REVIEW

TRANSDUCTION DIVERSITY IN OLFACTION

VINCENT E. DIONNE AND ADRIENNE E. DUBIN*

Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543, USA

Summary

Odors are powerful stimuli that can focus the attention, elicit behaviors (or misbehaviors) and even resurrect forgotten memories. These actions are directed by the central nervous system, but they depend upon the initial transduction of chemical signals by olfactory receptor neurons. Electrophysiological recordings suggest that the responses of olfactory receptor neurons to odors are more diverse than was initially believed, being mediated by effects on several different conductances. Both excitatory and inhibitory responses are produced by these effects and some, if not all, odors can affect more than one component of the membrane conductance. The extent of this diversity is reviewed here, and its impact on our understanding of odor discrimination is discussed.

Introduction

The olfactory systems of extant animals derive from the earliest chemical sensing systems. These senses were crucial to survival, providing organisms with an ability to find food and mates. The modern system displays a structure (Fig. 1) that is remarkably similar among different species and phyla, suggesting that this ancient sense depends upon certain robust, but still obscure, biological principles. In spite of its relatively simple architecture, we are still learning how the olfactory system acquires and processes sensory information. There are three main reasons for our poor understanding. First, there are no basic principles for organizing odors in terms of critical functional parameters. Second, except for the peripheral portion of each sensory neuron, most of the olfactory system is relatively inaccessible. Third, as discussed below, there is no universal mechanism for transducing odor signals; rather, several mechanisms are used. This diversity of transduction mechanisms may be necessary for olfactory discrimination. Several recent reviews provide additional information (Getchell, 1986; Reed, 1992; Shirley, 1992; Farbman, 1992; Scott, 1991; Kauer, 1991; Anholt, 1993; Trombley and Shepherd, 1993).

There is no unanimity of opinion about the mechanisms that underlie odor detection and transduction. In the recent literature, several mechanisms for odor transduction have

*Present address: Department of Biology, San Diego State University, San Diego, CA 92182, USA.

Key words: olfaction, odor transduction, olfactory receptor neurons, odor modulation.
been proposed, including direct and receptor-mediated indirect effects on ion channels and changes in membrane fluidity. Unarguably, odors can affect ion channels, membrane fluidity and enzyme activity in olfactory receptor neurons (ORNs). But non-olfactory cells also respond to odors, suggesting that not all odor responses mediate physiologically relevant olfactory transduction. The real question is whether any or all of the effects examined \textit{in vitro} underlie odor transduction \textit{in vivo}. Different investigators have seldom employed the same criteria to address this issue. We have used the following minimal requirements that any mechanism must satisfy if it is used for odor transduction. (1) An odorant should not elicit identical responses from all cells. If each odorant affected all ORNs identically, there could be no odor discrimination. (2) The response must occur at odor concentrations encountered in vivo. Responses that are elicited only by high concentrations of odor may not be mediated by mechanisms that underlie physiological odor transduction. (3) Exposure of only the apical membrane of an ORN to odor must be sufficient to elicit the response. Only the cilia and dendritic endings of ORNs are exposed to odors \textit{in vivo}. Tight junctions between the apical end of the dendrite and adjacent cells occlude odors from the basolateral space around the dendrite and soma. (4) The odor-induced response must affect neuronal excitability. A response that has no effect on excitability will not be communicated to the central nervous system. This requirement encompasses both intracellular and intercellular pathways, the latter communicated by electrical coupling (Schwartz Levey \textit{et al.}, 1992) or membrane-permeant chemical agents such as nitric oxide (Breer and Shepherd, 1993) and carbon monoxide (Ronnett and Snyder, 1992).
Detection thresholds for different odorants vary enormously among cells, depending on whether a particular odorant is the optimal stimulus for an individual ORN and on how the thresholds are measured. Thresholds measured in situ by single-unit recording or in vitro by intracellular and patch-clamp methods range from $10^{-12}$ to $10^{-5}\text{mol l}^{-1}$ (Sutterlin and Sutterlin, 1971; Suzuki and Tucker, 1971; Getchell, 1974; Caprio, 1978; Silver, 1982; Frings and Lindemann, 1990). This wide range of sensitivity may reflect the number of receptors of a particular type on different cells and may let the system monitor odor concentration as well as quality. An even wider range of thresholds has been reported using more integrative or behavioral assays (Passé and Walker, 1985). This broader range is not surprising, since threshold measurements using the intact olfactory system should reflect the most and least sensitive cells, which might not be assayed in single-cell studies. In air-breathing vertebrates, including humans, sensitivity thresholds are reported in the range from less than $10^{-13}$ to $10^{-4}\text{mol l}^{-1}$ with a mean in the picomolar range (Patte et al. 1975; Moulton, 1977; Amoore, 1982; Slotnick and
Schoonover, 1984). In fish, thresholds for bile acids are $10^{-11}$ to $10^{-5}$ mol l$^{-1}$ (Døving et al. 1980), whereas those for amino acids appear to be in the nanomolar to hundreds of micromolar range (Hara, 1973, 1976; Belghaug and Døving, 1977; Caprio, 1978; Døving et al. 1980). High concentrations of odorants can elicit various responses even from non-olfactory cells. At concentrations above 10–100 $\mu$mol l$^{-1}$, the commonly employed odorants citral, $\beta$-ionone, octanol and ethyl vanillin stimulate adenyl cyclase and cause dispersal of melanin when applied to the surface of non-neuronal melanophores (Lerner et al. 1988; Potenza and Lerner, 1992). Additionally, a variety of odorants at concentrations above 10 $\mu$mol l$^{-1}$ have been shown to alter membrane fluidity of lipid vesicles and N18 neuroblastoma cells (Kashiwayanagi and Kurihara, 1984, 1985). It is not clear whether odor concentrations above 10–100 $\mu$mol l$^{-1}$ are either normally encountered by air-breathing animals or required for odor detection, since at room temperature the concentration of a saturated vapor of most odorants (vapor pressures <2 mmHg) is less than 100 $\mu$mol l$^{-1}$.

