The migration of the VPCs during the L2 and L3 stage was observed at 2-hour intervals. Animals were kept at 10°C overnight. Cell migrations took place over 3 to 4 days under these conditions and were observed in two animals in each species.
12. Because the VPCs had to pass more posterior Pn.p cells during migration, the latter moved to a more dorsal position in the ventral cord.
13. We define the gonadal cell making the contact between vulva and uterus as the AC. As in *Caenorhabditis*, this cell is morphologically distinct from surrounding gonadal cells.
14. After ablation of M (five animals), V5 (two animals), P(9,10) (four animals), P(11,12) (three animals), B, F, U, and Y (three animals each), KL and KR (three animals), and F and U together (two animals), the vulva developed normally. Ablation of other combinations of these cells was always lethal.
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16. Criteria for the definition of cell fates were as follows. 1° cell fate: invagination at the four-cell stage; AC attachment between P6.pap and P6.ppa; final division pattern UTTU (U, undivided cell; T, transverse division). 2° cell fate: no invagination in the four-cell stage; only the outer cell undergoes a longitudinal third division (LUUU or UUUL; L, longitudinal division); no AC contact. Intermediate cell fate: no invagination in the four-cell stage; if the AC contacts the cells, the attachment is random; the lineage is variable. We observed LUUU, LLUU, and LLLU lineages; thus, five to seven cells are generated. The LLUU patterns occur most frequently. An invagination occurs after cell divisions are completed, whereas in the 1° fate, invagination occurs in the four-cell stage. In the intermediate lineage the distribution of the cells is random with respect to the invagination. Oblique division axis were observed with low frequency.
17. In 91 ablated animals (Table 1), there were only three cases in which P7.p or P8.p assumed the 1° cell fate.
18. In contrast, after ablation of P(5,6).p in *Cruznema*, P4.p or P7.p assumed the 1° fate (Fig. 3D). In these ablation experiments, the AC stops migration in the region of P4.p or P7.p. Because P4.p and P7.p are able to adopt the 1° cell fate, we conclude that VPCs have equivalent potentials in *Cruznema*. Thus, nonequivalence of VPCs is not required for posterior vulva formation in general. Whereas nonequivalent VPCs and gonad-independent vulva development in *Mesorhabditis* and *Teratorhabditis* are coincident, we have no evidence that they must coevolve.
19. A related phenomenon at the organismal level, called developmental constraint, is discussed in J. M. Smith *et al.* [*Q. Rev. Biol.* **60**, 265 (1985)] and S. J. Gould and R. C. Lewontin [*Proc. R. Soc. London Ser. B* **205**, 581 (1979)].
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22. We hypothesize that the positional information the VPCs receive early in development allows them to migrate to the more posterior position.
23. We thank L. Carta and D. Fitch for strains and E. Davidson, S. Fraser, E. Meyerowitz, L. Zimmer, and members of our laboratory for detailed discussion. P.W.S. is an investigator of the Howard Hughes Medical Institute. Supported by a Presidential Young Investigator Program award from the NSF (to P.W.S.). R.J.S. is supported by an EMBO long-term fellowship.

14 February 1994; accepted 10 May 1994

Suppression of Odorant Responses by Odorants in Olfactory Receptor Cells

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Odorants activate an inward current in vertebrate olfactory receptor cells. Here it is shown, in receptor cells from the newt, that odorants can also suppress this current, by a mechanism that is distinct from inhibition and adaptation. Suppression provides a simple explanation for two seemingly unrelated phenomena: the anomalously long latency of olfactory transduction and the existence of an "off response" at the end of a prolonged stimulus. Suppression may influence the perception of odorants by masking odorant responses and by sharpening the odorant specificities of single cells.

Odorants activate an inward (depolarizing) membrane current, termed the transduction current, in vertebrate olfactory receptor cells (*1–3*). This current is carried by second messenger–gated ion channels (*4, 5*) that are activated by enzymatic cascades consisting of receptor proteins (*6*), heterotrimeric guanosine triphosphate–binding proteins (G proteins) (*7*), and effector enzymes (*8*). However, these mechanisms cannot explain two prominent characteristics of olfactory responses: their long latencies, which can exceed 500 ms in amphibians and are only weakly dependent on odorant concentration (*9*), and the transient increase in membrane current after the end of a prolonged stimulus, termed an "off response" (*10–12*). Models of the activation kinetics of G protein cascades predict minimum latencies of less than 50 ms and a strong dependence of latency on odorant concentration (*13*). Off responses are not predicted by the G protein cascade model, nor are they observed in other signal-transducing cells. Consequently, the existence of these phenomena suggests fundamental differences between olfactory and other signal transduction mechanisms. Here we report that odorants can suppress the inward transduction current in solitary newt olfactory receptor cells, an effect that provides a simple explanation for these seemingly unrelated phenomena.

