

Selectivity and Response Characteristics of Human Olfactory Neurons

N. E. RAWSON,¹ G. GOMEZ,¹ B. COWART,¹ J. G. BRAND,^{1,3,4} L. D. LOWRY,^{1,2} E. A. PRIBITKIN,²
AND D. RESTREPO^{1,4}

¹Monell Chemical Senses Center, Philadelphia; ²Thomas Jefferson University, Philadelphia; ³Veterans Affairs Medical Center, Philadelphia; and ⁴University of Pennsylvania, Philadelphia, Pennsylvania 19104-3308

Rawson, N. E., G. Gomez, B. Cowart, J. G. Brand, L. D. Lowry, E. A. Pribitkin, and D. Restrepo. Selectivity and response characteristics of human olfactory neurons. *J. Neurophysiol.* 77: 1606–1613, 1997. Transduction mechanisms were investigated in human olfactory neurons by determining characteristics of odorant-induced changes in intracellular calcium concentration ($[Ca^{2+}]_i$). Olfactory neurons were freshly isolated from nasal biopsies, allowed to attach to coverslips, and loaded with the calcium-sensitive indicator fura-2. Changes in $[Ca^{2+}]_i$ were studied in response to exposure to individual odors, or odorant mixtures composed to distinguish between transduction pathways mediated by adenosine 3'5'-monophosphate (cAMP; mix A) or inositol 1,4,5-trisphosphate (InsP₃; mix B). Overall, 52% of biopsies produced one or more odorant-responsive olfactory neurons, whereas 24% of all olfactory neurons tested responded to odorant exposure with a change in $[Ca^{2+}]_i$. As in olfactory neurons from other species, the data suggest that odorant exposure elicited calcium influx via second-messenger pathways involving cAMP or InsP₃. Unlike olfactory neurons from other species that have been tested, some human olfactory neurons responded to odorants with decreases in $[Ca^{2+}]_i$. Also in contrast with olfactory neurons from other species, human olfactory neurons were better able to discriminate between odorant mixtures in that no neuron responded to more than one type of odor or mixture. These results suggest the presence of a previously unreported type of olfactory transduction mechanism, and raise the possibility that coding of odor qualities in humans may be accomplished to some degree differently than in other vertebrates, with the olfactory neuron itself making a greater contribution to the discrimination process.

INTRODUCTION

Human olfaction—the ability to detect many volatile chemicals in one's environment—appears to be a primitive system in an evolutionary sense, yet has proven to be biologically complex. It is commonly thought that olfaction is less developed in humans than most other species, yet we are able to detect and, with training, discriminate among thousands of odors at concentrations below the limit of detection of virtually any instrument (Amoore and Hautal 1983). On the basis of molecular biological studies of putative olfactory receptor genes (Buck and Axel 1991), it has been estimated that humans may express as many as 1,000 different G protein-linked olfactory receptors that can be grouped into a number of classes and subclasses on the basis of nucleotide sequence similarities (Lancet and Ben-Arie 1993). The mechanisms by which the olfactory system utilizes these receptors to detect and decode a multitude of odors have been studied in a variety of invertebrate and vertebrate systems, but comparatively few studies have addressed this question at the cellular level in human olfactory neurons.

Studies of olfactory neuron function in various animal models have led to a view of olfactory transduction that involves odorant activation of G protein-linked odorant receptors, followed by generation of the second messengers adenosine 3'5'-monophosphate (cAMP) and/or inositol 1,4,5-trisphosphate (InsP₃) (see reviews by Restrepo et al. 1996; Shepherd 1994). The opening of cAMP- or InsP₃-regulated cation channels results in influx of Ca²⁺ and a subsequent increase in intracellular calcium concentration ($[Ca^{2+}]_i$) (Restrepo et al. 1990, 1993a; Sato et al. 1991; Tareilus et al. 1995), leading to opening of Ca²⁺-activated Cl⁻ or K⁺ channels (Kleene and Gesteland 1991; Kurahashi and Yau 1993; Morales et al. 1995). In those cells in which the concurrent change in membrane potential is a depolarization, voltage-sensitive channels open, triggering action potentials that are carried along the axon to the olfactory bulb.

Studies in a variety of species have shown that single olfactory neurons often respond to more than one odorant and may even respond to odorants of different chemical or perceptual classes. In addition, a single olfactory neuron will often respond to structurally dissimilar odorants, and in some cases to two odorants known to stimulate different second-messenger pathways (Boekhoff et al. 1994; Firestein et al. 1993; Kang and Caprio 1995; Revial et al. 1982). This evidence that individual olfactory neurons are often nonselective has led investigators to propose that odor qualities are coded to a large extent by distributed patterns of neural activity interpreted at the level of the olfactory bulb (Kauer 1991).

The small amount of functional information available in humans suggests that human olfactory neurons respond to odorants similarly to olfactory neurons from other species (Doty et al. 1990; Restrepo et al. 1993b). However, a thorough study of the responsiveness of human olfactory neurons to odors has not been reported. In this manuscript we characterize the responses of human olfactory neurons isolated from olfactory tissue biopsies with the use of calcium imaging. Our studies indicate that, like olfactory neurons from other species, human olfactory neurons respond to odors utilizing at least two different second-messenger pathways. However, human olfactory neurons also respond to odorants with a decrease in $[Ca^{2+}]_i$, a response not observed in olfactory neurons from other species, and these cells appear to be more selective for odorants than are olfactory neurons from other species.

METHODS

Subjects and psychophysical measurements

Biopsies were obtained from volunteers ($n = 34$) who first completed a medical screening questionnaire and psychophysical

tests to assess olfactory function. Some biopsies were also obtained from surgery patients ($n = 10$) and are tabulated separately. Volunteers were recruited to include a cross section of the local population, age 18–64 yr, with 9 males and 20 females. Subjects provided informed consent by signing a document describing the nature and possible consequences of participation; those with potentially complicating diseases or medications were omitted from the study. Biopsies were obtained generally within 2 wk of initial evaluation. One compound from each of the two odorant mixtures used in the single-cell experiments was chosen for threshold testing: phenylethyl alcohol and lylal. These compounds appear to stimulate the olfactory system exclusively, because even at the highest concentrations tested they cannot be localized with the use of a lateralization test (C. Wysocki, personal communication) and are not detected by anosmics (Rawson et al. 1995). Threshold sensitivity was determined via a two-alternative, forced-choice staircase procedure described previously (Rawson et al. 1995). All subjects could detect both compounds on the side of the biopsy. Unilateral thresholds for phenylethyl alcohol on the biopsied side were 0.018–0.000024% vol/vol (median 0.00047%); for lylal, thresholds were 0.014%–0.0000042% vol/vol (median 0.00027%).