The cellular response to odors

Odors elicit responses in ORNs that alter neuronal firing to communicate the presence of odor to the brain. Both inhibitory and excitatory odor responses have been observed in ORNs from vertebrates (Gesteland et al. 1965; O’Connell and Mozell, 1969; Duchamp et al. 1974; Revial et al. 1978; Getchell and Shepherd, 1978a,b; Sicard and Holley, 1984; Dionne, 1992) and invertebrates (Derby and Harpaz, 1988; Derby et al. 1988; McClintock and Ache, 1989a,b), but until recently the inhibitory responses were generally discounted (discussed in Getchell, 1986; McClintock and Ache, 1989b). It is only with the use of modern recording methods and protocols that inhibitory odor responses have been consistently detected. Most olfactory responses appear to be produced by odor-elicited receptor potentials. Receptor potentials are induced by altering the membrane conductance for selected types of ion channels. Both depolarizing and hyperpolarizing receptor potentials have been observed, and both can produce excitation or inhibition depending on the resting state and the magnitude of the voltage change. Channels of more than one type are involved in odor transduction. In addition, some odor responses appear to occur independently of any receptor potential.

The cyclic-nucleotide-gated receptor potential in vertebrates

In vertebrates, the odor-modulated conductance most commonly associated with an olfactory receptor potential is produced by the cyclic-nucleotide-gated (CNG) cation channel. These channels, first identified in toad (*Bufo marinus*) ORNs (Nakamura and Gold, 1987), have been widely studied in salamanders, frogs and other species (see below). Nevertheless, odor modulation of the CNG cation channel has been demonstrated only in post-metamorphic amphibians, and the role of the channel in producing a receptor potential has been clarified only recently. The CNG cation channel in ORNs from the tiger salamander (*Ambystoma tigrinum*) can be activated by an odor mixture containing amyl acetate, acetophenone and cineole (Firestein et al. 1991a,b, 1993) with or without 2-hexylpyridine (Lowe and Gold, 1991). The cells respond with a depolarizing receptor
potential to high micromolar (Firestein and Werblin, 1989; Firestein et al. 1990) or millimolar (Lowe and Gold, 1991) concentrations of the mixture preferentially applied to their cilia, corresponding to the location of the CNG channels. Fifty to sixty per cent of the cells respond to these stimuli, a surprisingly high value in the light of the small percentage of cells that appear to harbor each putative odor receptor, but fewer cells respond to the individual components of the mixture (Firestein et al. 1993). Similarly, in ORNs isolated from newts (Cynops pyrrhogaster), cyclic nucleotides activate a cation conductance (Kurahashi, 1990) found primarily in the cilia (Kurahashi, 1989; Kurahashi and Kaneko, 1991); the conductance shows little selectivity among the monovalent, inorganic cations (Li+, Na+, K+, Rb+, Cs+) (Kurahashi, 1990). A similarly non-selective cation conductance can be activated in about 30% of the cells by 10 mmol l\(^{-1}\) amyl acetate (Kurahashi and Shibuya, 1990; Kurahashi, 1989) or a mixture containing several millimolar each of amyl acetate, isoamyl acetate and limonene (Kurahashi and Yau, 1993). Although it is a concern that many of these studies employed very high concentrations of odorant, the results otherwise indicate that these were transduction-related olfactory responses.

