Odor suppression of voltage-gated currents contributes to the odor-induced response in olfactory neurons

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Sanhueza, Magdalena, and Juan Bacigalupo. Odor suppression of voltage-gated currents contributes to the odor-induced response in olfactory neurons. *Am. J. Physiol.* 277 (*Cell Physiol.* 46): C1086–C1099, 1999.—Olfactory chemotransduction involves a signaling cascade. In addition to triggering transduction, odors suppress ion conductances. By stimulating with brief odorant pulses, we observed a current associated with odor-induced suppression of voltage-gated conductances and studied its time dependence. We characterized this suppression current in isolated *Caudiverbera caudiverbera* olfactory neurons. All four voltage-gated currents are suppressed by odor pulses in almost every neuron, and suppression is caused by odors inducing excitation and by those inducing inhibition, indicating a nonselective phenomenon, in contrast to transduction. Suppression has a 10-fold shorter latency than transduction. Suppression was more pronounced when odors were applied to the soma than to the cilia, opposite to transduction. Suppression was also present in rat olfactory neurons. Furthermore, we could induce it in *Drosophila* photoreceptor cells, demonstrating its independence from the chemotransduction cascade. We show that odor concentrations causing suppression are similar to those triggering chemotransduction and that both suppression and transduction contribute to the odor response in isolated olfactory neurons. Furthermore, suppression affects spiking, implying a possible physiological role in olfaction.

olfactory transduction; odor excitation; odor inhibition; olfactory cilia

VERTEBRATE OLFACTORY RECEPTOR NEURONS respond to odorants with changes in action potential firing. Excitatory responses, consisting of spiking rate increases, begin with a depolarizing receptor potential triggered by an inward transduction current (I_T). The mechanism underlying I_T involves a cAMP pathway that increases two ionic conductances, a nonselective cationic conductance gated by cAMP (20) and a Ca^{2+}-activated Cl^- conductance (12), both of which contribute to the net inward current. Inhibitory responses have also been reported in some vertebrate species and begin with a hyperpolarizing receptor potential responsible for a decrease in the spiking rate (5, 19; for reviews, see Refs. 2 and 21). The mechanism underlying odor inhibition remains largely unknown. In the Chilean toad *Caudiverbera caudiverbera* it has been studied in some detail (16–19) and shown to be due to a Ca^{2+}-activated K^+ conductance increase, which is a consequence of the activation of a Ca^{2+} conductance. A latency of hundreds of milliseconds precedes the inhibitory transduction current, revealing the participation of a second-messenger pathway. However, details of the second-messenger signaling pathway remain unknown.

All transduction conductances are confined principally to the olfactory cilia (7, 10, 13, 17). In contrast, voltage-gated conductances reside in the cell body. In *Caudiverbera*, four conductances are triggered by depolarization: a TTX-sensitive, inactivating Na^+ conductance; a voltage-dependent, noninactivating Ca^{2+} conductance; a delayed rectifier K^+ conductance; and a Ca^{2+}-activated K^+ conductance (4). In other vertebrate species, a similar array of conductances is present, with an additional inactivating K^+ conductance in some of them (see Ref. 21). When an olfactory cell is depolarized, either by an excitatory odorant or by current injection, all four voltage-gated conductances are activated and give shape to the response consisting of a train of action potentials.

It was previously shown that sustained bath odor exposure of isolated olfactory neurons suppresses the voltage-induced conductance changes (9). This effect was manifested as a reduction in both inward and outward currents triggered by depolarizing voltage steps applied in the presence of odorants. On the other hand, odorant pulses suppressed transduction currents with a very short latency (~20 ms) (11). In the present work we undertook a systematic study of the time dependence of odor suppression of the voltage-gated conductances, using brief odor pulses. Moreover, most electrophysiological studies on olfactory transduction have been conducted on isolated neurons using pulses of odorants. Because our results, like those of Kawai et al. (9), revealed that suppression and transduction share the same odor concentration thresholds, we expected that the effect of suppression should influence odor responses. Therefore, it seemed important to study odor suppression under experimental regimes similar to those typically used to study odor transduction. Here we applied the chemical stimuli onto the entire cell, rather than focusing them onto the olfactory cilia. Our experimental approach, which consisted of pulses of odorants [unlike bath applications that were used by Kawai et al. (9)], allowed us to quantify the contribution of odor suppression to the net odor-induced effect in those cells capable of transducing the applied odors. In this manner, we could evaluate with precision the contribution of both odor-induced effects (transduction and suppression) for every cell, allowing us to correct for suppression the measured current-voltage (I-V) curves and even the odor-triggered currents as a function of time. Our results indicate that suppression develops after a much shorter latency than that preceding the activation of I_T, suggesting that it results from a direct effect of odorants on the voltage-

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gated ion channels. We show that odor suppression of voltage-gated currents takes place in virtually every olfactory neuron in the toad and in the majority of these receptor cells in the rat, at the odor concentrations used in the present study. However, at higher concentrations, we observed that also all rat olfactory neurons displayed suppression. We also document that suppression occurs in nonolfactory cells as well, as in *Drosophila* photoreceptors, indicating that suppression is independent of the olfactory transduction cascade. We also show that suppression may be physiologically relevant in olfactory neurons, since it alters action potential firing activity.

MATERIALS AND METHODS

Cell Dissociation

Toad. Animals (*C. caudiverbera*) were cooled down to 0°C, killed, and pithed before dissecting out their olfactory epithelia. Olfactory receptor neurons were dissociated from the olfactory epithelium, as described by Morales et al. (19).

Rat. For obtaining dissociated olfactory neurons, Wistar rats were decapitated after exposure to CO_2. Olfactory epithelia dissected from the turbinates were cut into small pieces and mechanically dissociated, without the use of enzymes.

Drosophila. Omatidia were isolated from the eyes of Oregon-R flies, as described by Bacigalupo et al. (1). Single photoreceptor cells in isolated omatidia were whole cell voltage clamped.

Electrical Recordings and Stimulus Application

Electrical recordings were obtained with the patch-clamp technique (amplifier by Dagan), in its whole cell modality, as in our previous work (19). Experimental protocols and data analysis were conducted using pCLAMP 6.0 (Axon Instruments).

Odor stimulation was achieved with double-barreled puffer pipettes made of glass (tip diameter ~2 μm, Sutter Instruments). They were positioned 20 or 40 μm from the cell. Stimuli engulfed the entire cell, except in the localization experiments. Pressure pulses (range 2–14 lb/in.^{2}) were given to the pipettes with a picospritzer. We estimated that the delay of the puffing system was ~20 ms (delay of the current change induced by a H_2O pulse delivered ~5 μm from the recording pipette). Latencies were corrected for (see RESULTS). Odor concentrations at the cell level were estimated as in Firestein and Werblin (8).

Solutions

Toad. Extracellular solutions were normal Ringer (NR) solution containing (in mM) 115 NaCl, 1 $CaCl_2$, 1.5 $MgCl_2$, 2.5 KCl, 3 glucose, and 10 HEPES, pH 7.6; 0-Ca^{2+} Ringer solution containing 2 mM EGTA and enough Ca^{2+} to give pCa 8.0 (Win Max); and high-K^+ Ringer solution containing 100 mM KCl, 17.5 mM NaCl, and all other components as in NR. The internal solution contained (in mM) 120 KCl, 1 $CaCl_2$, 2 EGTA, 1 $MgCl_2$, 0.1 Na_2-GTP, 1 Mg-ATP, and 4 HEPES, pH 7.6, pCa 7.5.

Rat. The external solution contained (in mM) 1.3 $CaCl_2$, 1.0 $MgCl_2$, 0.7 $MgSO_4$, 5.4 KCl, 0.30 K_2HPO_4, 137 NaCl, and 1.2 Na_2HPO_4, pH 7.6. The internal solution was the same as for the toad.

Drosophila. The external solution contained (in mM) 20 NaCl, 10 HEPES, 8 $MgSO_4$, 5 KCl, 25 proline, 2.5 sucrose, and 1.5 $CaCl_2$, pH 7.5. The internal solution contained (in mM) 124 KCl, 10 HEPES, 2 $MgSO_4$, 1.1 EGTA, 0.1 $CaCl_2$, 0.5 GTP, and 2 ATP, pH 7.15, pCa 7.3.

Odorants

Mixture I contained (1 mM of each in the stimulus pipette) citralva (3,7-dimethyl-2,6-octadienenitrile), citronellal (3,7-dimethyl-6-octenal), and geraniol (3,7-dimethyl-2,6-octadien-1-ol). *Mixture II* contained (1 mM of each within the pipette) isovaleric acid (3-methylbutanoic acid), pyrazine (1,4-diazine), and triethylamine. Odors were prepared directly in Ringer solution, from 100 mM stocks prepared in distilled water. All chemicals were obtained from Sigma, except for citralva, which was kindly donated by D. Restrepo. All experiments were conducted at ~22°C.

RESULTS

Suppression by Odors of the Voltage-Gated Outward Currents in Olfactory Neurons

Suppression of the net outward current. If odorant-induced suppression results from an unspecific effect of odorants on ion channels, it should affect both transduction and voltage-gated currents in olfactory receptor neurons (9, 11). To test this idea we investigated the time dependence of the suppression effect on the voltage-gated outward currents, applying brief odor exposures to these sensory cells during depolarizing voltage steps.

