-activated currents in these two neurons, I-V relations showed obvious similarities.

Inward current. The chemical nature of odor stimuli used in the different experiments is an important consideration for the distinct kinetics of odor-induced currents in different species. Receptors which bind water-soluble odorants, such as amino acids, may be entirely unrelated in structure, mechanism and pharmacology to receptors which bind lipid-soluble molecules.

In some of the receptor cells in our study, the macroscopic kinetics of the amino acid-activated currents were more complex than would be obtained if the opening of
the channels were governed by a single mechanism. In these cells, the inward current activated normally during application of an odorant, but then instead of reaching a steady level, the current rose again. The secondary current increment emerged ~1 s after the initial activation of the response. Further, the amplitude of the amino acid-induced conductance often increased upon repeated stimulation with the same amino acid. The evidence for the augmentation of inward current for at least some receptor cells in our study indicates that in addition to the opening of transduction channels, amino acids trigger secondary intracellular processes affecting activation of these channels. The accumulation of Ca$^{2+}$ or other second messenger molecule in olfactory receptor neurons might be expected to account for the gradual increase in transduction currents. A regulatory function of intracellular Ca$^{2+}$ on cyclic nucleotide gated channels in olfactory receptor cells of salamander was recently described (Zufall, Shepherd, and Firestein, 1991). Ca$^{2+}$ influx through the odor-sensitive channels was suggested to stabilize a closed state of these channels and thus contribute to the decay of the inward current observed in amphibian olfactory neurons (Firestein et al., 1990; Kurahashi and Shibuya, 1990). A rise in internal free [Ca$^{2+}$] during application of amino acids to channel catfish olfactory neurons was detected (Restrepo, Miyamoto, Bryant, and Teeter, 1990; Restrepo and Boyle, 1991); however, as described above, the kinetics of the odor-induced currents in the different species were different, and the action of intracellular Ca$^{2+}$ ions on the functioning of the amino acid receptor channels is currently unknown.

**Voltage Dependence of the Odorant Responses**

Both linear and curvilinear $I$-$V$ relations were found for whole-cell currents induced by each of the four amino acids in different receptor cells. In addition, for some neurons, the amplitude of the amino acid-induced current either remained approximately constant or declined with hyperpolarization in the voltage range of ~50 to ~100 mV. However, repetitive amino acid stimulation could reduce or completely abolish rectification, and the $I$-$V$ curve became linear or exponential in the same

![Figure 13. Single-channel currents produced by application of 8-Br-cyclic GMP (100 μM) recorded in whole-cell voltage clamp from the cell in Figs. 11 D and 12 B. Holding potential was −82 mV. The openings to the largest conductance level of 275 pS accounted for the majority of the total open time. The traces were selected to illustrate the openings to subconductance levels of 110 pS (top trace) and 220 pS (middle trace) and the final closing of the channel (bottom trace). The currents were inward (the dots marked the closed state in each trace).](image-url)
voltage region. This atypical voltage-dependent activation of the receptor channels could not be attributed to one particular amino acid and appeared for all four stimuli. The currents were difficult to study at membrane potentials more positive than −40 mV, and our experiments left unanswered questions concerning the ionic selectivity of receptor channels activated by the different amino acids. Under the experimental conditions where we were able to measure it, the reversal potential was near 0 mV in receptor cells showing a linear \( I-V \) relation. A reversal potential of 0 mV is consistent with the opening of a nonselective channel permeable to monovalent and divalent cations. Extrapolation of exponential \( I-V \) curves, however, resulted in inward current at a membrane potential of 0 mV and therefore may reflect the opening of channels with a different ionic selectivity.