Whereas the CNG cation channel is about equally permeable to the small monovalent cations, it is only slightly permeable to divalent cations such as Mg\(^{2+}\) and Ca\(^{2+}\) (Kurahashi, 1990; Kolesnikov et al. 1990; Frings et al. 1992; Kolesnikov and Kosolapov, 1993). Physiological concentrations of Ca\(^{2+}\) partially block the channel (Nakamura and Gold, 1987; Kolesnikov et al. 1990; Kurahashi, 1990; Suzuki, 1990; Dhallan et al. 1990; Zufall et al. 1991; Frings et al. 1991; Kramer and Siegelbaum, 1992; Zufall and Firestein, 1993; Kolesnikov and Kosolapov, 1993). Despite this blockade, the Ca\(^{2+}\) influx through the CNG cation channel is sufficient to stimulate a Ca\(^{2+}\)-activated Cl\(^{-}\) conductance that can dominate the cyclic-nucleotide-stimulated response in frog, salamander, newt and rat ORNs (Kurahashi and Yau, 1993; Kleene, 1993a; Lowe and Gold, 1993a). The increase in Cl\(^{-}\) conductance, depending on intracellular buffering of Ca\(^{2+}\), appears to be responsible for half (Kurahashi and Yau, 1993) or more (Kleene, 1993a; Lowe and Gold, 1993a) of the CNG receptor potential. Studies conducted before the Cl\(^{-}\) dependence of the CNG receptor potential was recognized have characterized the potential as a depolarizing response produced by an increase in a non-selective cation conductance; however, those studies typically employed cation and Cl\(^{-}\) distributions both with equilibrium potentials near 0 mV (Nakamura and Gold, 1987; Firestein and Werblin, 1989; Firestein et al. 1990) or inadvertently obstructed the Cl\(^{-}\) current by omitting Ca\(^{2+}\) from the bath (Kurahashi, 1989). The normal intracellular concentration of Cl\(^{-}\) in ORNs is still uncertain for most species including tiger salamanders, newts, frogs and rats. Only in dissociated ORNs from the aquatic salamander Necturus maculosus has the Cl\(^{-}\) equilibrium potential been estimated. Its value (~45.5±2.5 mV, \(N=11\)) (Dubin and Dionne, 1994) corresponds to an intracellular [Cl\(^{-}\)] of 23.3±2.5 mmol l\(^{-1}\). If this value is typical of ORNs in other vertebrates, a moderate increase in Cl\(^{-}\) conductance would indeed cause a depolarizing receptor potential that was excitatory; however, a large conductance increase could depolarize the cell enough to inactivate Na\(^{+}\) channels and inhibit electrical activity. The apparent dependence of the vertebrate CNG receptor potential on...
a Ca$^{2+}$-activated Cl$^-$ conductance and the lack of data on the Cl$^-$ equilibrium potential warrant a careful reassessment of its physiological role.

Aside from tiger salamanders and newts, in most vertebrates the data implicating the CNG cation channel in odor transduction are incomplete. CNG cation channels have been found in ORNs from rat (Frings et al. 1992; Lowe and Gold, 1993a; Lynch and Lindemann, 1994), catfish (Miyamoto et al. 1992b), frog (Suzuki, 1990; Frings and Lindemann, 1991; Kolesnikov et al. 1990; Frings et al. 1992; Kleene, 1993b) and carp (Kolesnikov and Kosolapov, 1993) tissues, but these channels have not been shown to be activated by odors. While the precise subunit composition of the CNG cation channel is still unknown, a subunit has been cloned from rat (Dhallan et al. 1990), catfish (Goulding et al. 1992) and bovine (Ludwig et al. 1990) sources. When expressed in Xenopus oocytes (Altenhofen et al. 1991; Goulding et al. 1992) or 293 human embryonic kidney cells (Dhallan et al. 1990), this subunit forms functional channels with properties nearly, but not completely, identical to those of the native channel. In rod photoreceptors, where a homologous channel resides (Kaupp, 1991), at least one additional subunit of the CNG channel has been identified (Chen et al. 1993), suggesting that additional subunits of the olfactory channels may also exist. Fig. 2A illustrates the odor-modulated conductances in vertebrates.