Olfactory neurons are selectively sensitive to odors. When stimulated with micromolar concentrations of *mixtures I* or *II*, ~30% of the dissociated receptor cells are able to transduce one of the odorant mixtures (*I* or *II*), and a much smaller fraction of the cells (18%) responds to both of them (19). We examined the effect of *mixture I* on the net voltage-gated outward K^+ currents (I_o) in olfactory neurons that could not transduce this odorant mixture. Two examples are presented in Fig. 1, *A* and *C*, corresponding to two separate cells. Each was exposed to odorant puffs during a depolarizing step from a holding potential of −70 mV initiated 1 s before the onset of the chemical stimulus. The cell in Fig. 1*A* responded to a 1.5-s odor pulse with a rapid 35% reduction in the magnitude of the net I_o, activated by a step to 0 mV. *Mixture I* normally induces an excitatory I_T in responsive receptor neurons, with a reversal potential of 0 mV, as determined by local stimulation of the olfactory cilia (data not shown) (7, 10). Although I_T should be undetectable at 0 mV, one expects to observe an inward tail current (I_t) on repolarization to −70 mV if the cell is responsive to *mixture I*. The fact that no I_t developed in the response indicates that this neuron did not chemotransduce. Rather, the decline in I_o resulted from odor suppression of the voltage-gated current. We define the suppression current (I_S) as the difference between the net current and the control current measured during a puff of odorant-free Ringer solution. In the voltage range examined, the *I-V* relation for I_S (peak values) is a straight line that approaches 0 pA around −38 mV (Fig 1*B*). I_S is zero at greater negative voltages, a range at which all voltage-gated channels are virtually closed. Figure 1*C* shows another nontransducing olfactory neuron (note the

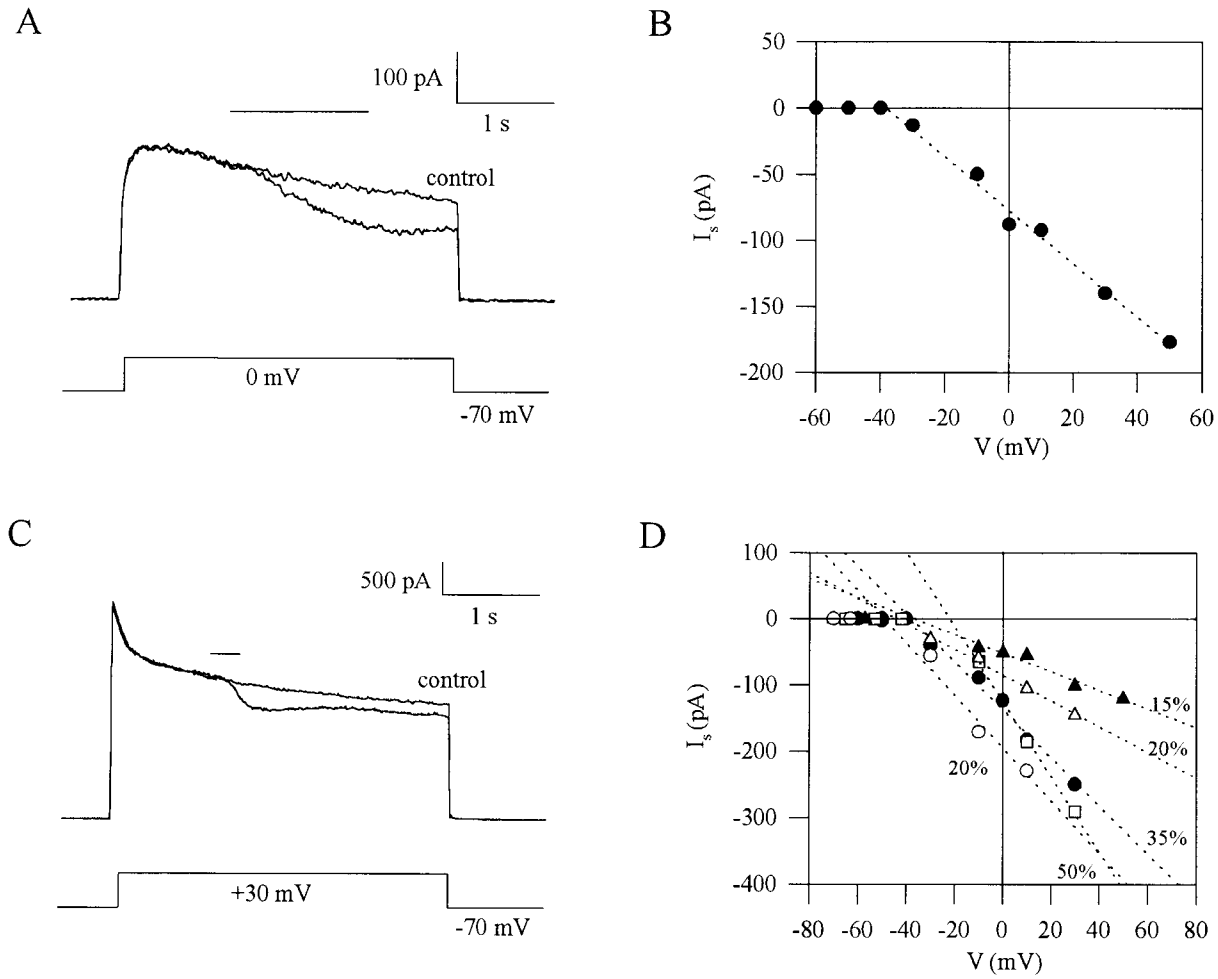

Fig. 1. Suppression of the voltage-gated net outward current (I_o) by odorants of *mixture I*. *A*: this cell was under voltage clamp [holding potential (V_{hold}) = −70 mV]. An odorant stimulus was given (bar, 100 μM) during a step to 0 mV. I_o rapidly decayed by 35% (peak) of control level. Superimposed to it is the control current, obtained with an identical protocol, except that the puff consisted of odorant-free Ringer solution. Note the absence of a tail current (I_t), indicative of the inability of this cell to transduce *mixture I*. *B*: current-voltage (*I-V*) relation for the suppression current (I_S) was built with peak values of odor-induced currents after subtraction of control currents. Experimental points from −38 mV up were fitted with a straight line. *C*: suppression of net outward voltage-gated current in an olfactory neuron missing its cilia. Note that voltage was pulsed to +30 mV, and odor puff (50 μM) was shorter than in *A*. *D*: I_S *I-V* relations from 5 cells. Curves obtained from other 7 cells were all similar to those shown here but were left out for clarity. Average 0-current potential: −39 ± 11 mV (means ± SD). Percent suppression of I_o is indicated beside each line.

absence of I_t). This neuron had lost its chemosensory cilia, which are essential for chemotransduction (for a review, see Ref. 21). We used a similar protocol with a shorter odor pulse (0.3 s), and the voltage was stepped to +30 mV in this case. At such voltage an outward current would be expected if transduction channels were open. In this example peak I_S was ~15% of I_o. We investigated the *I-V* relations for I_S in 12 olfactory neurons that were equally unresponsive to *mixture I*. In all cases they could be fitted to a straight line; for clarity, only five of them are superimposed in Fig. 1*D*. The potential value at which I_S departs from zero ranged from −60 to −22 mV, with an average value of −39 ± 11 mV (mean ± SD). The percentage of the suppressive effect on I_o varied from cell to cell. This variation was likely due to differences in odorant concentration, in addition to other factors (see DISCUSSION).

The shape of the I_S *I-V* curve differs greatly from both the excitatory (6, 7, 10) and the inhibitory transduction *I-V* curves (17, 19), and it does not resemble either of the *I-V* curves associated with the voltage-gated ionic currents present in *C. caudiverbera* olfactory neurons. Rather, the shape of the suppression *I-V* relation is consistent with a reduction in the net outward current.

Dose-response relation of suppression. Suppression was dose dependent. Figure 2*A* illustrates a family of whole cell currents induced in a nontransducing olfactory neuron (note absence of I_t) by identical voltage steps from −70 mV (holding potential) to −10 mV, each one associated with an odorant puff of different intensity (including a control consisting of a puff of odorant-free Ringer solution). The dose-response relation for this experiment is presented in Fig. 2*B*. Odorant concentrations ≥40 μM produced a maximal I_o suppression of 50% (Fig. 2*B*, closed circles). The concentrations at

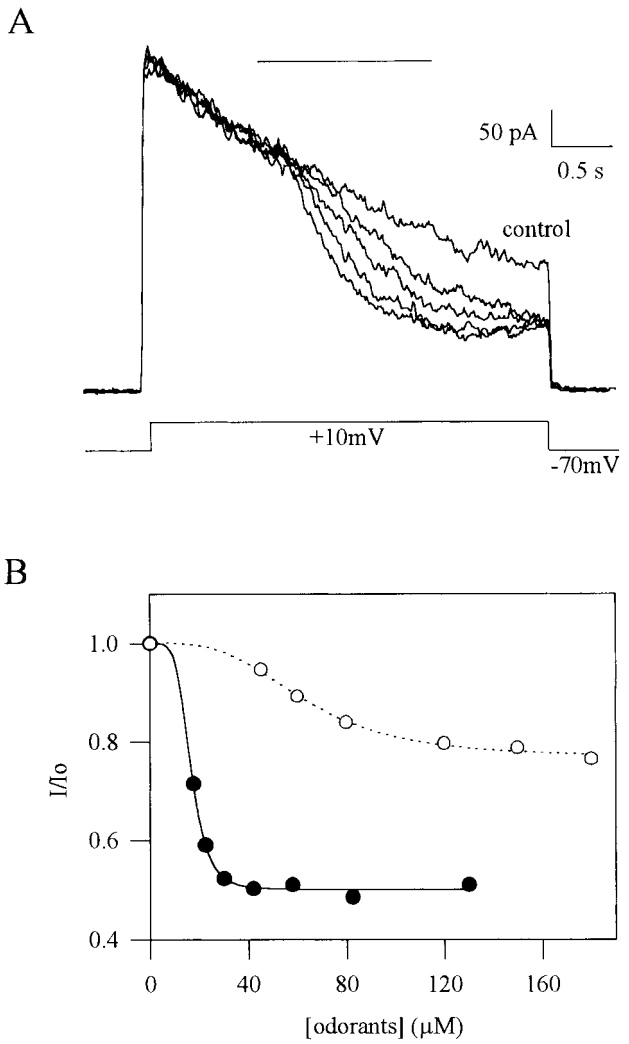

Fig. 2. Dose-response relation of the suppression effect on I_o for a nontransducing cell. *A*: family of currents displaying the suppression induced by odorant stimuli of increasing concentrations, obtained by incrementing the pressure on the stimulus pipette. *B*: dose-response curve built with the suppressed currents at the points of maximal effect, relative to the control current (●). Experimental data were fitted to a Hill equation $I/I_o = (1 - \text{min})/(1 + C/I_{50})^n + \text{min}$, where $I_{50} = 17$ μM, $n = 5.5$, min = 0.51 (min = asymptotic value of I/I_o due to suppression). ○, Another example ($I_{50} = 64$ μM, $n = 3.2$, min = 0.75).

which *mixture I* started to induce suppression varied from cell to cell, ranging from 5 to 40 μM. The percentage of the outward current suppressed by odors varied widely, virtually from 0 to 100%, when measured at the moment of maximal suppression. In the majority of cases, suppression reached intermediate values. Figure 2*B* includes another example, from a different cell, to illustrate this variability (open circles). In this case, maximal reduction was 25% of I_o, at odorant concentrations ≥120 μM.