**Spontaneous Fluctuations of Odorant-induced Membrane Currents**

Amino acid-induced currents were differentiated according to the amplitude of the spontaneous fluctuations of the channels contributing to these currents. Although we referred to two types of responses termed large noise and small noise because of their resemblance to glutamate responses found in cerebellar granule neurons (Cull-Candy et al., 1988), for any amino acid in our study both types of responses were represented in the olfactory neurons. The stationary current records in our experiments were short (3–10 s); nevertheless, the time constants and the mean single channel conductances of the amino acid receptor channels in one of the olfactory neurons showing both large and small noise types of responses were derived using fluctuation analysis. Mean single-channel conductances of 3.5, 7.7, 23.6, pS, and 360 fS were estimated for the openings produced by alanine, glutamate, norvaline, and arginine, respectively (assuming a reversal potential of 0 mV). Similar time constants were obtained from the single Lorentzian spectra of both alanine- \((\tau_{\text{noise}} = 2.1 \text{ ms})\) and arginine-induced fluctuations \((\tau_{\text{noise}} = 2.9 \text{ ms})\), as well from the high frequency component of the glutamate-induced noise \((\tau_{\text{noise}} = 2.1 \text{ ms})\). However, it was not possible to be certain whether the two-component nature of the glutamate spectrum resulted from the gating kinetics of a single type of channel or arose from two separate populations of glutamate-receptor channels, one also activated by alanine or arginine. The channel conductances estimated from the amino acid-induced fluctuations were consistently smaller than the conductance of the smallest 8-Br-cyclic GMP-evoked openings resolved directly in catfish olfactory receptor neurons, implying that amino acids may activate transduction channels different from the cyclic nucleotide-gated channels. One of the amino acid receptor channels appeared to have a unitary conductance in the femtosiemens range. The relatively large amplitudes of the small noise whole-cell currents suggest that these channels are expressed in high density in olfactory receptor neurons.

A maximal conductance of 55 pS was obtained in inside-out patches for the cloned olfactory cyclic nucleotide gated channels of the catfish expressed in oocytes (Goulding et al., 1992). However, for olfactory receptor neurons in the present report, 8-Br-cyclic GMP produced openings to levels of 110, 220, and 275 pS. The transitions from the largest conductance level to the closed level appeared to be direct, and transitions to subconductance levels were infrequent; therefore, it seems likely that the 275 pS events observed here represent openings of a single large conductance channel rather than the simultaneous openings of five independent 55
pS channels. One possibility to explain this discrepancy in the maximal single-channel conductance is to suggest that cyclic nucleotide gated channels form clusters.

**Odorant Receptor Distribution Among Individual Olfactory Receptor Neurons**

Arginine, glutamate and either alanine or norvaline, which primarily activate different receptor sites (Caprio and Byrd, 1984; Bruch and Rulli, 1988), induced responses in the same olfactory neurons. This observation is consistent with previous evidence from extracellular, single-unit recordings in other vertebrates which indicated that individual olfactory receptor neurons respond to qualitatively different odorants (reviewed in Getchell, 1986). However, it is unknown whether different odorants open the same transduction channels in olfactory receptor cells. While a recent study of olfactory receptor neurons of salamander provided the first direct evidence that both an odor mixture and the elevation of cyclic nucleotide intracellular concentration activated the same 40 pS transduction channels (Firestein, Zufall, and Shepherd, 1991), the present study reveals a much larger diversity in signaling pathways. Three observations in the present experiments are difficult to reconcile with the simple idea that different amino acid odorants activate a single receptor type coupled to the same sensory channel. The first concerns the variability in the current fluctuations and differences in the unitary conductances determined from noise analysis of whole-cell currents considered above. The second observation concerns the dependence of the time course of the odorant response on the type of amino acid stimulus and raises the question of how a single second messenger cascade might give rise to both monophasic and biphasic inward currents that were observed in the same cells. Finally, the relative proportions of current amplitudes produced by alanine, norvaline, arginine, and glutamate were different in different cells under similar experimental conditions. If the whole-cell currents represent activation of the same population of receptors, then it is reasonable to expect that under similar conditions one odorant would be the most effective and the proportions of current amplitudes produced by the other stimuli would be approximately the same across different cells. In fact, not only did the most effective stimulus vary from cell to cell, but the relative amplitudes of the responses differed. It thus seems likely that several different receptor types are present in individual olfactory receptor neurons and that these receptors activate more than one population of sensory-receptor channels. Further, because natural odors are generally complex mixtures rather than individual compounds, several different receptor types may contribute to the total depolarization of a single sensory neuron.

In addition to the most common odor-induced excitation, amino acid-induced suppression of spontaneous activity of olfactory receptor neurons were previously reported for both invertebrates (Michel, McClintock, and Ache, 1991) and vertebrates (Dionne, 1992). Two of the amino acids examined in the present study, L-arginine and L-glutamate, elicited both excitatory and inhibitory responses in different olfactory receptor cells of the channel catfish. The mechanism of the inhibitory responses, however, is currently unknown because the amino acids failed to activate directly a current which tends to hyperpolarize the sensory neurons diminishing spontaneous firing. The dialysis of an essential metabolite required for the normal functioning of the amino acid receptors underlying inhibitory responses could be one explanation for the lack of such current in the whole-cell recordings.
Alternatively, arginine, and glutamate were shown to increase a transient voltage-gated potassium current in olfactory receptor neurons of the same species (Ivanova and Caprio, 1992), and this modulation of K channels could affect membrane repolarization during the action potential, thus modifying spontaneous neural activity.

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