Excitatory and inhibitory receptor potentials in lobsters

An odor-evoked, cyclic-nucleotide-responsive conductance has been observed in ORNs from lobsters (Panulirus argus); but, in contrast to that in vertebrates, the conductance is K$^+$-selective (Michel et al. 1991; Michel and Ache, 1992), and whether it is directly gated by cyclic nucleotides or modulated by enzymatic action is uncertain. In lobster ORNs, the cyclic-nucleotide-responsive K$^+$ conductance can be activated by odor mixtures and single amino acids applied apically at micromolar concentrations; in cultured neurons, the responses are dose-dependent with thresholds of 10–100 nmol l$^{-1}$ (Fadool et al. 1993). The conductance can be activated by an individual odorant in but a fraction of odor-sensitive cells. Activation produces a hyperpolarizing receptor potential that is inhibitory (Michel et al. 1991). Interestingly, the cyclic-nucleotide-responsive K$^+$ conductance was detected in only about 50% of cells tested with membrane-permeable analogs of cyclic adenosine 5$'$-monophosphate (cyclic AMP) or cyclic guanosine 5$'$-monophosphate (cyclic GMP), forskolin or the phosphodiesterase inhibitor isobutyl methylxanthine (IBMX) (Michel and Ache, 1992), suggesting substantial heterogeneity among receptor cells.

Lobster ORNs also respond to odors with depolarizing receptor potentials that are
Transduction diversity in olfaction

A

Increase [NSC+] Increase Ca^{2+} - activated Cl\(^{-}\) conductance

Depolarization

Tiger salamander, newt, frog, rat

B

Increase [Ca^{2+}] Increase [NSC^{+}]

Depolarization

Lobster

C

Increase [Cl^{-}] Decrease [Cl^{-}]

Depolarization

Mudpuppy

Fig. 2
excitatory (Schmiedel-Jakob et al. 1989, 1990), and these responses are mimicked by cultured lobster ORNs (Fadool et al. 1991, 1993; Fadool and Ache, 1992). Like the inhibitory responses, these responses are elicited by complex odor mixtures as well as by single amino acids applied to the apical dendrite (Schmiedel-Jakob et al. 1989, 1990), but single-channel measurements indicate that the responses are produced by two different channel types, a non-selective cation channel and a Ca$^{2+}$ channel, both of which are directly gated by intracellular inositol 1,4,5-trisphosphate (Ins1,4,5P$_3$) (Fadool and Ache, 1992; Ache et al. 1993; Ache, 1994). In addition, there is a third type of channel that is directly gated by inositol 1,3,4,5-tetrakisphosphate (Ins1,3,4,5P$_4$) that also depolarizes the cell and can interact in a reciprocal fashion with the Ins1,4,5P$_3$-gated channels either to potentiate their activity or to be inhibited by them (Fadool and Ache, 1994).

Although the pathways for excitatory and inhibitory odor responses in lobster ORNs are separate, they occur in the same cell (McClintock and Ache, 1989b; Michel et al. 1991; Michel and Ache, 1992; Fadool et al. 1993). Individual neurons can be excited by one odorant and inhibited by another. In addition, the same single odorant can elicit excitatory and inhibitory responses from different ORNs (Michel et al. 1991; Michel and Ache, 1992), and some odorants can elevate both cyclic AMP and Ins1,4,5P$_3$ concentrations (Ache et al. 1993). Thus, neither the receptor neurons nor the odorants can be subdivided into excitatory and inhibitory types. Such diversity of odor responses suggests that there may be more than one receptor type for each odorant or that specific receptors may couple (possibly through different G-proteins) to different transduction pathways in different cells. Fig. 2B illustrates the transduction pathways in lobster ORNs.