Suppression and transduction currents contribute to the net odor response. The range of odorant concentrations at which suppression operates overlaps with that at which isolated olfactory neurons transduce odorants (Fig. 2) (7, 19). This is best illustrated in cells that responded with an I_T to odorant puffs that engulfed the entire neuron. Figure 3*A* shows currents induced by an odorant puff at −30, 0, and +30 mV (from a holding potential of −70 mV) after subtracting the control currents. All three traces exhibited tail currents, revealing that the cell transduced the odors. It is also clear that in this cell the odors suppressed I_o because an inward current was observed during the stimulus at 0 mV (the reversal potential of I_T) and, therefore, the reduction of the net I_o was due entirely to suppression (Fig. 3*A*, *middle trace*). It is expected, therefore, that at voltages other than 0 mV, I_S and I_T should overlap. In cases where the kinetics of I_S and I_T are not substantially different and $I_T >> I_S$, as in this particular

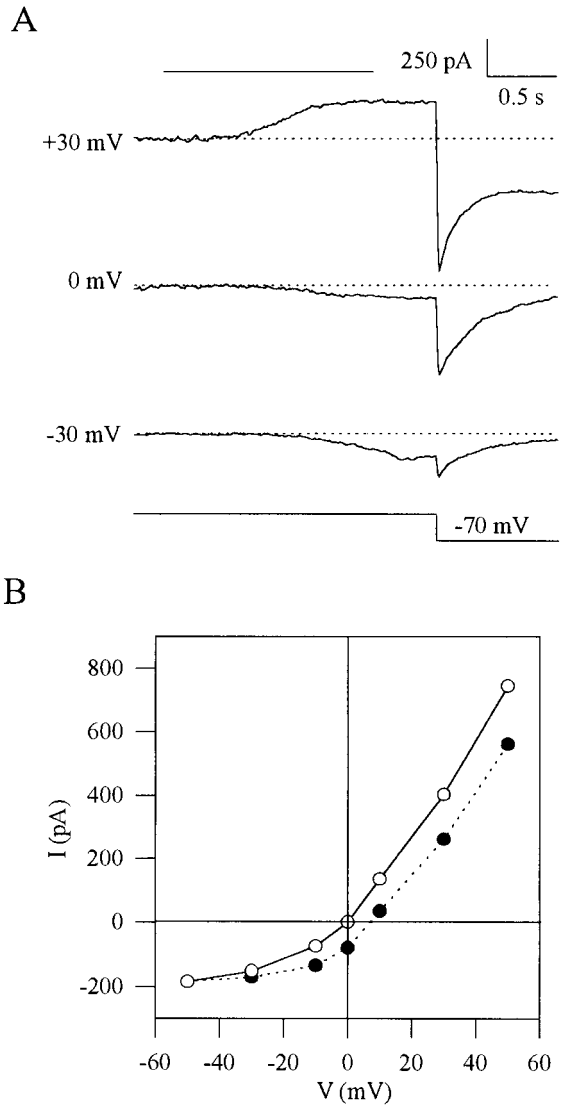

Fig. 3. Suppression in an olfactory neuron transducing *mixture I*. *A*: odor-induced currents for 3 different voltages, after subtracting control currents. Odor stimuli are indicated by bar. *B*: *I-V* curve built from peaks of odor-induced (experimental) currents (●). ○, Obtained after correcting each of the experimental values for suppression. The correction considered the facts that at 0 mV the odor-dependent current corresponds solely to I_S and that the I_S *I-V* is linear (Fig. 1*B*). We calculated I_S at any given voltage (V) by the equation $I_S = aV + b$, where $a = -b/39$ (39 mV is average 0-current potential; see legend to Fig. 1*D*) and b is I_S at $V = 0$ mV. The corrected *I-V* curve was obtained by subtracting I_S from the experimentally measured current (*method I*).

example, the *I-V* curve for the odor-induced current should be somewhat shifted with respect to that of I_T, without a major distortion in its shape (Fig. 3*B*). In contrast, in cases in which $I_T \approx I_S$ and their time courses are markedly different, the *I-V* curve shape may be considerably distorted (see below). The closed circles in Fig. 3*B* correspond to the measured peak values of the odor-induced currents and the open circles correspond to I_T, after correcting for I_S. In making this correction, we reasoned that, since I_S is linearly related to voltage (see Fig. 1*B*) and the total value of the odor-induced current equals I_S at 0 mV, we could estimate the value of I_S at each voltage and make the appropriate correction to the experimental values to obtain I_T at every voltage (*method I*; see legend to Fig. 3).

Two further examples are presented in Fig. 4. In both cells it is possible to distinguish both I_T and I_S as two coexisting, but clearly distinguishable currents. The presence of I_T was confirmed by I_t in both cases. In each cell, I_S could be observed in isolation at the reversal potential of I_T, 0 mV (Fig. 4*A*, *bottom trace* and 4*D*, *middle trace*). In the cell in Fig. 4*A*, I_S clearly preceded I_T in such a manner that, at positive potentials, the inward I_S component was followed by the outward I_T component (Fig. 4*A*, *middle trace*). This situation was more dramatic at further positive potentials, where the odor-induced currents became clearly biphasic (Fig. 4*A*, *top trace*). The *I-V* curve, built from the maximal values of the net current at each potential (which in this case coincided with the end of the pulse), is depicted by the closed circles in Fig. 4*B*. The cell in Fig. 4*D* had a larger I_S relative to I_T. At −30 mV, I_S preceded I_T, the latter one expressed as a further inward current before the end of depolarization. At +30 mV, voltage at which I_T is outward, the magnitude of this current was insufficient in this case to overcome I_S, resulting in a net inward current at the end of depolarization (Fig. 4*D*, *top trace*). The net current *I-V* relation for this cell is shown in Fig. 4*E* (closed circles). Both experimental curves (closed circles in Fig. 4, *B* and *E*) are remarkably distorted from the expected *I-V* relation shape, as a result of suppression.

The more complex odor-induced currents, apparent in the two cells, enabled us to validate our I_S-correction procedure by comparison with another method (*method II*). Figure 4*B* plots the *I-V* built from the experimental points (closed circles) and the I_T *I-V* curve after correcting for I_S by *method I*, as explained above (open circles). Taking advantage of the fact that I_S can be distinguished within the odor-induced current, because it developed earlier than I_T, we attempted a different manner of correcting for I_S (*method II*). We plotted the peak I_S vs. voltage (triangles) and subtracted these values from those of the odor-induced currents at the end of the depolarization. This method yielded an *I-V* curve (squares) virtually indistinguishable from the one obtained by correcting with *method I* (open circles). *Method I* is of general use, because it can be applied to any olfactory neuron responding with a transduction current; whereas *method II* can only be applied to those cells in which I_S had fully developed before I_T became evident. In the present example, I_S was sustained at 0 mV (Fig. 4*A*, *bottom trace*). Considering that I_S had the same behavior at the other voltages (as we have observed in nontransducing olfactory neurons), we fitted a Boltzmann function to I_S in each case (Fig. 4*C*, broken lines) and subtracted the calculated currents from the recorded odor-induced currents (I_{OD}, same as in Fig. 4*A*) to obtain I_T as a function of time in isolation.

For the second example (Fig. 4*E*), we corrected the experimental *I-V* curve (closed circles) by *method I* (open circles) and by method II (squares) using peak I_S (triangles). The curve crosses 0 mV and displays the characteristic shape of the I_T *I-V* curve. Despite the pronounced distortion exhibited by the experimental *I-V* curve, both methods corrected the curve quite well. The two methods gave slightly different values only on the two points taken at the most positive potentials. We attribute this small mismatch principally to the rundown of the voltage-dependent currents, which in this particular cell was larger than usual. Rundown principally affected the two positive values, because they were taken at the end of the experiment, when the rundown effect was more pronounced.

We examined a total of 17 transducing neurons in which odor stimuli were applied to the cilia. The two components, I_T and I_S, could be clearly discriminated in 15 of such cases; in all of them I_S preceded I_T. In the two remaining neurons, both currents had indistinguishable time courses (Fig. 3 and see DISCUSSION).

In conclusion, our results indicate that, no matter how anomalous the experimental *I-V* relation for odor-induced currents was, its shape and reversal potential became quite close to the expected after correcting for I_S by either method.