Biopsy and cell dissociation procedures

Biopsies of $\sim 1 \text{ mm}^3$ were obtained from the high middle turbinate and apposed septum from subjects after local anesthetic as described (Lowry and Pribitkin 1995; Rawson et al. 1995). For surgery patients, epinephrine was injected locally in addition to the general anesthetic and a single biopsy was obtained from the inferior half of the middle turbinates being resected for sinus surgery. It has traditionally been thought that the olfactory epithelium is located predominantly within the olfactory cleft (Lanza and Clerico 1995). The higher risk of obtaining tissue from this region because of its proximity to the cribriform plate led us to obtain biopsies from the middle turbinate and apposing septum, a region not generally characterized as containing olfactory epithelium. Nonetheless, nearly 50% (14 of 34) of the biopsies obtained from this region produced one or more odorant-responsive olfactory neurons. This finding is consistent with early reports suggesting that olfactory epithelium may also be present in the superior aspect of the middle turbinate (see Lanza and Clerico 1995 for discussion). Biopsies obtained from surgery patients were generally 2–3 times as large and produced odorant-responsive olfactory neurons 90% of the time. Cells were dissociated by brief incubation in cation-free mammalian Ringer solution containing 12–15 U/ml papain, as described previously (Restrepo et al. 1993b).

Measurement of $[Ca^{2+}]_i$ and odorant exposure

Changes in $[Ca^{2+}]_i$ were observed by loading cells with fura-2 and observing fluorescence emitted in response to dual excitation at 340 nm (Ca^{2+} sensitive) or 360 nm (Ca^{2+} insensitive). Details have been published elsewhere (Restrepo et al. 1993a,b). Cells attached to coverslips were superfused with Ringer solution and exposed to odor mixtures or single odorants via the superfusion buffer or via a computer-controlled Solution Changer (Bio-Logic, RSC-100, Pullman, WA). Cells were tested with two odorant mixtures, mix A (hedione, geraniol, phenylethyl alcohol, citralva, citronellal, eugenol, and menthone) and mix B (lylal, lilyal, triethylamine, ethylvanillin, isovaleric acid, and phenylethyl amine), or with individual odorants dissolved in Ringer solution at 1 or 100 μM as indicated. Some olfactory neurons were tested to determine the extent of calcium uptake following a challenge with the calcium ionophore ionomycin (5 μM). The $[Ca^{2+}]_i$ of these olfactory neurons ($53.6 \pm 14.9 \text{ nM}$, mean \pm SE) increased to $206.7 \pm 15.7 \text{ nM}$ after ionomycin [$t(9) = 6.58$, $P < 0.05$; $n = 10$]. Baseline and

ionomycin-stimulated $[Ca^{2+}]_i$ were similar in responding and non-responding olfactory neurons. Baseline $[Ca^{2+}]_i$ did not differ between olfactory neurons responding to mix A and mix B, those from males and females, or those from subjects and surgery patients. Depolarizing cells by increasing extracellular K^+ leads to an influx of Ca^{2+} in $>90\%$ of rat olfactory neurons tested because of activation of voltage-sensitive calcium channels (Restrepo et al. 1993b; Tareilus et al. 1995). In contrast, depolarizing human olfactory neurons by replacing extracellular Na^+ in the superfusion buffer with K^+ produced an increase in $[Ca^{2+}]_i$ in only two of eight olfactory neurons tested ($\chi^2 = 15.40$, $P < 0.001$ vs. rat).

Statistics

Only responses that began within 100 s of exposure, returned toward baseline after removal of the odorant, and could be repeated after washout were included in the analyses. Statistical comparisons were performed with the use of paired t -tests for baseline versus stimulated values and independent t -tests for comparisons of baseline values between groups with Statistica software (StatSoft).

RESULTS

Response rates to odorant stimuli

Psychophysical tests showed that all subjects were able to detect odorous compounds included in the mixtures used to stimulate isolated olfactory neurons (see METHODS).

The overall response rate for olfactory neurons from all subjects was 24%. Response rates and olfactory thresholds were not correlated. Similar response rates were obtained from smokers ($n = 7$ biopsies) and nonsmokers ($n = 27$) and from females (27%) and males (20%). The average age of subjects whose biopsies produced one or more odor-responsive cells (“responders”) was similar to that of “non-responders” (31.7 vs. 30.6 yrs). A higher response rate was seen in cells from surgery patients versus subjects. This may be because of the larger size of the biopsy or differences in anesthetics (general vs. local). However, it seems unlikely that these anesthetics influenced response characteristics, because similar response types were seen in neurons obtained from subjects and surgery patients despite the different molecular targets of the anesthetics. An insufficient number of nonwhite subjects was biopsied to permit analysis of ethnic differences (23 white, 6 black, 1 Hispanic, 3 Asian-Indian).

Human olfactory neurons respond to stimulation with odorant mixtures with either increases or decreases in $[Ca^{2+}]_i$

Changes in $[Ca^{2+}]_i$ in response to odorant stimuli were measured in 179 human olfactory neurons (Table 1) identified by the presence of a single dendrite and olfactory knob with attached cilia. As shown previously (Restrepo et al. 1993b), human olfactory neurons exhibiting an increase in $[Ca^{2+}]_i$ in response to a mixture of odorants known to stimulate cAMP formation in rat (“mix A,” 100 μM) were observed (Fig. 1, A and B; Table 1). Baseline and stimulated $[Ca^{2+}]_i$ for these olfactory neurons was 37.2 ± 9.6 (SE) nM and 81.8 ± 21.0 (SE) nM, respectively [$t(15) = 3.83$, $P < 0.01$]. Responses were also observed to lower concentrations of mix A (1 and 10 μM) that in some cases were of similar magnitude, possibly because of saturation of the response.