Receptor potentials and ‘silent’ responses in Necturus

The multiplicity of odor-elicited responses seen in lobsters is not unique to invertebrates. Similarly diverse responses were first described using extracellular recording methods in frogs (Gesteland et al. 1965; O’Connell and Mozell, 1969; Revial et al. 1978) and tiger salamanders (Getchell and Shepherd, 1978a; Sicard and Holley, 1984). In ORNs from Necturus maculosus (the mudpuppy), odors can elicit both depolarizing and hyperpolarizing receptor potentials with varying ionic dependencies, and odors can also alter neuronal excitability without producing a detectable receptor potential (Dionne, 1992; Dubin and Dionne, 1993, 1994) (Fig. 2C). The odor-elicited receptor potentials satisfy the four criteria for transduction: only a small fraction of chemosensitive cells appears to respond to any particular odorant; responses are induced at odor concentrations as low as 10–100 nmol l$^{-1}$; responses are elicited preferentially to odors applied on the ciliated end of the cell (Dionne, 1992); and neuronal excitability is excited by depolarizing receptor potentials of modest size, but inhibited by large depolarizing potentials and by hyperpolarizing receptor potentials (Dubin and Dionne, 1993). The ionic bases of the receptor potentials in mudpuppy are complex: odorants can increase or decrease a Cl$^{-}$ conductance; increase a non-selective cation conductance; or decrease a specific K$^+$ conductance (Dubin and Dionne, 1993). The same odorant can elicit each of these conductance changes in different cells, and different odorants can cause different types of response in the same cell.

Olfactory responses seen as a change in excitability not accompanied by a receptor
potential or a change of input impedance have been termed ‘silent responses’ (Dionne, 1992; Dubin and Dionne, 1994). Silent responses were detected in Necturus ORNs as changes in the rate of spontaneous firing and as a change in the frequency or duration of firing when a neuron was driven with depolarizing current pulses; the frequency may be more than doubled or reduced to less than half. The responses appear to satisfy the criteria for transduction, although the preferential association with apical membrane has not yet been tested. Silent odor responses have been detected in cells that also responded to different odorants with a receptor potential (Dionne, 1992), suggesting that individual ORNs may have several odor transduction pathways. Although the mechanisms that produce silent responses have not been fully characterized, they may involve either small local conductance changes that are not detected in whole-cell measurements or changes in the gating properties of specific types of Na⁺, K⁺ or Ca²⁺ channels. In mudpuppy ORNs, action potentials can be generated in the dendrite, conferring upon the cell an enhanced sensitivity to small odor-modulated conductances (Dubin and Dionne, 1994).

All chemosensitivity in isolated mudpuppy ORNs washes out rapidly in the normal whole-cell recording mode (Dionne, 1992; Dubin and Dionne, 1993). This loss of responsiveness includes odor-elicited receptor potentials as well as silent responses and supports the idea that soluble constituents in the cytoplasm constitute part of each transduction pathway. Although the intracellular mediators of the odor responses in Necturus have not yet been identified, increases in the Cl⁻ and cation conductances in these cells can be elicited by cyclic AMP (A. E. Dubin and V. E. Dionne, unpublished observations). Fig. 2C illustrates the transduction pathways in Necturus ORNs.

**Diverse odor responses in other species**

**Catfish**

In 20% of isolated ORNs tested from channel catfish (*Ictalurus punctatus*), a mixture of L-arginine, L-alanine and L-norleucine with or without L-glutamate (100 μmol l⁻¹ each) activated receptor currents of three different types, based upon voltage- and ion-dependence, time course, reversal potential and sensitivity to amiloride (Miyamoto et al. 1992b). In about 40% of the chemosensitive cells, the response occurred with a 50 ms latency, was reversibly decreased by removal of extracellular Ca²⁺, and reversed polarity at +23 mV under ionic conditions where the equilibrium potentials for Cl⁻ (E_Cl) and non-specific cation conductances (E_NSC) were both about 0 mV. In another 30% of the cells, a short-latency outwardly rectifying response reversed polarity near 0 mV, was insensitive to amiloride, but was blocked by forskolin; inward, but not outward, currents were blocked by extracellular Ba²⁺. In the remaining 30% of chemosensitive cells, a short-latency response was amiloride-sensitive and reversed polarity at ~15 mV. Miyamoto et al. (1992b) suggested that at least two different conductances in different populations of cells were activated by the odorants, but the ionic bases of the responses were not fully characterized. In a separate study, internal perfusion with either Ins1,4,5P₃ or cyclic AMP caused a transient depolarization of the receptor neurons (Miyamoto et al. 1992a); the responses were sustained when extracellular Ca²⁺ was removed or the intracellular Ca²⁺ buffering capacity was elevated, but otherwise the responses had dissimilar pharmacologies. The authors suggested that Ca²⁺ influx through Ins1,4,5P₃- or cyclic-
AMP-induced conductances curtailed the response by activating a Ca\(^{2+}\)-dependent K\(^+\) conductance. Ca\(^{2+}\)-activated K\(^+\) currents have been described in ORNs from catfish (Miyamoto et al. 1992b) and other species (Trotier, 1986; Firestein and Werblin, 1987; Maue and Dionne, 1987; Schild, 1989; Suzuki, 1990; Nevitt and Moody, 1992; McClintock and Ache, 1989a), and l-alanine-stimulated Ins1,4,5P\(_3\) production in catfish olfactory cilia is GTP-dependent (Restrepo et al. 1993).