Suppression Takes Place Independently of the Transduction Cascade

Suppression of the voltage-gated conductances takes place in 94% of isolated olfactory neurons (n = 121), including those missing their chemosensory cilia. Transduction currents (I_T), on the other hand, are triggered only in a fraction of these sensory cells (30%) (19) and never in neurons missing their cilia. This suggests that suppression is independent of the transduction cascade, which is localized in the cilia (7, 10, 13, 17). An additional important distinction between transduction and suppression is that I_T exhibits rundown until it eventually disappears, whereas I_S persists for as long as there are voltage-gated currents. The voltage-gated currents are susceptible to rundown, although considerably less than transduction currents. The lower sensitivity to rundown of I_S compared with I_T is in agreement with the notion that I_S is not dependent on second messengers, in contrast to I_T. Three additional lines of evidence further support the view that suppression is independent of the transduction cascades: *1*) the latencies of I_S are much shorter than those of I_T, *2*) odors are more effective on activating I_S in the nontransducing cellular regions, and *3*) there is further evidence supporting the notion that I_S activation is nonspecific.

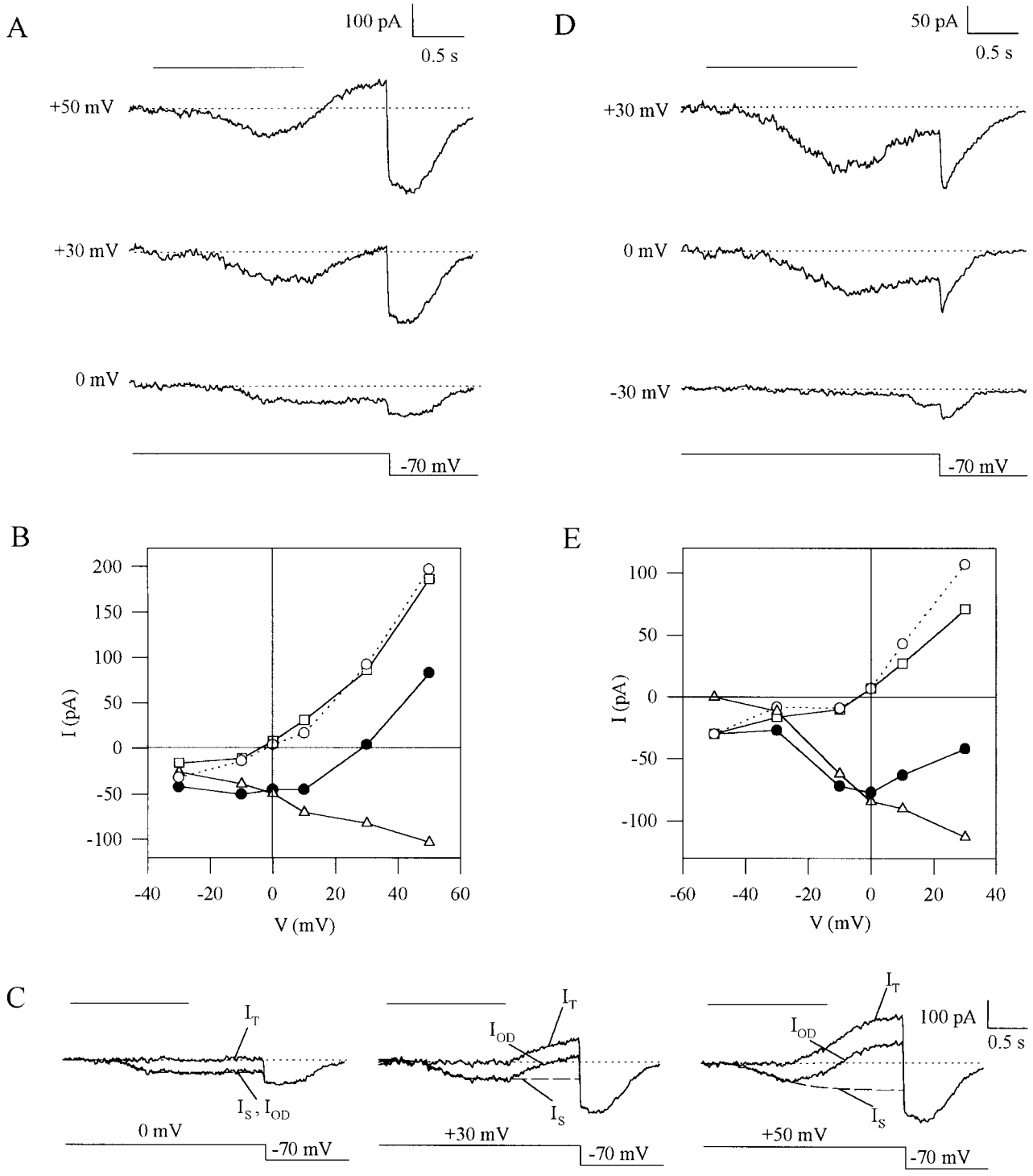

Fig. 4. Suppression in olfactory neurons having I_S and transduction current (I_T) with clearly distinguishable time courses. *A*: odor-induced currents at 3 different voltages. I_S precedes I_T. At 0 mV, I_T is 0 (its reversal potential). *B*: *I-V* relations of odor-induced currents measured at end of voltage steps (●), of peak I_S values (△), of I_T after correcting for I_S by *method I* (○), and of I_T after correcting for I_S by *method II* (□; see below). *C*: odor-induced current (I_{OD}), I_S, and I_T in isolation. I_S at 0 mV (*left*) was well fitted by a Boltzmann equation [dashed line; $I_{OD} = A/[1 + B\exp(t/q)]$, where $A = 39$ pA, $q = 85$ ms, $B = 0.02$ pA]. The same function (+30 mV: $A = 72$ pA, $q = 141$ ms, $B = 0.03$ pA; +50 mV: $A = 112$ pA, $q = 283$ ms, $B = 0.18$ pA) predicts an I_S shape for the other 2 voltages (*middle* and *right traces*, respectively). Dotted line, zero-current level. *D*: current recordings from a separate olfactory neuron, using same protocol as in *A*. *E*: *I-V* curves for the cell in *D*; symbols are equivalent to those used in *B* for the first cell. *Method II* involves subtracting peak I_S value (△) from maximal value of net odor-induced current (●) at every voltage.

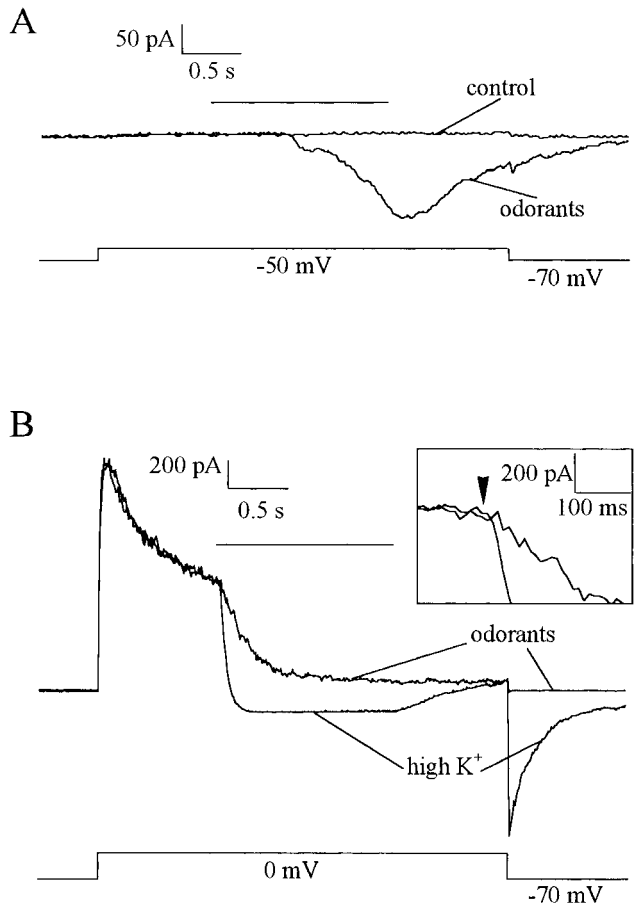

Fig. 5. Latencies of transduction and suppression currents. *A*: representative recording of a transduction current triggered by *mixture I*. Latency = 560 ms. *B*: superimposed currents induced by puffs of odorant and high-K^+ Ringer solution (100 mM K^+, different cell to that in *A*). *Inset*: detail of both currents; arrowhead, stimuli onset. Latencies were 10 and 30 ms for high-K^+ and odor stimuli, respectively.

Suppression latency. If suppression results from a direct effect of odorants on the voltage-gated channels, unlike transduction, which is a cascade process, its latency (the time between the puff onset and the moment at which the current induced by the puff develops) should be substantially shorter than that of the transduction response. Figure 5 shows that this was indeed the case. The olfactory neuron in Fig. 5*A* exhibited a typical transduction response to *mixture I*, with a latency of 560 ms, when a localized stimulus was delivered 20 μm away from the olfactory cilia. The cell in Fig. 5*B*, incapable of chemotransducing *mixture I*, was used to test the delay of the suppressive effect. Because I_S latency is strongly dependent on stimulus strength (being longer for lower pressure pulses; Fig. 2*A*), we chose a pressure value (10–14 lb/in.^{2}) for which the latency was minimal. We stimulated the neurons with a double-barreled pipette, one barrel of which contained *mixture I* while the other was filled with high-K^+ Ringer solution (100 mM K^+). The high-K^+-induced current is presented superimposed on I_S in Fig. 5*B*. The odor puff induced I_S with a 30-ms latency. A high-K^+ puff applied with the same pressure and from the same location also induced an inward current, but with a somewhat shorter latency of 10 ms (Fig. 5, *inset*). The high-K^+ puff is followed by an inward tail current, reflecting the fact that the high-K^+ concentration favored the influx of this cation after shifting the voltage back to −70 mV, until the excess of K^+ diffused away.

The latency of the suppression effect is one order of magnitude shorter than that typical of I_T. In a total of 15 experiments in which an identical protocol was used (pressures 10–14 lb/in.^{2}), we found similar results, with an average latency of 19 ± 13 ms (means ± SD). These results are consistent with the notion that suppression directly affects the voltage-gated channels without involving a signaling process.