TABLE 1. Response rates for biopsies and individual olfactory neurons

Group	n	Average Number ONs Tested/ Biopsy	Biopsies Responding/ Number Tested	Positive Responses/Number of ONs Tested		
			Response to mix A or mix B	Mix A	Mix B	Mix A or Mix B
Subjects	29	4.1	14/34 (41)	14/134 (10)	11/129 (8)	25/142 (18)
Surgery	10	3.4	9/10 (90)	11/34 (32)	7/37 (19)	18/37 (49)
All subjects	39	3.9	23/44 (52)	25/168 (15)	18/166 (11)	43/179 (24)

Response rates from volunteers also given psychophysical tests ("subjects") or from surgery patients. Values in column 3 are the number of biopsies yielding ≥ 1 responsive olfactory neuron/number of biopsies tested. Numbers in parentheses in columns 3–6 are percentages. Five subjects donated biopsies twice. One hundred fifty-five olfactory neurons were tested with both mixtures; 11 with mix A only, 13 with mix B only. To control for nonspecific effects of odorant mixes A and B, we tested odorant responsiveness of respiratory epithelial cells from the same biopsies. Mix A and B (100 μM) did not elicit changes in intracellular calcium concentration in 12 respiratory epithelial cells (different from olfactory neurons, $\chi^2 = 3.89$, $P < 0.05$). ONs, olfactory neurons.

In contrast with studies in rat showing that all responsive olfactory neurons respond to odorants with an increase in $[\text{Ca}^{2+}]_i$ (Restrepo et al. 1993a; Tareilus et al. 1995), a substantial number of human olfactory neurons (9 of 25) responded to mix A with a decrease in $[\text{Ca}^{2+}]_i$ (Fig. 1C). The $[\text{Ca}^{2+}]_i$ in these olfactory neurons decreased from a baseline

of $67.9 \pm 22.6 \text{ nM}$ to $52.3 \pm 17.4 \text{ nM}$ after odorant exposure [$t(8) = 5.06$, $P < 0.001$].

Olfactory neurons responding to a mixture of odorants known to stimulate InsP_3 formation in rats ("mix B," 100 and 10 μM) were also found. These cells responded with increases in $[\text{Ca}^{2+}]_i$ that in some cases were spatially homo-

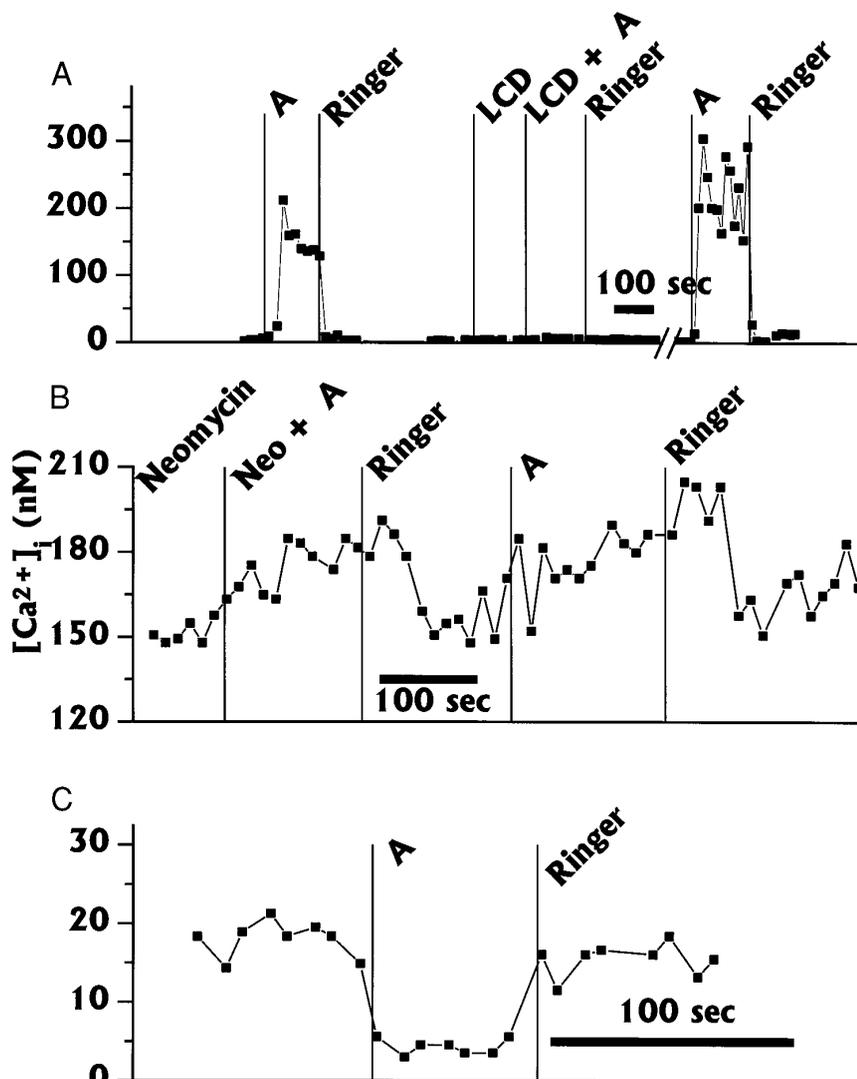


FIG. 1. Intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) responses of human olfactory neurons to a mixture of odorants shown to elicit increases in adenosine 3'5'-monophosphate (cAMP) in isolated olfactory cilia (Breer and Boekhoff 1991) (mix "A"). A: record from an olfactory neuron that exhibited an increase in $[\text{Ca}^{2+}]_i$ in response to mix A (100 μM) that was blocked by L-cis-diltiazem (LCD, 20 μM). B: neomycin (1 mM) fails to block the rise in $[\text{Ca}^{2+}]_i$ in another cell. C: record from an olfactory neuron that exhibited a decrease in $[\text{Ca}^{2+}]_i$ in response to mix A (100 μM).

geneous and in others were localized apically where the receptor and transduction machinery is thought to be located (Fig. 2, A–C). Baseline and stimulated $[Ca^{2+}]_i$ was 60.2 ± 15.4 (SE) nM and 104.8 ± 31.6 (SE) nM, respectively [$t(13) = 2.88$, $P < 0.05$].

In addition, of the 18 olfactory neurons responding to mix B, 4 exhibited a decrease in $[Ca^{2+}]_i$ (Figs. 2, D and E, and 3). Baseline and stimulated $[Ca^{2+}]_i$ for these olfactory neurons was 82.3 ± 41.1 (SE) nM and 59.2 ± 29.6 (SE) nM, respectively [$t(3) = 4.98$, $P < 0.01$].