**Toad**

Excitatory and inhibitory odor responses have been observed in toad (*Caudiverbera caudiverbera*) ORNs and reported briefly (Bacigalupo et al. 1993). An odor mixture containing isovaleric acid, triethylamine and pyrazine elicited an outward (hyperpolarizing) current in ORNs, causing inhibition, whereas a mixture containing citralva, citronellal and geraniol caused an inward (depolarizing) current and excitation. Three groups of cells were described, one showing the inhibitory response only, one the excitatory response only, and the other showing a mixed response. In membranes from rat olfactory tissue, the odorants in the inhibitory mixture stimulate the production of Ins1,4,5P\(_3\) whereas those in the excitatory mixture stimulate cyclic AMP (Boekhoff et al. 1990).

**Squid**

Various chemical stimuli applied to the olfactory organ of squid (*Loligo opalescens*) elicit an escape response (Gilly and Lucero, 1992); these responses can be produced by two different and opposing effects on the membrane conductance. Several naturally occurring compounds, including squid ink, L-Dopa and dopamine, activate the escape response in the animal and reversibly increase the membrane conductance (possibly for K\(^+\) or Cl\(^-\)) in isolated ORNs, causing the cells to hyperpolarize and inhibiting firing. Other compounds that are not natural odorants, including propyl paraben, tetrabutylammonium, tetraethylammonium, 4-aminopyridine and methadone, also elicit the escape response, but at the same concentrations at which they block K\(^+\) channels in the neurons, causing them to depolarize and increasing their excitability (Lucero et al. 1992). Chemosensitivity was detected using the perforated patch method (Horn and Marty, 1988), but was not seen under normal whole-cell recording conditions.

**Mechanisms underlying odor transduction**

As summarized above, odor transduction utilizes several different pathways, more than one of which may be found in each ORN, but a single odorant appears to activate only one transduction pathway in any cell. In some cases, it is clear that odor-elicited effects on neuronal excitability are mediated by second messengers. Is this always the case? Since numerous compounds with diverse physico-chemical properties are perceived as odorants, it is entirely possible that more than one mechanism is involved in odor transduction. In this section, after evaluating three classes of mechanisms that have been proposed to underlie odor transduction, we conclude that the known transduction pathways belong to the class of receptor-coupled, indirect mechanisms.
Direct gating of ion channels

Some odors may directly gate ion channels, acting like various neurotransmitters. Several studies employing reconstitution into artificial membranes reported direct, odor-induced activation of single channels (Vodyanoy and Murphy, 1983; Labarca et al. 1988), and in lobster ORNs histamine appears to gate a somatic Cl⁻ channel directly (McClintock and Ache, 1989c). However, the data fail to demonstrate that odors are transduced by a direct gating mechanism.

Vodyanoy and Murphy (1983) reconstituted membrane vesicles prepared from a crude homogenate of rat olfactory epithelium into phosphatidylethanolamine planar bilayers; nanomolar concentrations of diethyl sulfide or (–)-carvone, but not a saturated aqueous solution of camphor, activated a K⁺-selective channel with particularly slow kinetics: the odorant was reported nearly to double the unstimulated mean open time (29 s) of the channel. Although the response seemed to be specific for the olfactory epithelium, since it was not observed with vesicles prepared from respiratory epithelia, no similar channel or response has been reported in rat ORNs. Later studies reported that diethyl sulfide activated a membrane conductance only in the presence of ATP and GTP, and that cyclic AMP alone induced a similar but smaller response, suggesting an indirect effect of odor on membrane conductance (Vodyanoy and Vodyanoy, 1987). Using vesicles prepared from cilia-enriched membranes of bullfrog (Rana catesbeiana) olfactory epithelia, Labarca et al. (1988) found that nanomolar concentrations of isobutyl methoxy-pyrazine (bell pepper odor) and citralva activated a multistate channel of indeterminate ion selectivity (symmetrical solutions were used). Similar channels were not observed with vesicles from cilia-enriched preparations of respiratory epithelia, but since vesicles were obtained from crude membrane preparations or preparations partially enriched in cilia, and since ORNs are not the only ciliated cells in these epithelia, the origins of the relatively few channels that were examined are unknown. Furthermore, there is a precedent for channel properties being altered by reconstitution (Miller, 1987), raising the possibility that the responses observed in vitro might not occur in vivo. Finally, it cannot be ruled out that the reconstituted vesicles did not include other proteins that indirectly mediated the response between odor and channel; however, odor-induced effects in the absence of exogenous ATP, GTP and other second messengers were observed (Labarca et al. 1988).