Localization of I_S. Although transduction channels are mostly confined to the olfactory cilia, voltage-gated channels are localized in the cell body and dendrite of olfactory receptor neurons (7, 10, 13, 17). Using locally applied odorant puffs in cells that did not respond to *mixture I* (note the absence of I_t in Fig. 6*A*), we found that suppression of voltage-gated currents by local application to the cell body was significantly larger than that induced by application to the cilia (Fig. 6*A*). In the typical example illustrated in Fig. 6 (n = 4), odors suppressed the outward current by 8% when directed to the cilia and by 20% when addressed to the cell body, while the membrane potential was held at

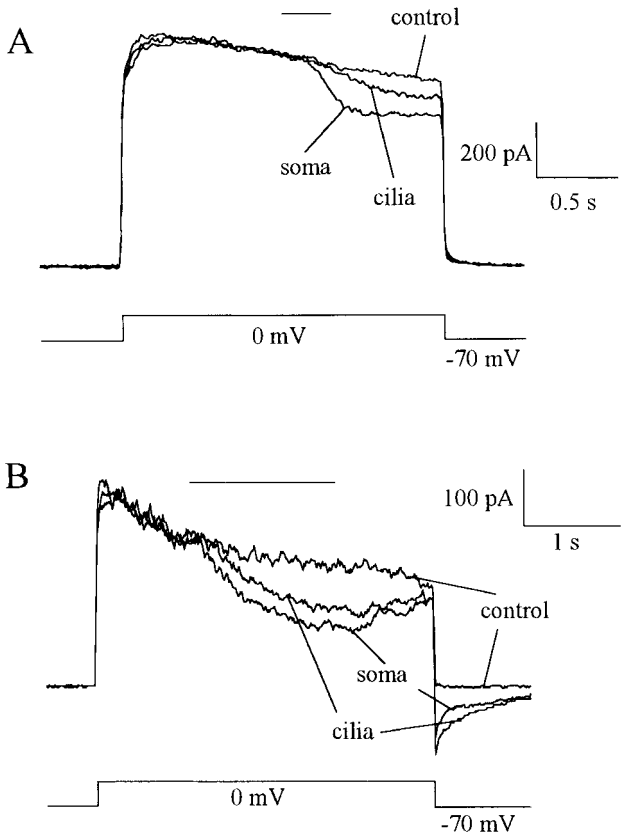

Fig. 6. Localization of suppression. *A*: an olfactory neuron unable to transduce *mixture I* was stimulated locally with identical odor puffs on its cilia, and subsequently on its cell body, during a step to 0 mV. *B*: a similar experiment in an olfactory neuron capable of transducing *mixture I*. Control traces consist of odorant-free puffs.

0 mV. Most likely, suppression observed when stimulating the cilia was due to odorants that diffused to the dendrite and cell body. The longer latency and slower time course of the effect when the odors were applied to the cilia compared with the cell body support this interpretation. This observation is contrary to what would be expected if suppression had an effect on the transduction channels or on any of the upstream cascade components. In a second example (Fig. 6*B*), from a neuron responding to the odorants with a transduction current (note the presence of I_t) also held at 0 mV, I_o was reduced by 34% when odors were directed to the cilia and by 50% when the cell body was stimulated. In contrast, it is worth noting that the I_t of the response obtained by cilia stimulation was larger than the I_t of the response to the stimulus directed to the soma, in agreement with the transduction conductance being localized to the cilia.

Nonspecificity of suppression. Suppression is not only induced by odorants that excite *Caudiverbera* olfactory neurons but also by odorants that inhibit these cells (*mixture II*) (2, 19). Figure 7A shows an example (n = 20) of suppression caused by *mixture II*, which was not different from suppression by *mixture I*, shown in the previous examples.

Suppression by *mixture I* also takes place in rat isolated olfactory neurons. In this case, depolarizing steps, from −70 to +30 or to 0 mV, induced pronounced early transient outward currents (14), followed by a slowly decaying outward current (Fig. 7*B*). The puff of *mixture I* reduced the outward current by ~38% in a manner virtually identical to that in the toad. Within the concentration range of 1–150 μM, only 45% of all the rat olfactory neurons (n = 66) were suppressed by *mixture I*. However, 500 μM odors suppressed ~80% of the cells. Somewhat higher concentrations suppressed nearly every olfactory neuron, supporting the notion that the mechanism underlying suppression is nonspecific. *Mixture II* also suppressed the voltage-gated currents in the rat, although requiring two- or threefold higher concentrations than *mixture I* (not shown).

The fact that suppression is induced by odorants that excite *Caudiverbera* olfactory neurons as well as by those that inhibit those cells (*mixture II*) and that it also occurs in rat olfactory receptor neurons strongly supports the notion that suppression is a nonspecific effect of odorants on the voltage-gated ion channels.

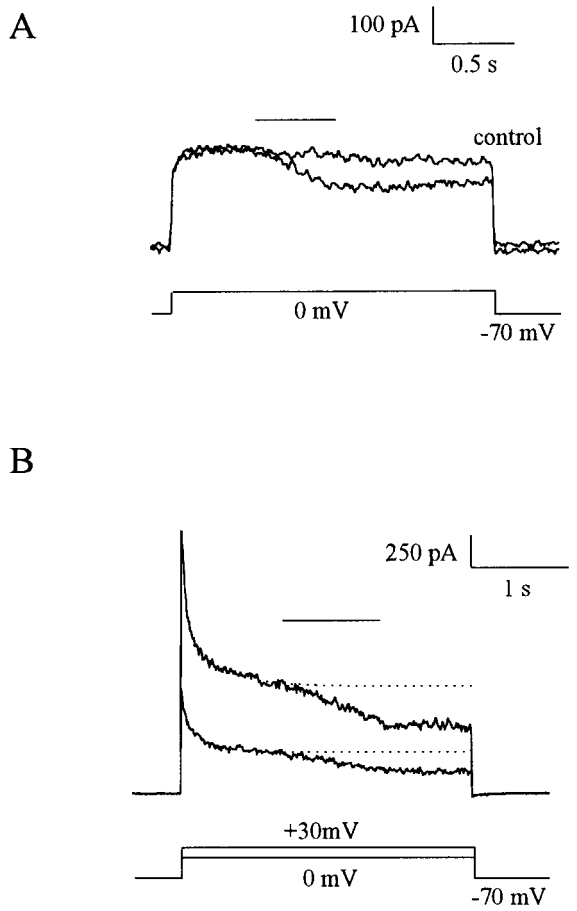

Fig. 7. Suppression by *mixture II* in *Caudiverbera* and by *mixture I* in rat olfactory neurons. *A*: a toad olfactory neuron exhibiting suppression by putrid odors (80 μM). Control: puff of odorant-free Ringer solution. *B*: *mixture I* also suppressed a rat isolated olfactory neuron (100 μM). Superimposed are 2 recordings obtained at 0 and +30 mV. Dotted lines correspond to single exponential fits to I_o before odor stimulation.

Suppression by Brief Odor Pulses Affects All Voltage-Gated Conductances

We examined suppression on each of the four relevant voltage-gated currents in *Caudiverbera*, the delayed rectifier K^+ current [$I_{K(V)}$], the Ca^{2+}-dependent K^+ current [$I_{K(Ca)}$], the Na^+ current (I_{Na}), and the Ca^{2+} current (I_{Ca}). We showed above how the net outward current (I_o) was suppressed by odors. To investigate whether each of the individual voltage-gated K^+ current components in these cells was a target of suppression, we examined the direct effect of brief odorant pulses on each component in isolation. For this purpose, we temporarily abolished $I_{K(Ca)}$ by briefly exposing the olfactory neurons to 0-Ca^{2+} Ringer solution, as illustrated by Fig. 8*A*. Both superimposed currents were induced by a voltage step from −70 to +30 mV, under normal and 0-Ca^{2+} Ringer solution. The *I-V* curves for the families of currents measured at the end of the steps are presented in Fig. 8*B* (normal Ringer solution, closed circles; 0-Ca^{2+} Ringer solution, open circles). In this cell, suppression of I_o under normal Ringer solution was of 700 pA (Fig. 8*C*). During exposure to 0-Ca^{2+} Ringer solution, we were able to measure odor suppression of $I_{K(V)}$ in isolation, using 0-Ca^{2+} Ringer solution as the carrier solution in the pipette. This current was suppressed by 500 pA (Fig. 8*C*). If rundown of the currents is ignored, the difference between suppression of I_o and suppression of $I_{K(V)}$ corresponds to suppression of $I_{K(Ca)}$. Accordingly, for the cell in Fig. 8*C*, suppression of $I_{K(V)}$ accounted for ~70% of the suppression of the net outward current, the other ~30% corresponding to suppression of $I_{K(Ca)}$. Similar results were obtained in four other cells, although the percentage of suppression, as well as the relative contribution of each component to the net outward current, was different in every case. Figure 8*D* illus-