Of the 43 cells responding to either mixture, 30% showed a decrease in $[Ca^{2+}]_i$ and an individual olfactory neuron exhibited only one type of response (i.e., either increase or decrease). Neither the morphology nor the baseline $[Ca^{2+}]_i$ differed between olfactory neurons exhibiting decreases and those exhibiting increases. Decreases in $[Ca^{2+}]_i$ tended to occur throughout the cell, in some cases peripherally as shown in Fig. 3. The olfactory transduction mechanisms mediating increases in $[Ca^{2+}]_i$ have been localized to the

cilia and apical, dendritic portion of the cell, and consistent with this, odorant-induced increases in $[Ca^{2+}]_i$ often occur initially or primarily in this region (Fig. 2B). Clearly, further work will be needed to understand the mechanism for odorant-induced decreases in $[Ca^{2+}]_i$ and their localization.

All responding human olfactory neurons discriminated between mix A and mix B odorants

In previous experiments in our laboratory (Restrepo et al. 1993a), we found that one third of responding rat olfactory neurons responded to both mixes A and B at a concentration of $100 \mu\text{M}$. Similar results were obtained in independent experiments in rat olfactory neurons (Tareilus et al. 1995) with the use of similar odorants. These observations are consistent with electrophysiological investigations of odorant responses in other species showing that single olfactory neurons often respond to more than one odorant and may even respond to odorants of different chemical or perceptual

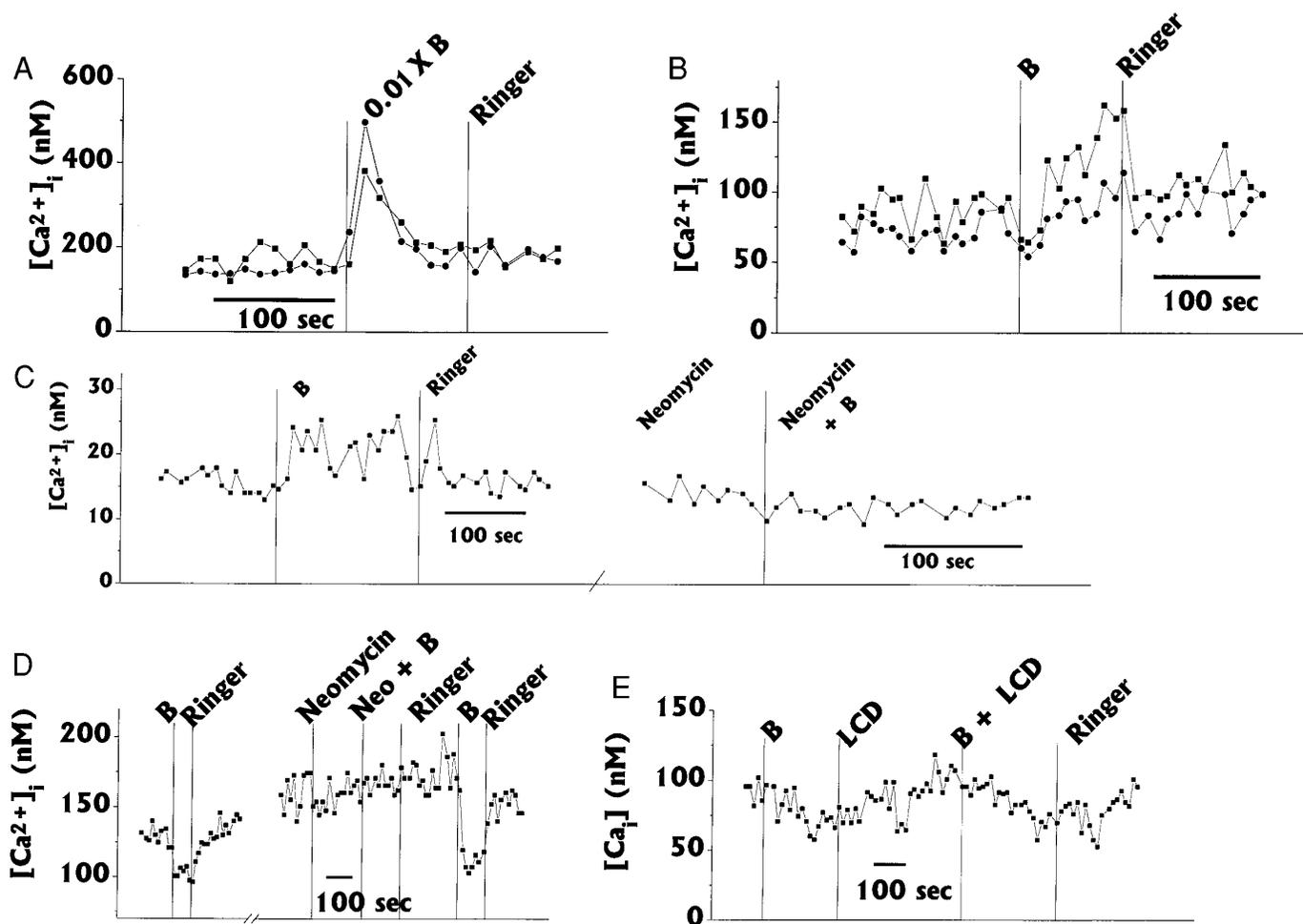


FIG. 2. Responses of human olfactory neurons to a mixture of odorants known to stimulate inositol 1,4,5-trisphosphate (InsP_3) production in isolated olfactory cilia (Breer and Boekhoff 1991) (mix "B"). A: record from a cell that responded to $1 \mu\text{M}$ mix B with a homogeneous rise in $[Ca^{2+}]_i$. B: data from an olfactory neuron that responded to $100 \mu\text{M}$ mix B with an apically localized increase in $[Ca^{2+}]_i$. Filled circles: cell body. Filled squares: apical, dendritic portion. C: neomycin prevents the rise in $[Ca^{2+}]_i$ induced by mix B ($100 \mu\text{M}$). D: neomycin also prevents the decrease in $[Ca^{2+}]_i$ induced by mix B ($100 \mu\text{M}$) in another cell. E: blocking the cyclic nucleotide-gated channel with LCD ($20 \mu\text{M}$) does not affect the decrease in $[Ca^{2+}]_i$ elicited by mix B ($100 \mu\text{M}$) in another cell. Mix B was applied with the use of the rapid solution exchanger in A and B, and by superfusion in the other figures.