McClintock and Ache (1989c) found ion channels on the somata of lobster ORNs that appeared to be directly gated by histamine. Since histamine applied to lobster ORNs elicits a behavioral response, it was considered that the channels might mediate odor transduction. A careful search for histamine-gated channels on dendritic membranes of lobster ORNs revealed none, however (Bayer et al. 1989; McClintock and Ache, 1989c), making it unlikely that the somatic-type channels are involved in odor transduction.

Membrane fluidity

Airborne odorants are volatile compounds that dissolve readily in nonpolar environments, including lipid membranes. Such compounds can alter both the membrane fluidity and membrane potential in liposomes (Kashiwayanagi et al. 1990; Nomura and
Kurihara, 1987), in cultured cells (Kashiwayanagi and Kurihara, 1984, 1985) and in dissociated olfactory epithelial cells (Kashiwayanagi et al. 1987). Changes in fluidity could affect protein–lipid interactions and have been proposed as a mechanism for odor transduction (Nomura and Kurihara, 1989; Kurihara, 1990). Since these responses appear to be independent of specific ions (Shoji and Kurihara, 1991; Kashiwayanagi et al. 1991), it has been proposed that odors alter the membrane potential through an effect on the phase boundary potential rather than by modulating ion conductances. In ORNs, odor-induced changes in membrane fluidity have been reported with thresholds only in the $10^{-3}$ to $10^{-5}$ mol l$^{-1}$ range (Kashiwayanagi et al. 1987). These values border the range of odor detection thresholds and cannot account for olfactory transduction under most conditions. The three criteria not involving odor thresholds (see above) have not been tested. Although odorous compounds can affect the fluidity of membranes, the data do not yet support the hypothesis that this is a mechanism that underlies odor transduction.

Receptor-mediated transduction

The preponderance of recent biochemical and electrophysiological data suggest that odors modulate neuronal excitability indirectly through receptor proteins linked to second-messenger-dependent pathways. Several different types of evidence support this conclusion.

1. **Washout of odor responses in whole-cell recordings.** The whole-cell patch-clamp method employs an electrode sealed tightly to the cell membrane, but allows free exchange between the intracellular saline and the pipette solution (Hamill et al. 1981). The chemosensitivity of isolated neurons studied with this method is labile, appearing to fade or wash out, presumably because important intracellular constituents diffuse away into the pipette (Trotier, 1986; Maue and Dionne, 1987; Frings and Lindemann, 1988; Dionne, 1992). The loss of chemosensitivity is especially rapid when the recording pipette is sealed to the dendrite close to the chemosensitive apical membrane, but can be retarded if resistive methods that reduce fluid exchange are used (Dubin and Dionne, 1993). Slower washout is seen when the pipette is sealed to the soma relatively far from the apical surface, and studies recording from the somata of ORNs with especially long dendrites (e.g. from lobsters) often find no washout (Schmiedel-Jakob et al. 1989; Michel and Ache, 1992). Typically, neither MgATP alone nor in combination with GTP rescues cells from washout (Frings and Lindemann, 1988; Dionne, 1989), but cyclic GMP may slow the rate of washout (Lischka and Schild, 1993).

2. **The latency between odor application and the cellular response.** The response of ORNs to odors occurs with a latency of several hundred milliseconds in adult tiger salamanders (Firestein and Werblin, 1989; Firestein et al. 1990). This would be long enough to involve a multistep pathway in the transduction process, even in poikilotherms such as these, where enzyme kinetics are likely to be slower than in mammals.