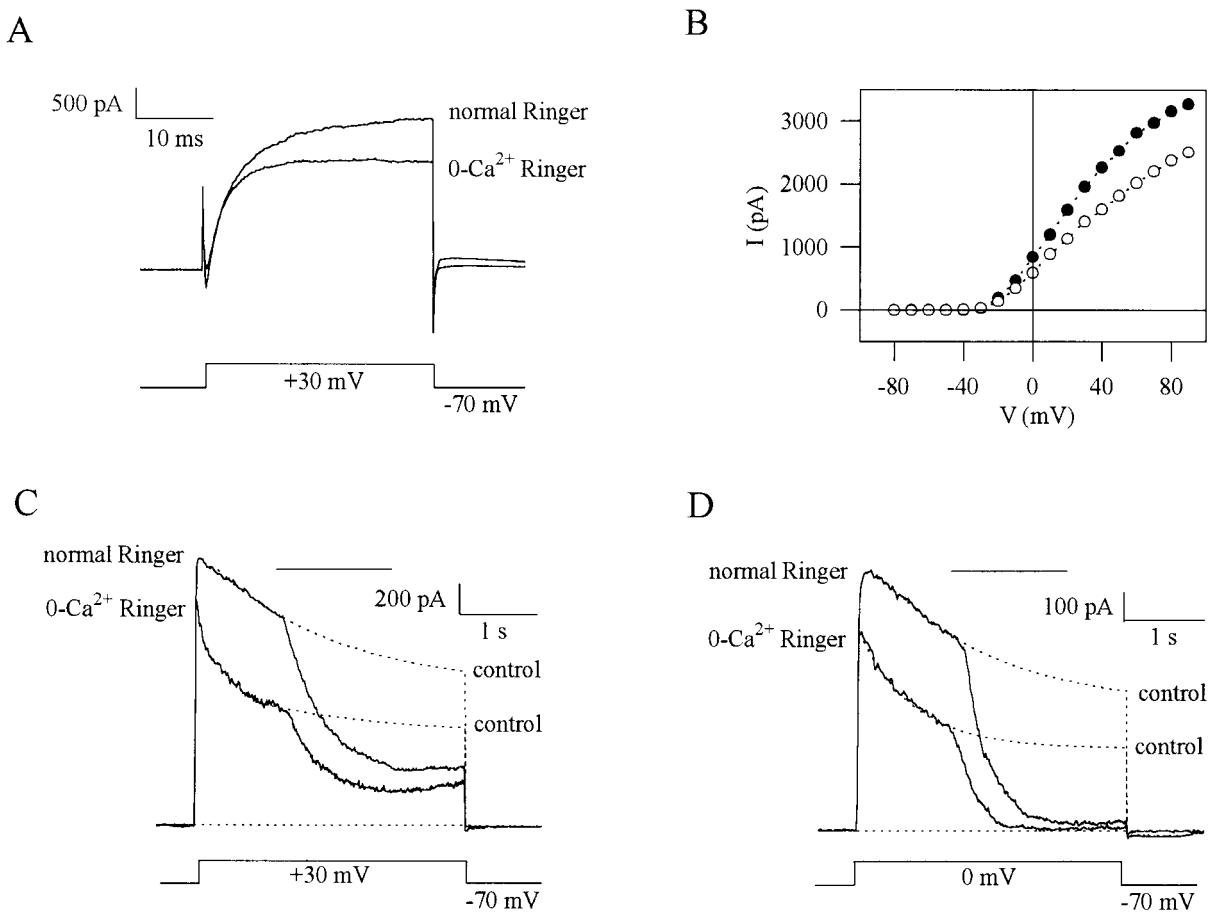

Fig. 8. Suppression affects both I_o components, delayed rectifier K^+ current [$I_{K(V)}$] and Ca^{2+}-dependent K^+ current [$I_{K(Ca)}$]. *A*: whole cell current under normal Ringer solution (I_o) and under 0-Ca^{2+} Ringer solution [$I_{K(V)}$], induced by steps from −70 mV (V_{hold}) to +30 mV. *B*: *I-V* curves built from families of currents (same experiment as in *A*), induced by a series of voltage steps (20-mV increments), and measured at the end of the steps. ●, Normal Ringer solution; ○, 0-Ca^{2+} Ringer solution. *C*: identical odor stimuli (*mixture I*) suppressed I_o in normal Ringer solution and under 0-Ca^{2+} Ringer solution. *D*: another example, illustrating complete suppression of both I_o components.

trates an extreme case in which odors completely suppressed both components of I_o, $I_{K(V)}$, and $I_{K(Ca)}$.

I_{Na} and I_{Ca} were both odor suppressed as well. Because I_{Na} rapidly inactivates, we used a different protocol for this study. In this case, the odorant puff was initiated 450 ms before the depolarizing step, lasting to the end of it. In addition, the pipette was filled with Cs^+ internal solution to abolish I_o. It can be observed that depolarization from −70 to −10 mV induced the transient I_{Na} followed by the sustained I_{Ca}. Both currents were suppressed by odorants in a dose-dependent fashion (Fig. 9*A*). The dose-suppression curve for I_{Na} is presented in Fig. 9*B*. Suppression of I_{Na} reached a maximum of ~70% in this particular cell. In general, odorant concentrations ≥5 μM were required to induce an observable effect, and the average maximum suppression was 72 ± 18% (means ± SD; $n = 12$). Although I_{Ca} was also suppressed in a dose-dependent fashion, it was difficult to build a dose-suppression curve due to the small magnitude of this current and to the possible contribution of other small current components (possibly carried by Cs^+ or other ions; Madrid and Bacigalupo, unpublished results, and see Ref. 4). For these reasons, we were able to clearly resolve I_{Ca} in only 3 of the 12 cells examined, all of which exhibited suppression.

Suppression by Odors in Nonolfactory Cells

If suppression is a nonspecific phenomenon on the voltage-gated ion channels of the olfactory receptor neurons, independent of the transduction cascades existing in this sensory cell (3), it might affect ion channels of cells other than these chemoreceptors. To test this prediction, we applied odorant puffs (*mixture I*) to *Drosophila* photoreceptor cells and examined their effect on voltage-dependent currents. *Mixture I* reduced the net outward current activated by depolarizing the membrane from a holding potential of −70 to 0 mV (in 2 out of 2 cells tested), as in olfactory neurons (Fig. 10). This result suggests that odor suppression is a nonspecific effect of odor molecules on voltage-gated channels, regardless of cell type. Moreover, it demonstrates that suppression is independent of olfactory transduction cascades.

Physiological Significance of Suppression

An important question regarding the phenomenon of suppression is whether it contributes to the odorant

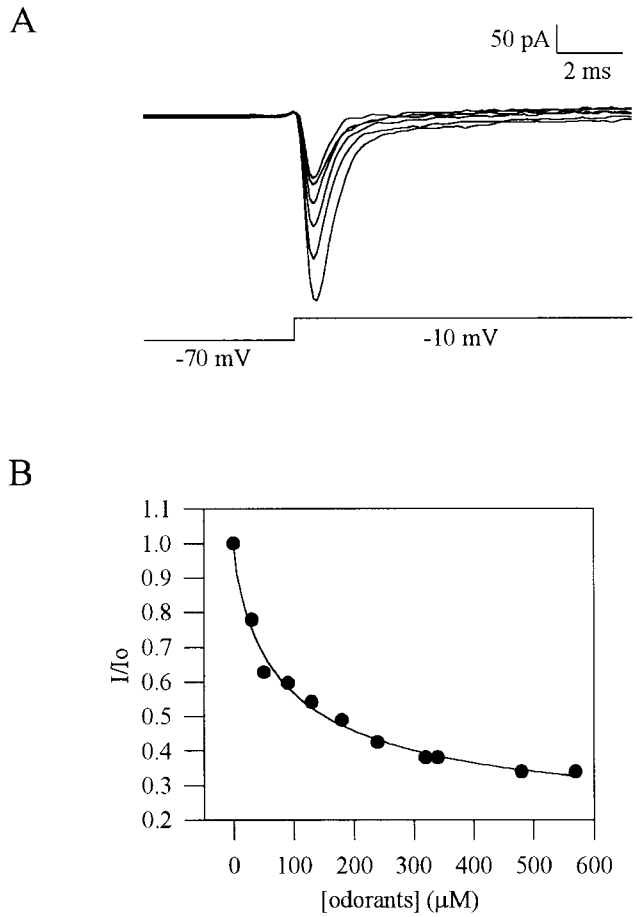

Fig. 9. Suppression of voltage-gated inward currents. *A*: because of Na^+ channel inactivation, odor exposures were initiated 450 ms before the depolarizing step, ending after the presented traces. In addition, Cs^+ replaced K^+ in the internal solution. Superimposed are currents obtained by 6 steps from −70 (V_{hold}) to −10 mV, accompanied by puffs of increasing concentrations of *mixture I*. *B*: dose-response curve for peaks of Na^+ current in *A*. Data were fitted to same function as in Fig. 2, with I_{50} = 92 μM, n = 0.8, and min = 0.16.

responses. To investigate the physiological effect of suppression on these receptor cells, we undertook current-clamp experiments and monitored the behavior of the membrane potential. For this study we only used olfactory neurons that did not transduce *mixture I*.

Figure 11*A* shows an olfactory neuron with a rather high spontaneous spiking rate. The *inset* to Fig. 11*A* indicates that *mixture I*, although producing suppression, did not trigger a transduction response in this cell. Under current-clamp conditions, we gave successive 1.5-s puffs of odorant-free Ringer solution and of increasing concentrations (60–80 μM) of *mixture I*. The firing rate was not altered by a puff of Ringer solution. In contrast, the odorant puffs transiently depolarized the cell, increased the firing rate, and decreased the size of the spikes (*middle traces*). During the strongest stimulus, the action potentials suddenly ceased (*bottom trace*), presumably because the Na^+ channels were suppressed or the level of depolarization reached the threshold for Na^+ channel inactivation, which appears to be by itself modified by odors (9). After spiking resumed, repetition of the same experimental protocol gave identical results.

Another example, corresponding to a separate olfactory neuron, shows that in some cases the end of firing induced by an odor stimulus (similar to those used in Fig. 11*A*) appears to result from a hyperpolarization rather than a depolarization (Fig. 11*B*). The same odorant stimulus that ended spiking activity induced a hyperpolarization from −70 to −80 mV, when presented after the interruption of the action potentials.

We investigated the behavior of the receptor potential induced by identical odorant stimuli applied at different membrane potential values under current-clamp conditions. Odorant stimuli either depolarized or hyperpolarized the cell, depending on where the membrane potential was set. The voltage value at which the odor effect reversed varied across cells, and it was usually different from the cell resting potential. This is illustrated by Fig. 12 for two separate olfactory neurons. In one of the cells (Fig. 12*A*; same cell as in Fig. 11*A*), with a resting potential of −65 mV, the polarity of the effect reversed at −80 mV (Fig. 12*B*), and in the other cell (Fig. 12*C*) with a resting potential of −60 mV, it reversed at −55 mV (Fig. 12*D*).