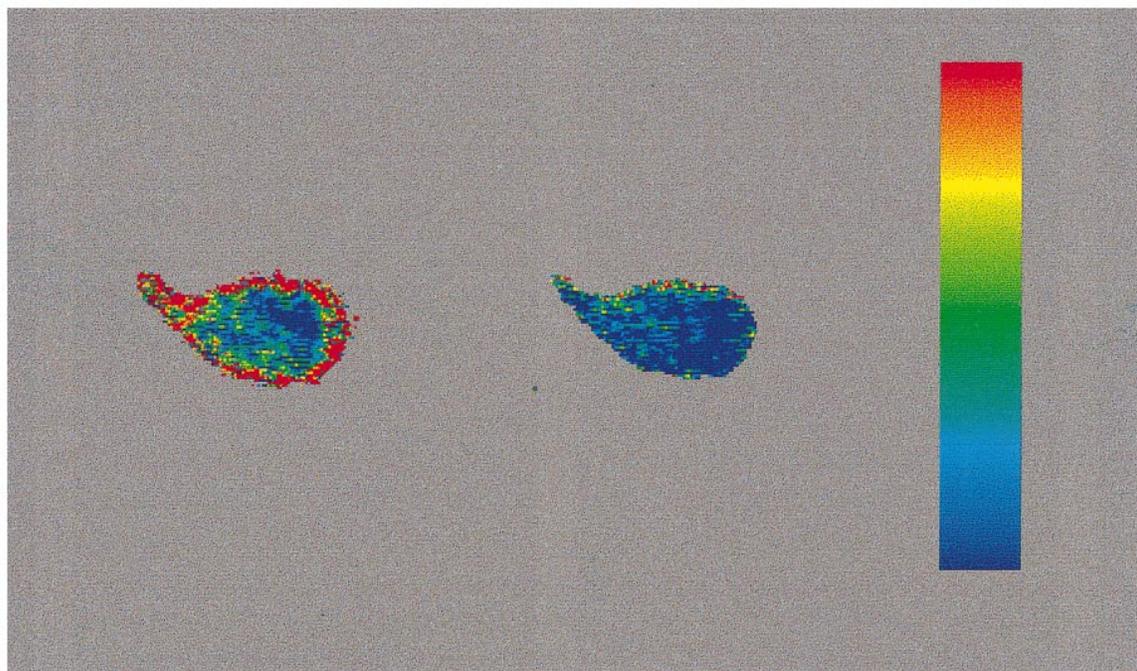


FIG. 3. Pseudocolor image displaying the spatial distribution of the decrease in $[Ca^{2+}]_i$ elicited by mix B in a human olfactory receptor neuron. $[Ca^{2+}]_i$ is color coded linearly by the color palette displayed in the rainbow. In this image red corresponds to 200 nM $[Ca^{2+}]_i$, whereas blue corresponds to 100 nM $[Ca^{2+}]_i$. *Left*: olfactory neuron before stimulation. *Right*: olfactory neuron 10 s after stimulation with 100 μ M mix B. This cell responded repeatedly to mix B with a decrease in $[Ca^{2+}]_i$, and this response was inhibited by 1 mM neomycin.

classes (Boekhoff et al. 1994; Kang and Caprio 1995; Revial et al. 1982). In contrast, in 155 human olfactory neurons tested with odorant mixes A and B, 43 responded to either mixture, but not one responded to both.

Responses of human olfactory neurons to single odorants

Olfactory neurons ($n = 16$) from five subjects were tested with a set of individual odorants. As expected, few olfactory neurons responded to an individual odorant; however, olfactory neurons responding repeatedly to lialil (1 of 16) or isovaleric acid (2 of 16) were observed (Fig. 4). All responding olfactory neurons tested with more than one individual odorant responded to one odorant only (3 of 18).

Two distinct transduction pathways are involved in human olfactory transduction

To investigate the transduction pathways involved in the observed calcium responses, inhibitors were used in conjunction with odorants. *L-cis*-diltiazem (LCD; 20 μ M) was used to block the cyclic nucleotide-gated channel (Kolesnikov et al. 1990), and neomycin (1 mM) was used to inhibit phospholipase C activity (Strigrow and Bohensack 1994) and $InsP_3$ production. As found previously, LCD reversibly inhibits the rise in $[Ca^{2+}]_i$ induced by mix A (Fig. 1A) (Restrepo et al. 1993b). Similar to rat olfactory neurons (Restrepo et al. 1993a; Tareilus et al. 1995), activation of adenylate cyclase with forskolin elicited an increase in $[Ca^{2+}]_i$ that could be blocked with LCD (data not shown). Addition of neomycin to inhibit $InsP_3$ production did not alter the increase in $[Ca^{2+}]_i$ in response to mix A (Fig. 1B;

$n = 3$). LCD alone did not affect resting $[Ca^{2+}]_i$, nor did it block the decrease in $[Ca^{2+}]_i$ observed in response to mix A in some cells ($n = 3$; not shown).

As expected for odorants presumed to stimulate phospholipase C, neomycin reversibly inhibited the responses of olfactory neurons to mix B, regardless of whether the effect was an increase ($n = 4$) or a decrease ($n = 2$) in $[Ca^{2+}]_i$ (Fig. 2, C and D). Accordingly, the cAMP-gated channel inhibitor LCD did not affect the changes in $[Ca^{2+}]_i$ elicited by mix B ($n = 3$; not shown) or the mix B odorant isovaleric acid (Fig. 4, B and C). LCD did not affect the response to mix B odorants, regardless of the direction of change (Fig. 2E).