Odorant suppression of the transduction current was shown by the application of a brief odorant pulse during a cell's response to an earlier pulse of the same odorant (Fig. 1A) (*14*). A single pulse of the odorant amyl acetate caused a transient inward current that began with a latency of 280 ms (trace 1) (*15*). Such a response is typical of whole-cell recordings from vertebrate olfactory receptor cells (*1–3*). However, a second odorant pulse that was timed to begin at the peak of the response to the first pulse caused an immediate decrease in membrane current (trace 2; latency, 20 ms). This

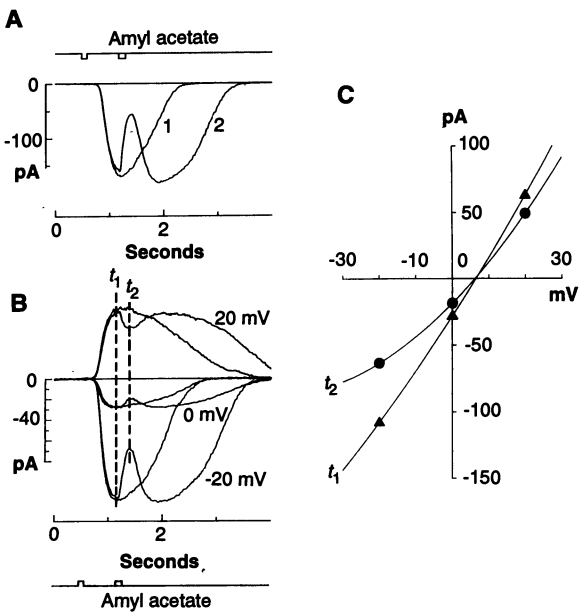

Fig. 1. Suppression of an odorant response by a pulse of odorant. (**A**) Trace 1, response to a single pulse of amyl acetate (10 psi; 100 ms in duration, starting at 0.5 s); trace 2, response to two pulses of amyl acetate, the first pulse identical to the pulse in trace 1 and the second starting at 1.2 s (10 psi, 120 ms in duration). Holding potential, −40 mV. (**B**) The same experiment as in (A), repeated at the holding potentials indicated; t_1 (1.1 s) was the time at the peak of the current indiced by the first pulse; t_2 (1.4 s) was the time at maximal suppression. (**C**) Current-voltage relations for the traces in (B), measured at t_1 (triangles) and t_2 (circles). The curves are second-order regression.

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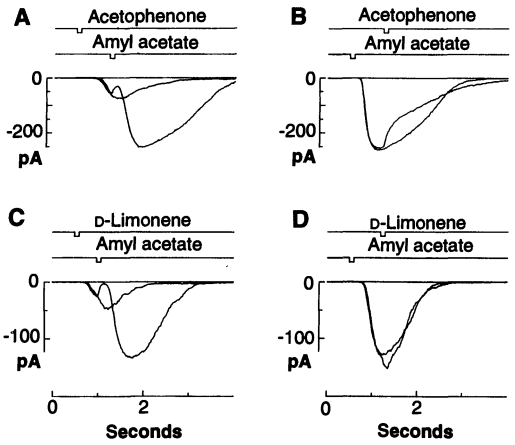

Fig. 2. Suppression of odorant responses by different odorants. In each panel, the first odorant pulse activated the transduction current and the second odorant pulse caused suppression. (**A**) and (**B**) were from one cell; (**C**) and (**D**) were from another. In each panel, the different odorants were delivered by different stimulus pipettes having the same tip opening. In (D), D-limonene caused a small transduction current but no suppression; the smaller size of the current increment, compared with the response in (C), may be due to response saturation.

decrease was not a stimulation artifact, because an identical pulse of odorant-free Ringer solution had no effect on membrane current. The initial decrease in membrane current was followed by a more prolonged increase that was presumably due to further activation of the inward transduction current by the second stimulus.

We investigated the ionic mechanism of the decrease in membrane current caused by the second odorant pulse by repeating the double pulse experiment at several membrane potentials (Fig. 1B). The transduction current (the peak amplitude of the current caused by the first stimulus) and the decrement in current caused by the second odorant stimulus both reversed at the same membrane potential (Fig. 1C), which indicates that the decrement in current resulted from suppression of the inward current rather than activation of an outward, or inhibitory, current. Consequently, we refer to this phenomenon as suppression to distinguish it from inhibition, which has been demonstrated in lobster olfactory receptor cells (*16*). Suppression also differs from adaptation because adaptation develops more slowly (*17*). An effect similar to suppression has been observed in vivo with extracellular field recording (*11*). However, the equivalence of this phenomenon to suppression cannot be established from existing data.