Our results indicate that odors affect the spiking activity of isolated olfactory receptor neurons not only by triggering the transduction cascade, but also by suppressing ion conductances.

DISCUSSION

In this work we have studied odor-induced suppression of the voltage-gated conductances in voltage-clamped or current-clamped isolated olfactory neurons, using short odorant exposures. Suppression appeared as a transient reduction of the whole cell membrane currents. Our results indicate that it is a nonspecific effect on voltage-gated ion channels occurring in almost

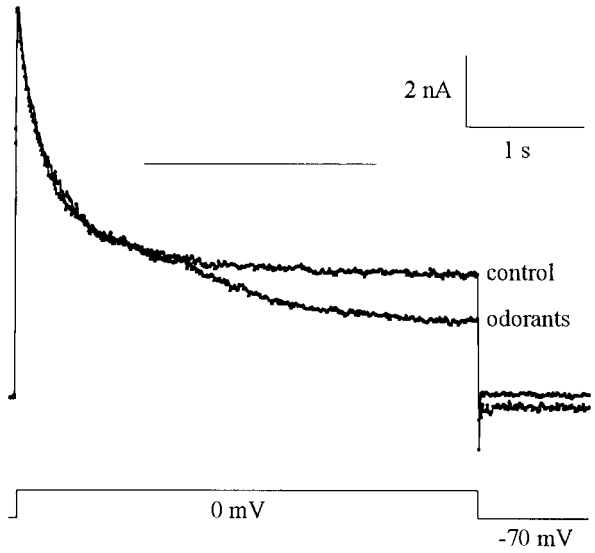

Fig. 10. Odor suppression of voltage-gated ion conductances of *Drosophila* photoreceptor cells. An odor stimulus (30 μM *mixture I*) was applied (bar) to voltage-clamped *Drosophila* photoreceptor cells in isolated omatidia, during a step to 0 mV (V_{hold} = −70 mV). Control: odorant-free puff.

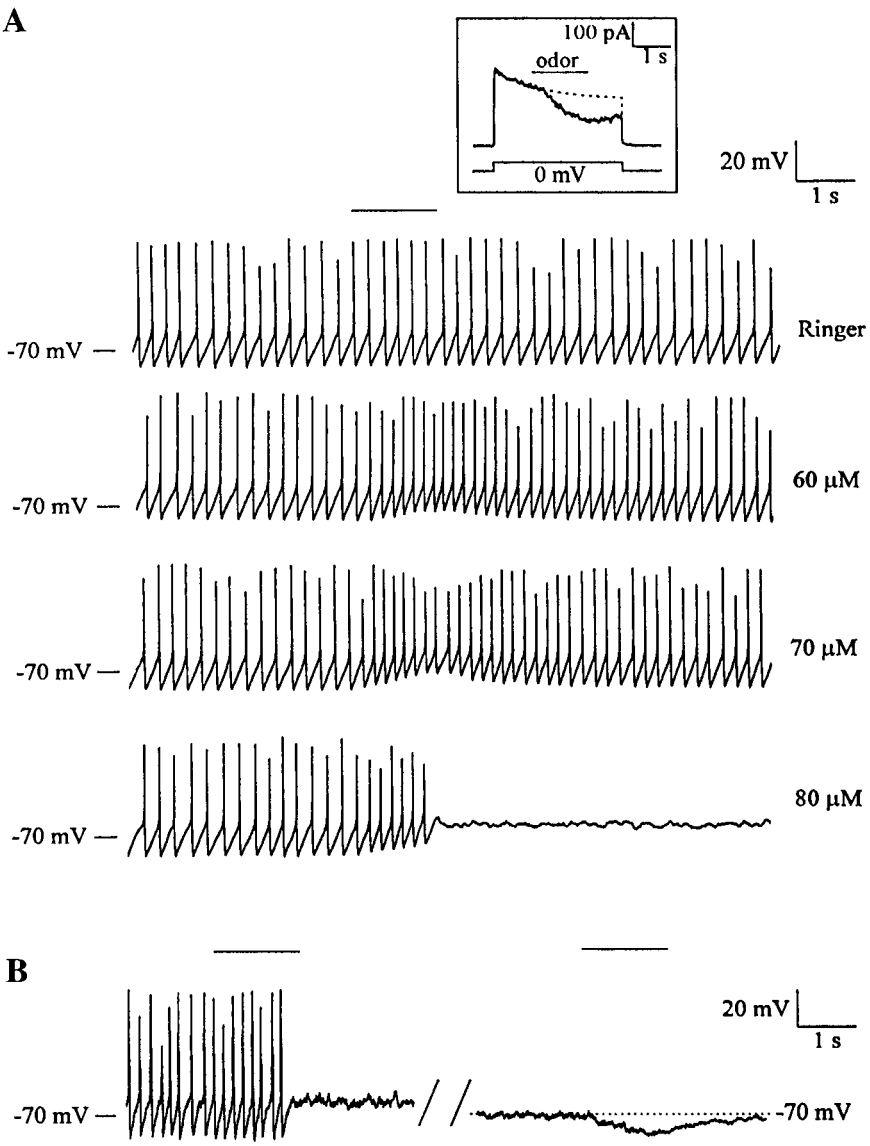

Fig. 11. Suppression affects action potential firing by depolarization or hyperpolarization. *A*: spiking olfactory neuron not transducing *mixture I* (*inset*). Subsequent traces, under current clamp, show firing behavior in response to puffs of odorant-free Ringer solution and of increasing *mixture I* concentrations (60, 70, and 80 μM). *B*: a separate olfactory neuron, also incapable of transducing *mixture I* and with a resting potential (V_{rest}) of −70 mV, was given an 80 μM puff of *mixture I* during spiking and again during the silent period, inducing a hyperpolarization (time interval between traces ≈ 20 s).

every tested olfactory neuron. Suppression contrasts with odor transduction, which is a rather specific physiological phenomenon. Only a small fraction of all olfactory neurons responds with a transduction current to a particular odorant; the underlying conductances are triggered by a signaling cascade (2, 21). We demonstrated that suppression and transduction are different phenomena, both of which contribute to shaping odorant-induced responses, at least in isolated olfactory neurons. Furthermore, our current-clamp measurements suggest that suppression is physiologically significant in olfactory neurons. Because odor suppression and odor transduction are induced by odor pulses of similar concentration and duration, it seems likely that both I_S and I_T effectively contribute to the electrical response to odors in the olfactory epithelium.

Characterization of Odor Suppression

Odor suppression of voltage-gated currents was previously investigated in the Japanese newt, by examining the effect of prolonged bath applications of odors on the voltage-gated currents (9). Chronic odor application reversibly suppressed all voltage-gated currents present in those sensory receptors. However, those results did not shed light over the time course of the suppression effect and they did not allow an evaluation of its possible contribution to the cell responses to brief odor exposures.

Much of what we know about odor transduction comes from electrophysiological studies of isolated olfactory receptor cells, stimulated with odorant pulses. To determine whether suppression contributes to the odor-induced responses, it was important to characterize suppression under a similar experimental regime. The most straightforward approach was to study olfactory neurons that were incompetent to transduce the utilized odorant stimuli. The absence of transduction and tail currents was taken as an indication of the inability of a cell to chemotransduce.

For convenience, we characterized suppression of the net outward current because this is a large sustained

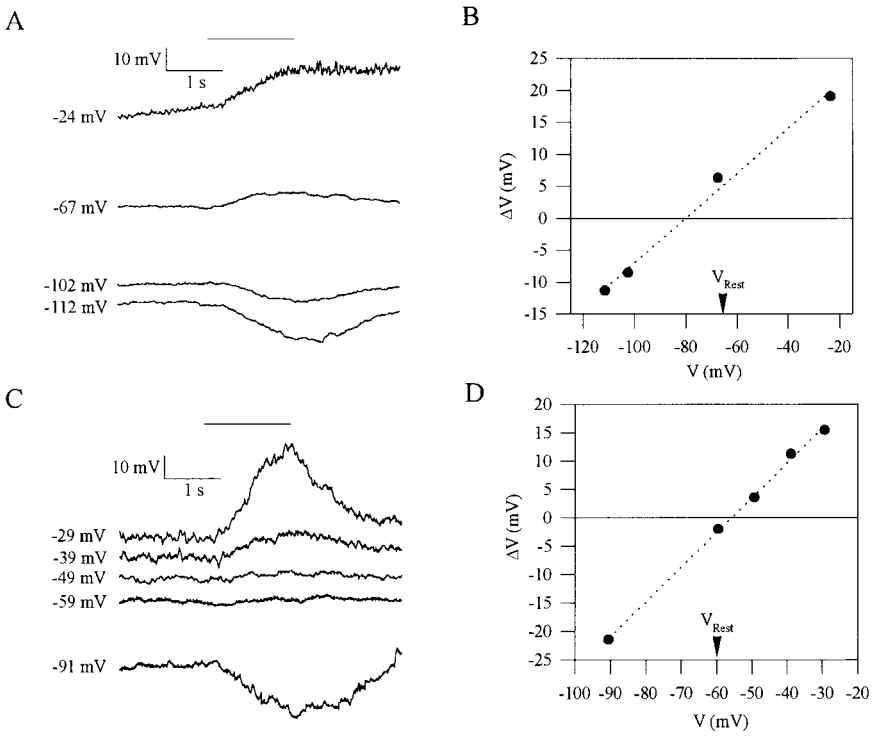

Fig. 12. Suppression as a function of membrane potential. *A*: responses at 4 different membrane potentials (indicated beside each trace) under current clamp. Membrane potentials were set by current injection (same cell as in Fig. 11*A*). *B*: odor-induced voltage changes (peaks) as a function of membrane potential (measured before stimulus application) for cell in *A*. *C*: another example, from a separate cell. *D*: odor-induced voltage responses vs. membrane potential for the cell in *C*.

current. The magnitude of suppression (percent reduction of I_o) varied widely from cell to cell. We attribute this large variation to various factors, among which are the following: the different odor concentrations used in our experiments, unequal relative contribution of both K^+ currents and their different sensitivities to odors (see Ref. 9), and geometrical problems associated with the way odors were applied to the cells. The latter factor relates to the fact that the puffer pipette was positioned on one side of the cell, making it likely that the concentration of odorants was not homogenous around the entire neuron. This lack of homogeneity depended on differences in size and shape of the cells (including the dendrite length, which is greatly variable), in the position of the pipette with respect to the cell, and slight differences in pipette shape. Despite these variations, the experimental conditions remained constant during each experiment, permitting us to draw important conclusions from the kinetics and voltage-dependence relation of suppression.