DISCUSSION

Some of our results are similar to those obtained in previous studies with olfactory neurons from other vertebrate as well as invertebrate species. For example, as found in other species (see reviews by Breer et al. 1994; Restrepo et al. 1996; Shepherd 1994), our experiments indicate that at least two distinct second-messenger pathways mediate the odorant-induced changes in $[Ca^{2+}]_i$ in human olfactory neurons. The presence of separate transduction pathways is evidenced by inhibition of responses to odorant mix B by the phospholipase C inhibitor neomycin, but not by the cAMP-gated channel blocker LCD, and inhibition of the responses to odorant mix A by LCD, but not by neomycin. This differential inhibition clearly shows that two pharmacologically distinct pathways are involved, and the known specificity of the inhibitors suggests that the second messengers cAMP and $InsP_3$ are involved in human olfactory transduction. These results are

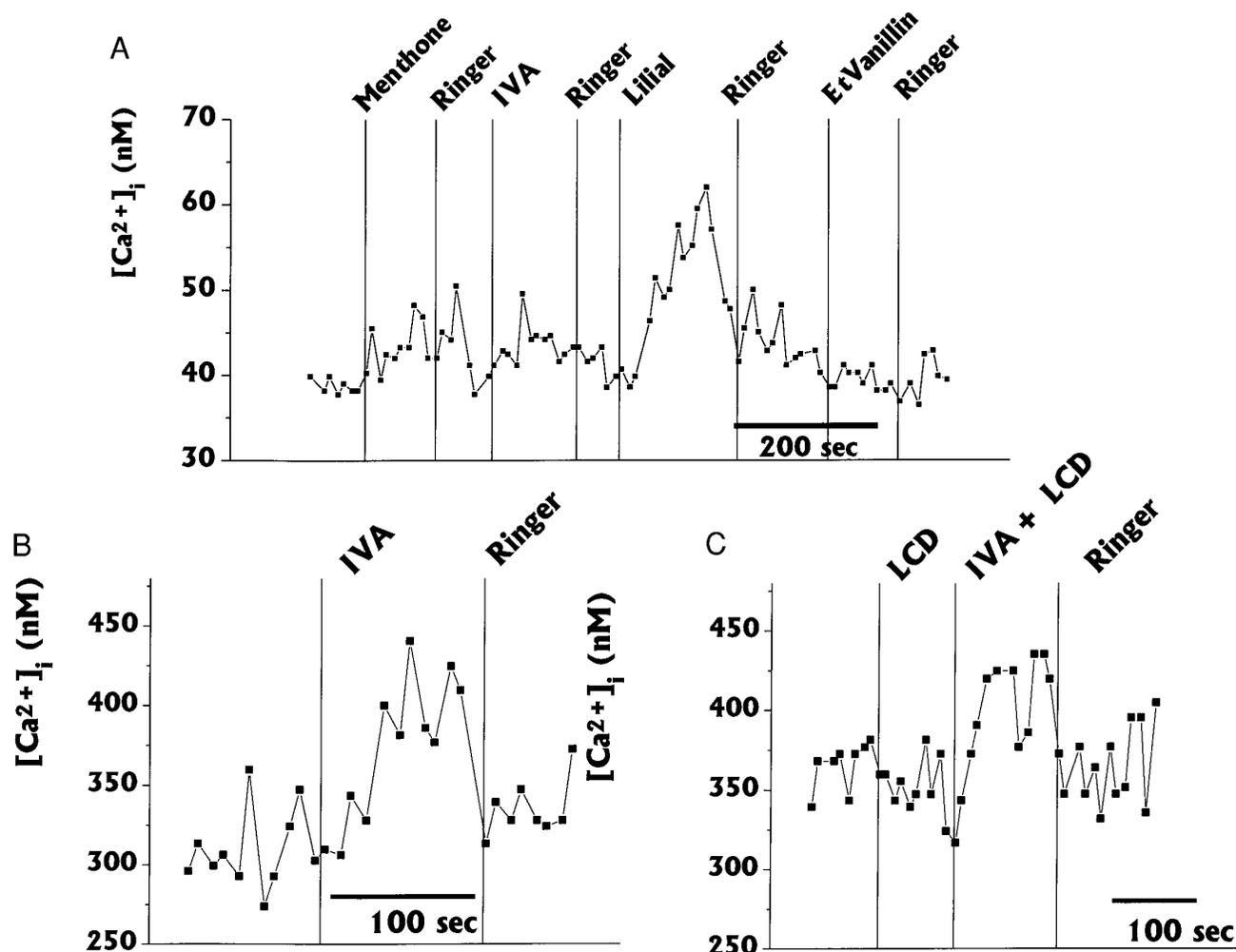


FIG. 4. $[Ca^{2+}]_i$ responses of human olfactory neurons to individual odorants. *A*: portion of a record from a cell that responded to linal (100 μ M), a component of mix B. This olfactory neuron responded to linal several times, but not to isovaleric acid (IVA) or ethylvanillin, also components of mix B, or menthone, a component of mix A. *B*: another cell responds to IVA (100 μ M). *C*: cAMP-gated channel inhibitor LCD (20 μ M) does not affect the increase in $[Ca^{2+}]_i$ elicited by 100 μ M IVA (*B* and *C* are from the same cell). A total of 18 cells was tested with individual odorants at a concentration of 100 μ M. The responsiveness to these odorants was as follows: ethyl vanillin (0 of 16 cells responding), linal (1 of 16), isovaleric acid (2 of 16), menthone (0 of 11), β -ionone (0 of 18), hedione (0 of 17).

consistent with previous pharmacological studies in rat (Tareilus et al. 1995).

There were, however, striking differences between human olfactory neurons and olfactory neurons from other species that raise important issues concerning the study of olfactory transduction. First, the finding that a significant number of human olfactory neurons responded to odorant stimulation with a decrease in $[Ca^{2+}]_i$ was particularly unexpected because this type of response has never been reported in similar studies with rat olfactory neurons (Restrepo et al. 1993a; Tareilus et al. 1995). Odor-induced decreases in $[Ca^{2+}]_i$ have not been described in studies in amphibians (Nakamura et al. 1994; Sato et al. 1991), and are rare in catfish olfactory neurons (1 of 140 catfish olfactory neurons) (Restrepo and Boyle 1991). Current models of olfactory transduction do not provide a mechanistic explanation for the odorant-induced decrease in $[Ca^{2+}]_i$ reported here. In all current models, odorants elicit an increase in $[Ca^{2+}]_i$. Although not sufficient to propose a

model accounting for the odorant-induced decreases in $[Ca^{2+}]_i$, our data can be used to rule out plausible models. For example, high basal levels of either cAMP or $InsP_3$ in some olfactory neurons might cause the second-messenger (cAMP or $InsP_3$)-gated channels to be open at steady state such that odorants trigger channel closing leading to a decrease in Ca^{2+} influx. However, in mix A-responsive olfactory neurons, the observed effect is not due to inhibition of the cyclic nucleotide-gated channel, because LCD alone did not affect $[Ca^{2+}]_i$ in cells that responded to mix A with a decrease in $[Ca^{2+}]_i$ (data not shown). Furthermore, it is unlikely that an increase in cytosolic Ca^{2+} buffering or in uptake of Ca^{2+} by intracellular organelles mediates the odorant-induced decreases in $[Ca^{2+}]_i$, because these decreases were not transient, persisting throughout stimulation (Figs. 1C and 2, D and E). Therefore the most likely explanations are either an increase in Ca^{2+} efflux or a decrease in influx through a pathway other than the second-messenger-gated channels.