We also tested whether one odorant could suppress a response to another odorant. Amyl acetate suppressed a response to acetophenone (Fig. 2A), acetophenone suppressed a response to amyl acetate (Fig. 2B) (*18*), and amyl acetate suppressed a response to D-limonene (Fig. 2C). However, D-limonene did not suppress a response to amyl acetate (Fig. 2D). Thus, suppression can occur with some but not all of the odorants tested, and an odorant can suppress a response to itself as well as a response to another odorant.

The existence of odorant suppression provides a likely explanation for the anomalously long latency of the transduction current. In previous measurements, the time course of the odorant stimulus overlapped the rising phase of the transduction current (*1–3*). Consequently, suppression could have attenuated the rising phase of the response, increasing the apparent latency. We tested this hypothesis by observing the effect of stimulus duration on response latency. Latency increased when we increased the duration of the stimulus by changing the duration of the odorant pulse (Fig. 3A) or by following the odorant pulse with a pulse of odorant-free Ringer solution to remove the odorant more rapidly than by diffusion alone (Fig. 3B) (*19*). These effects are the opposite of those expected from temporal integration of the stimulus (*9*) but support the hypothesis that much of the response latency is due to suppression of the transduction current by the stimulus itself (*20*). Furthermore, even the shortest latency in Fig. 3 (180 ms) may overestimate the true latency of olfactory transduction, because amyl acetate is hydrophobic and therefore may have remained at a high concentration in the cell membrane for some time after the extracellular odorant concentration was reduced. Previously, the long latency of olfactory transduction and its weak dependence on odorant concentration have been interpreted as evidence of mechanistic differences between olfactory and other G protein–dependent transduction mechanisms (*9*). Our data indicate that existing latency measurements cannot provide information about the transduction mechanism unless odorant suppression is taken into account (*21*).

Odorant suppression also provides a likely explanation for the off response that can occur at the end of a prolonged odorant pulse (*10–12*), because the removal of suppression could cause a transient increase in membrane current. We tested this hypothesis by comparing the voltage dependence of the off response to that of the initial transduction current. A 5-s odorant pulse induced an inward current that decayed initially because of ad-

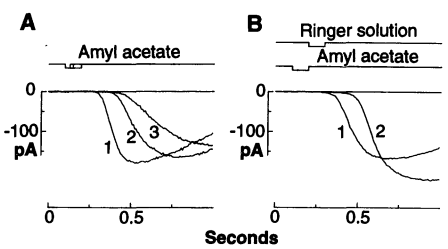

Fig. 3. Effect of stimulus duration on the latency of the transduction current. (**A**) Effect of changing the duration of the pressure pulse. Amyl acetate was applied to the cell for 50, 100, and 200 ms, causing responses 1, 2, and 3, respectively. (**B**) Effect of accelerated odorant removal. Trace 1, stimulation with a 100-ms pulse of amyl acetate followed immediately by a 100-ms pulse of Ringer solution; trace 2, stimulation with a 100-ms pulse of amyl acetate.

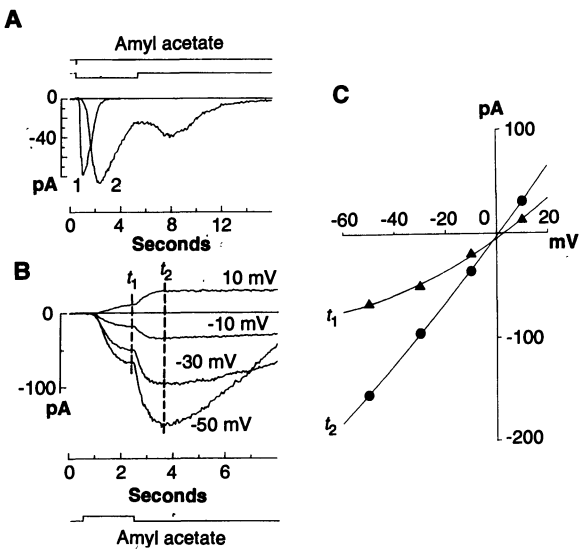

Fig. 4. Off responses observed at the end of prolonged stimulation with amyl acetate. (**A**) Trace 1, current induced by a brief pulse of amyl acetate (30 ms); trace 2, current induced by a prolonged pulse of amyl acetate (5 s). (**B**) Responses to a long pulse of amyl acetate (2 s) at the holding potentials indicated; t_1 (2.4 s) was the time at the peak of the initial inward current; t_2 (3.6 s) was the time at the peak of the off response. (**C**) Voltage dependence of the inward current (triangles) and of the off response (circles), measured as the current in (B) at times t_1 and t_2, respectively.

aptation (*17*) but increased transiently after the end of the stimulus (Fig. 4A, trace 2). A 30-ms odorant pulse induced a transient inward current that rose more rapidly and had a shorter latency (trace 1), presumably because the briefer stimulus caused less suppression. The voltage dependence of the off response was similar to that of the inward transduction current (Fig. 4, B and C), which indicates that the off response was caused by the removal of suppression that occurred during the long odorant pulse.