I_S is a linear function of voltage. The voltage range in which I_S is detectable coincides with that where voltage-gated currents were observed, as expected if I_S is due to a suppression of these currents. The *I-V* curve for I_S differs from that of any of the known conductances from these cells. Rather, its sign and shape (a straight line with a negative slope), in addition to the common origin of the I_S and I_o *I-V* curves, are consistent with a reduction of the net K^+ conductance (Fig. 8*B*).

The slope of the I_S *I-V* curve changed from cell to cell, most likely due to the same factors that explain the differences in the magnitude of suppression (see above), in addition to the important variations in the size of the ionic currents across cells.

I_S latency was also highly variable among neurons due to differences in stimulus strength (pressure and distance) and to the geometrical factors described above. When stimulating by pressure pulses, as in our case, the time it takes to reach the minimal odor concentration needed for causing suppression is longer for low pressures (Fig. 2*A*). Because we used different pressures, we attribute the variability of the latency to this reason. In the cases (2 of 17) in which I_S and I_T latencies were similar (Fig. 3), the cells had long dendrites, and odor puffs were applied to the cilia with particularly low pressures (3–4 lb/in.^{2}). We therefore used relatively high pressure stimuli (typically 10–14 lb/in.^{2}) in those experiments addressed to investigate suppression latency. The minimal I_S latency was shorter than I_T latency by one order of magnitude or more, and it was comparable to the latency of the inward current induced by a high-K^+ puff. Together, the results indicate that, unlike transduction, odors suppress I_o by a direct action on the underlying ion channels, without mediation of second messengers. This action seems likely to take place within the membrane, due to the hydrophobic nature of odorant molecules. Consistent with this notion, the mechanism of suppression is voltage independent, as indicated by the linearity of the I_S *I-V* curve. The fact that I_S latency is somewhat longer than that preceding the high-K^+-induced current may be related to the fact that volatile odors are liposoluble, and it may take longer for these molecules to partition into the membrane to cause their effect than for replacement of K^+ at the extracellular side of the membrane. Alternatively, it is possible that odors cause suppression by acting on a separate molecule intimately related to the ion channels, thus increasing the latency.

However, this possibility seems unlikely, because suppression is a nonselective process, and the putative intermediary molecule would have to be associated with all ion channels suppressed by odors and to present an extremely wide spectrum of interactions with odorants.

Localization studies suggest that the suppression mechanism is entirely independent of the transduction cascade. I_S was larger and faster when the odor pulses were directed to the cell body, where the voltage-gated channels reside, than when directed to the cilia, which contain the transduction channels. On the contrary, the magnitude of I_t, which is associated with transduction, was larger when odors were applied to the cilia.

The observation that I_S was considerably less affected by rundown than I_T is in agreement with the view that suppression involves a direct effect on ion channels, in contrast to transduction, which is mediated by a cascade mechanism.

The dose-response relation of suppression indicates that odor concentrations causing suppression are similar to those that trigger transduction in olfactory neurons (7, 19). We applied chemical stimuli to the entire cell to examine to what extent I_S interfered with I_T by performing studies on neurons capable of transducing *mixture I*. We corrected the odor-induced response by subtracting the effect of suppression. After this correction was done, the shape of the *I-V* curve became virtually indistinguishable from that reported for the odor-induced current in various species, where the chemical stimuli were focused on the olfactory cilia (Refs. 6, 7, 10, and see Refs. 16 and 21). According to our study, variations on the reversal potential of the transduction current are expected to occur, depending on the extent that odors reach the nontransducing plasma membrane in each particular experiment (soma and dendrite). When the differences in time courses between both odor effects were not large enough to allow a clear distinction between the two current components (I_S and I_T), we were able to correct the odor-induced currents only by *method I*. The validity of *method I* is supported by the application of *method II*, which could be used only in cases in which I_S and I_T were clearly separated in time. In such cases, both methods gave virtually identical results. In some cases, rundown accounted for small differences between the corrected *I-V* curves. Furthermore, we were able to separate the two components (I_S and I_T) of the net odor-induced current as a factor of time (Fig. 4*E*).

The degree of distortion in the *I-V* curve depends, to a large extent, on the relative magnitudes of I_S and I_T, being more pronounced the closer I_S was to I_T.

Localization of the chemical stimulus to the cilia of isolated olfactory neurons is, therefore, important for diminishing the interference of odor suppression when studying transduction currents at potentials at which the voltage-gated channels are open.

We show that brief odor pulses suppress all four voltage-gated conductances in *Caudiverbera*. Suppression is induced by both odorant mixtures, *I* and *II*, in *Caudiverbera* and in rat olfactory neurons, including those receptor neurons incapable of transducing such odors. We observed that the fraction of olfactory neurons suppressed by odor *mixture I* was smaller in the rat than in *C. caudiverbera*, in the concentration range of 1–150 μM (45% in the rat, compared with 94% in *Caudiverbera*). This difference may be due to the fact that the magnitude of the voltage-gated K^+ currents is considerably smaller in the rat than in the toad, making the resolution of the suppression effect more difficult. Indeed, we observed that, at higher odor concentrations, the percentage of suppressed rat olfactory neurons increased significantly. Additional reasons for the lower suppression effect observed in the rat, as possible differences in the nature of the K^+ channels of the olfactory neurons from both species, cannot be ruled out.

Suppression can also be induced in nonolfactory cells, e.g., *Drosophila* photoreceptor cells, where it reduced the voltage-gated currents, showing that odor suppression is independent of chemotransduction.

Our results demonstrate that, in contrast to odor transduction, suppression is a nonspecific phenomenon directly affecting ion channels, without requiring a signaling cascade. This possibility was previously suggested by Kawai et al. (9), who reported suppression of voltage-gated currents activated by depolarizing pulses applied during odor exposure. Based on the rapid suppression of the transduction currents by odor pulses reported by Kurahashi et al. (11), Kawai and coworkers proposed that a similar direct mechanism may underlie suppression of the voltage-gated currents. In the present work, we directly measured the latency of the suppression effect on the voltage-gated currents and demonstrated its independence of the transduction cascade.

Physiological Role of Suppression

The observation of the dual effect of volatile odorants, of triggering transduction and nonspecifically suppressing ion channels, raises the question of the actual significance of suppression in the normal physiology of olfactory neurons. Previously, Kurahashi et al. (11) demonstrated that odorants had a dual effect on odorant-induced transduction currents. One such effect was to trigger the transduction cascade, leading to the activation of the transduction current. The other effect was to suppress the transduction current, apparently by directly affecting the underlying ion channels. Suppression took place in 20 ms, comparable to the latency that we found for suppression on the voltage-gated channels. Those authors attributed the remarkably long latency (hundreds of milliseconds) that normally precedes the odor-induced transduction current to this suppressive effect. It is likely that the same mechanism underlies odor suppression in both the transduction and the voltage-gated channels.

The response induced by excitatory odorants on olfactory receptor neurons consists of a depolarizing receptor potential accompanied by an increase in action potential firing. The shape of this response is determined by the orchestration of the transduction conductances, a nonselective cationic cAMP-gated conductance and a Ca^{2+}-activated Cl^- conductance, and the

voltage-gated conductances. Therefore, to understand the physiological role of suppression, it is essential not only to consider suppression of the transduction conductances but also to establish how suppression alters the voltage-gated conductances in a time-dependent fashion.

To gain insight into whether odor suppression of the voltage-gated conductances may influence the response to excitatory odorants, we examined whether odorant pulses affected action potential firing in current-clamped olfactory neurons. We found that suppression can induce either a depolarizing or a hyperpolarizing membrane potential change, depending on the particular neuron, and that there was no apparent correlation between the polarity of the suppression-induced voltage change and the value of the cell resting potential. The type of effect of odor suppression in a given neuron depends on its particular pool of voltage-gated ion channels, whose relative densities vary from cell to cell (Ref. 15 and Madrid and Bacigalupo, unpublished observations), and on the membrane potential at the time of stimulation.

Our results indicate that suppression of voltage-gated channels is an important factor in determining the response of an olfactory neuron to odor stimulation and has to be taken into account to fully characterize the odor-induced response. It should be kept in mind, however, that our experiments could not address this question thoroughly, because in isolated olfactory neurons the odor stimuli reach the soma to an extent that depends on how the stimulus is applied. In situ, however, olfactory receptor neurons form part of the olfactory epithelium and odorants are presented by nature to the mucosal surface of this tissue. Tight junctions among the epithelial cells constitute a diffusion barrier for odorants toward the basolateral membranes. Nevertheless, the fact that volatile odorants can partition into the lipid bilayer of the plasma membrane and diffuse through it, as shown by Lowe and Gold (13), opens the possibility that, also in vivo, odorants may suppress ion channels present in the basolateral membrane of olfactory neurons. If this were the case, suppression would have a physiological role in olfaction that deserves to be properly evaluated. A definitive answer to this problem demands further studies that are beyond the scope of the present paper.

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