Ca^{2+} can be removed from olfactory neurons via either an $\text{Na}^+/\text{Ca}^{2+}$ antiporter or a Ca^{2+} ATPase. Activation of the $\text{Na}^+/\text{Ca}^{2+}$ antiporter is unlikely to account for the decrease in $[\text{Ca}^{2+}]_i$, because this transporter acts in the dendrite and is apparently active only at higher $[\text{Ca}^{2+}]_i$ (Jung et al. 1994). The decreases we observed did not tend to be localized to the dendrite, and baseline $[\text{Ca}^{2+}]_i$ did not differ significantly between cells exhibiting increases and those exhibiting decreases. A Ca^{2+} ATPase has been implicated in the hyperpolarization induced by the chemoattractant cAMP in paramecia (Wright et al. 1993), but the role of such a pump in vertebrate chemoreception has not been investigated. It is possible that the decrease in $[\text{Ca}^{2+}]_i$ is occurring after an increase that is brief and transient, faster than the first image taken after stimulation (typically 7–10 s). However, even if this were true, the mechanism for such an odorant-stimulated decrease in $[\text{Ca}^{2+}]_i$ and its possible role in olfactory transduction in humans remain to be investigated.

Second, it is notable that of 155 olfactory neurons tested with both odorant mixtures in this study, of the 43 that responded, not one responded to both mixtures. By contrast, a significant proportion (33–50%) of olfactory neurons from rats tested with the same or similar odorant mixtures responded indiscriminately and with responses of similar magnitude to both mixtures (Restrepo et al. 1993a; Tareilus et al. 1995). Indeed, studies with invertebrate and vertebrate species (Boekhoff et al. 1994; Firestein et al. 1993; Kang and Caprio 1995; Restrepo et al. 1993a; Revial et al. 1982; Tareilus et al. 1995) suggest that many olfactory neurons are not narrowly selective, but respond to qualitatively different odorants.

The finding that human olfactory neurons were able to discriminate two odorant mixtures that cannot be discriminated by a substantial number of individual rat olfactory neurons suggests that human olfactory neurons may be more selective. However, other explanations are also possible. It may be suggested that neurons are more likely to respond to suprathreshold concentrations of odorants nonselectively, and that if rat neurons are more sensitive than human olfactory neurons, they will be more likely to respond indiscriminately at the odorant concentrations used. However, the percentage of human olfactory neurons responding to either mixture is similar to the percentage of rat olfactory neurons responding to either mix A only or mix B only. The generally higher overall response rate seen in studies of calcium responses in rat olfactory neurons appears to be due to an additional population of cells responding to both odorant mixtures (Restrepo et al. 1993a; Tareilus et al. 1995).

Alternatively, the differences in responses between rat and human olfactory neurons could reflect differences in prior experience with the odors used in these studies. Although it is not clear to what extent experience influences odorant sensitivity or neuronal response characteristics, effects have clearly been shown in some cases (e.g., androstenone) in both humans and rats (Wang et al. 1993). Although rats can detect at least some odorants used in physiological studies (Slotnick et al. 1991), most animal studies do not combine behavioral and cell biological methods to establish the sensitivity of the same animals to the odorants used. It is unlikely that the rats raised in a laboratory and fed only rat chow

had prior experience with odors like those used in our and other cell biological studies. Thus it might be that the response characteristics of rat olfactory neurons to odorants to which the animals had prior exposure would be more like those we have seen in human olfactory neurons when odorant mixtures are used that are part of a typical human environment.

The apparently greater selectivity of these human olfactory neurons raises the possibility that coding of odor qualities by the human olfactory system is accomplished somewhat differently than in other vertebrates studied, with a greater role being played by the olfactory neuron itself. The unusual response types suggest that it is necessary to reevaluate current models of olfactory transduction. Whatever the reason for these differences, our results underscore the importance of the interplay between human research and research in animal model systems.

The authors thank E. Varga, L. Roben, and H. Rees for excellent technical assistance and G. Beauchamp, S. Kemp, J. Pierce, S. Snyder, J. Teeter, and C. Wysocki for valuable suggestions and discussions.

These studies were supported by grants from the National Institutes of Health, the Human Frontier Science Program, and by a grant from the Veterans Affairs Department.

Address for reprint requests: N. E. Rawson, Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104-3308.

Received 13 June 1996; accepted in final form 19 November 1996.

NOTE ADDED IN PROOF

Shortly after this manuscript was accepted for publication, a publication from Brunet et al. (1996) appeared in press. Based on general anosmia of knockout mice with a disruption in the olfactory cyclic nucleotide-gated channel, Brunet and co-workers conclude that cAMP is the sole second messenger mediating olfactory transduction. The studies of the pharmacology of odorant responsiveness of human olfactory neurons presented in this manuscript and similar studies in rat (Restrepo et al. 1993a; Tareilus et al. 1995) are difficult to reconcile with a single second messenger pathway. The discrepancy between the results of Brunet and co-workers and the present work may stem from species differences or from pleiotropic effects of the disruption of the cAMP-gated cation channel on the InsP_3 pathway.