We have shown that suppression of the transduction current is caused by some, but not all, odorants and that suppression occurs at the same odorant concentrations as those that cause transduction in single cells. Thus, suppression must be considered in quantitative analyses of the transduction current, particularly when prolonged stimuli are used to study adaptation. The mechanism or mechanisms of suppression are not revealed by our experiments. However, the shorter latency of suppression compared with that of the inward transduction current suggests that suppression is due to a direct effect of odorants on ion channels or on second messenger metabolism, rather than to an effect on olfactory receptor proteins. Traditionally, olfactory transduction has been viewed solely in terms of receptor protein–mediated effects that occur at threshold odorant concentrations. However, the existence of suppression emphasizes the fact that olfactory transduction, when studied electrophysiologically or behaviorally, reflects all mechanisms by which odorants can affect membrane potential.

It is likely that suppression occurs in vivo, because the effects described here on isolated receptor cells are similar to those apparent in extracellular field recordings in vivo (*10, 11, 21*). Therefore, suppression may influence the perception of odorants. For example, suppression could improve odorant discrimination by preferentially attenuating small responses generated in cells that are relatively insensitive to a particular odorant (*20*). Suppression may also contribute to odor masking (*22*) because it enables one odorant to attenuate responses to other odorants.

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14. Odorant-induced currents were recorded in the whole-cell configuration from solitary olfactory receptor cells from the newt (*Cynops pyrrhogaster*). All experimental manipulations were as described (*1*); solutions were as described in (*5*), except that the Ringer solution contained 90 mM NaCl. The holding potential was −50 mV unless indicated otherwise. The odorants were applied to receptor cells by pressure ejection from a micropipette (pressure 10 psi unless indicated otherwise). All pipette solutions contained the specified odorant at a concentration of 0.5 μl/ml. The odorant concentration at the cell surface was estimated to be less than one tenth the concentration in the micropipette (*2*). In all figures, traces superimposed on a single baseline were obtained from the same cell. All figures illustrate experiments carried out in triplicate.
15. Latency is defined as the time interval between the beginning of the pressure pulse and a definite change in current away from the baseline.
16. An odorant-activated inhibitory conductance has been demonstrated in lobster olfactory receptor cells [W. C. Michel, T. S. McClintock, B. W. Ache, *J. Neurophysiol.* **65**, 446 (1991)]. Hyperpolarizing odorant responses have also been reported in vertebrate species (*11*) [T. V. Getchell and G. M. Shepherd, *J. Physiol. (London)* **282**, 541 (1978); V. E. Dionne, *J. Gen. Physiol.* **99**, 415 (1992)]; however, the ionic mechanisms of these responses were not determined, so it is not certain whether they reflect inhibition or suppression.
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18. The smaller secondary rise in current that was caused by acetophenone presumably reflected the smaller transduction current induced by this odorant (Fig. 2, A and B).
19. As expected from the fact that D-limonene did not cause suppression (Fig. 2D), the latency of D-limonene responses was relatively short (150 ms) and was comparable to the shortest latency observed for an amyl acetate stimulus when the stimulus was removed by a pulse of Ringer solution (Fig. 3B).
20. The increase in response latency indicates that suppression of the transduction current is nonlinear, with small currents being attenuated more than large currents.
21. In previous studies on salamanders, increasing the odorant concentration had variable effects on latency, depending on the experimental preparation. For in vivo extracellular single-unit recordings, a decrease in latency was observed [T. V. Getchell and G. M. Shepherd, *J. Physiol. (London)* **282**, 521 (1978)]; for recordings of extracellular field potential, the latency decreased over a lower odorant concentration range then increased over a higher odorant concentration range [A. H. Arzt, W. L. Silver, J. R. Mason, L. Clark, *J. Comp. Physiol. A* **158**, 479 (1986), figure 5]; for whole-cell recordings on dissociated receptor cells, the latency remained approximately constant in one study (*9*) but decreased in another study [S. Firestein, C. Picco, A. Menini, *J. Physiol. (London)* **468**, 1 (1993)]. These diverse effects can be understood by considering the opposing effects of increasing odorant concentration on the latency of second messenger production and on suppression. The sensitivity of the cells and the absolute odorant concentration will determine which effect will dominate or whether they will mutually cancel.
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23. We thank B. G. Green for helpful discussions concerning odor masking and G. K. Beauchamp, L. M. Masukawa, and E. N. Pugh Jr. for critical comments on the manuscript. Supported by a grant from NIH and a fellowship from the Monell Chemical Senses Center to T.K.

22 December 1993; accepted 26 April 1994