REFERENCES

- AMOORE, J. E. AND HAUTAL, E. Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 chemicals in air and water dilution. *J. Appl. Toxicol.* 3: 272–290, 1983.
- BOEKHOFF, I., MICHEL, W. C., BREER, H., AND ACHE, B. W. Single odors differentially stimulate dual second messenger pathways in lobster olfactory receptor cells. *J. Neurosci.* 14: 3304–3309, 1994.
- BREER, H. AND BOEKHOFF, I. Odorants of the same odor class activate different second messenger pathways. *Chem. Senses* 16: 19–29, 1991.
- BREER, H., RAMING, K., AND KRIEGER, J. Signal recognition and transduction in olfactory neurons. *Biochim. Biophys. Acta* 1224: 277–287, 1994.
- BRUNET, L. J., GOLD, G. H., AND NGAI, J. General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron* 17: 681–693, 1996.
- BUCK, L. AND AXEL, R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65: 175–187, 1991.
- DOTY, R. L., KREISS, D. S., AND FRYE, R. E. Human odor intensity perception: correlation with frog epithelial adenylate cyclase activity and trans-epithelial voltage response. *Brain Res.* 527: 130–134, 1990.
- FIRESTEIN, S., PICCO, C., AND MENINI, A. The relation between stimulus and response in olfactory receptor cells of the tiger salamander. *J. Physiol. Lond.* 468: 1–10, 1993.
- GOMEZ, G., RESTREPO, D., RAWSON, N., LOWRY, L. D., KEANE, W. M., AND ROTHSTEIN, J. L. Induction of differentiation of human olfactory

- neuroblastoma cells into odorant-responsive cells. *Neuroscience* 74: 567–577, 1996.
- JUNG, A., LISCHKA, F. W., ENGEL, J., AND SCHILD, D. Sodium/calcium exchanger in olfactory receptor neurones of *Xenopus laevis*. *Neuroreport* 5: 1731–1744, 1994.
- KANG, J. AND CAPRIO, J. In vivo responses of single olfactory receptor neurons in the channel catfish, *Ictalurus punctatus*. *J. Neurophysiol.* 73: 172–177, 1995.
- KAUER, J. S. Contributions of topography and parallel processing to odor coding in the vertebrate olfactory pathway. *Trends Neurosci.* 14: 79–85, 1991.
- KLEENE, S. J. AND GESTELAND, R. C. Calcium-activated chloride conductance in frog olfactory cilia. *J. Neurosci.* 11: 3624–3639, 1991.
- KOLESNIKOV, S. S., ZHAINAZAROV, A. B., AND KOSOLAPOV, A. V. Cyclic nucleotide-activated channels in the frog olfactory receptor plasma membrane. *FEBS Lett.* 266: 96–98, 1990.
- KURAHASHI, T. AND YAU, K.-W. Co-existence of cationic and chloride components in odorant-induced current of vertebrate olfactory receptor cells. *Nature Lond.* 363: 71–74, 1993.
- LANCET, D. AND BEN-ARIE, N. Olfactory receptors. *Curr. Biol.* 3: 668–674, 1993.
- LANZA, D. C. AND CLERICO, D. M. Anatomy of the human nasal passages. In: *Handbook of Olfaction and Gustation*, edited by R. L. Doty. New York: Dekker, 1995, p. 53–73.
- LOWRY, L. D. AND PRIBITKIN, E. A. Collection of human olfactory tissue. In: *Experimental Cell Biology of Taste and Olfaction*, edited by A. I. Spielman and J. G. Brand. Boca Raton, FL: CRC, 1995, p. 47–48.
- MORALES, B., LABARCA, P., AND BACIGALUPO, J. A ciliary K^+ conductance sensitive to charibdotoxin underlies inhibitory responses in toad olfactory receptor neurons. *FEBS Lett.* 359: 41–44, 1995.
- NAKAMURA, T., TSURU, K., AND MIYAMOTO, S. Regulation of Ca^{2+} concentration by second messengers in newt olfactory receptor cell. *Neurosci. Lett.* 171: 197–200, 1994.
- RAWSON, N. E., BRAND, J. G., COWART, B. J., LOWRY, L. D., PRIBITKIN, E. A., RAO, V. M., AND RESTREPO, D. Functionally mature olfactory neurons from two anosmic patients with Kallmann syndrome. *Brain Res.* 681: 58–64, 1995.
- RESTREPO, D. AND BOYLE, A. G. Stimulation of olfactory receptors alters regulation of $[Ca_i]$ in olfactory neurons of the catfish (*Ictalurus punctatus*). *J. Membr. Biol.* 120: 223–232, 1991.
- RESTREPO, D., MIYAMOTO, T., BRYANT, B. P., AND TEETER, J. H. Odor stimuli trigger influx of calcium into olfactory neurons of the channel catfish. *Science Wash. DC* 249: 1166–1168, 1990.
- RESTREPO, D., OKADA, Y., AND TEETER, J. H. Odorant-regulated Ca^{2+} gradients in rat olfactory neurons. *J. Gen. Physiol.* 102: 907–924, 1993a.
- RESTREPO, D., OKADA, Y., TEETER, J. H., LOWRY, L. D., AND COWART, B. Human olfactory neurons respond to odor stimuli with an increase in cytoplasmic Ca^{++} . *Biophys. J.* 64: 1961–1966, 1993b.
- RESTREPO, D., TEETER, J. H., AND SCHILD, D. Second messenger signaling in olfactory transduction. *J. Neurobiol.* 30: 37–38, 1996.
- REVIAL, M. F., SICARD, G., DUCHAMP, A., AND HOLLEY, A. New studies on odour discrimination in the frog olfactory receptor cells. I. Experimental results. *Chem. Senses* 7: 175–190, 1982.
- SATO, T., HIRONO, J., TONOIKE, M., AND TAKEBAYASHI, M. Two types of increases in free Ca^{2+} evoked by odor in isolated frog olfactory receptor neurons. *Neuroreport* 2: 229–232, 1991.
- SHEPHERD, G. M. Discrimination of molecular signals by the olfactory receptor neuron. *Neuron* 13: 771–790, 1994.
- SLOTNICK, B. M., KUFERA, A., AND SILBERBERG, A. M. Olfactory learning and odor memory in the rat. *Physiol. Behav.* 50: 555–561, 1991.
- STRIGGOW, F. AND BOHNENSACK, R. Inositol 1,4,5-trisphosphate activates receptor-mediated calcium entry by two different pathways in hepatocytes. *Eur. J. Biochem.* 222: 229–234, 1994.
- TAREILUS, E., NOE, J., AND BREER, H. Calcium signaling in olfactory neurons. *Biochim. Biophys. Acta* 1269: 129–138, 1995.
- WANG, H.-W., WYSOCKI, C., AND GOLD, G. H. Induction of olfactory receptor sensitivity in mice. *Science Wash. DC* 260: 998–1000, 1993.
- WRIGHT, M. V., ELWESS, N., AND VAN HOUTEN, J. Ca^{2+} transport and chemoreception in *Paramecium*. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 163: 288–296, 1993.