3. **Odor responses depend on G-protein function.** G-proteins are heterotrimeric proteins that mediate GTP-dependent responses elicited by bioactive ligands and which act as intermediaries between ligand receptors and targets such as adenyl cyclase and phospholipase C. A third or more of the known G-protein α-subunits have been detected in rat olfactory epithelia (Jones and Reed, 1989; Jones et al. 1990; Mania-Farnell and
Farbman, 1990; Shinohara et al. 1992), as have the $\beta\gamma$-subunits (Shinohara et al. 1992; Mania-Farnell and Farbman, 1990). Although $G_S\alpha$ expression occurs predominantly in non-neuronal support cells (Jones et al. 1988), $G_{OLF}\alpha$ expression occurs specifically in ORNs and depends on receptor cell innervation of the olfactory bulb (Jones and Reed, 1989). Odor-activated, GTP-dependent elevations of cyclic AMP (Pace et al. 1985; Sklar et al. 1986; Shirley et al. 1986; Breer et al. 1990; Boekhoff et al. 1990) and Ins1,4,5P$_3$ (Huque and Bruch, 1986; Bruch and Kalinowski, 1987; Breer et al. 1990; Boekhoff et al. 1990) concentrations indicate that G-proteins mediate the odor-elicited stimulation of second-messenger systems. Pertussis and cholera toxins, which ADP-ribosylate different but specific G-$\alpha$ subunits and alter their activity, differentially and predictably, affect the odor-sensitive increases in cyclic AMP and Ins1,4,5P$_3$ concentrations in rat olfactory membranes (Boekhoff et al. 1990). In addition, in cilia-enriched membranes from catfish olfactory tissue, GTP analogues modulate the binding affinities of receptors for L-alanine and L-arginine in a manner suggesting that G-proteins couple to these receptors (Bruch and Kalinowski, 1987). Furthermore the odor-sensitive current is predictably altered by non-hydrolyzable guanosine nucleotide analogs in tiger salamander ORNs (Firestein et al. 1991a).

(4) Intracellular compounds and second messengers. The diffusible second messengers cyclic AMP, Ins1,4,5P$_3$ and Ca$^{2+}$ act directly on specific types of ion channels in ORNs, thereby altering membrane excitability and providing several putative pathways for olfactory transduction. Odor-induced effects on cyclic-nucleotide-sensitive channels can be mimicked by application of exogenous compounds (Kurahashi, 1990; Firestein et al. 1991a) or by manipulation of endogenous second messenger levels using an adenylyl cyclase activator (forskolin) or a phosphodiesterase inhibitor (IBMX) (Frings and Lindemann, 1991; Firestein et al. 1991b; Michel and Ache, 1992). Ins1,4,5P$_3$ directly activates cation channels in lobsters and rats (Fadool and Ache, 1992; Restrepo et al. 1990; Restrepo and Boyle, 1991). Ins1,4,5P$_3$-sensitive channels in excised patches from cultured lobster ORNs are activated when the patch is crammed into the cytoplasm of a different cell and food odor is applied (Fadool and Ache, 1992). Ca$^{2+}$ activates Cl$^-$ (Kleene and Gesteland, 1991) and K$^+$ (Maue and Dionne, 1987; Firestein and Werblin, 1987; Nevitt and Moody, 1992) channels. Local odorant-induced increases in intracellular [Ca$^{2+}$] at the apical end of intact cultured rat ORNs have been observed using Ca$^{2+}$ imaging techniques (Restrepo et al. 1993). Second messengers may also alter ion channel gating indirectly by modulating enzyme activity (Reed, 1992; Wegener et al. 1993). Other compounds, including cyclic GMP, diacyl glycerol, Ins1,3,4,5P$_4$, NO and CO, have also been implicated in olfactory transduction (Zufall and Hatt, 1991; Ronnett and Snyder, 1992; Breer et al. 1992; Breer and Shepherd, 1993; Lischka and Schild, 1993; Verma et al. 1993; Fadool and Ache, 1994). NO and CO are membrane-permeable and may provide an intercellular communication pathway.

(5) G-protein-coupled receptors. Since odor modulation of second messenger levels is G-protein-dependent, it was widely postulated that odor receptors might belong to the seven-helix family of G-protein-coupled receptors (see Lancet and Ben-Arie, 1993; Nef, 1993, for recent reviews). Guided by this idea, Buck and Axel (1991) cloned 18 different seven-helix receptors from rat olfactory tissue. They proposed that these proteins were...
odor receptors, and estimated from screening a genomic library that there may be hundreds of structurally similar receptors in olfactory tissue. Others (Selbie et al. 1992; Raming et al. 1993; Ngai et al. 1993b) have extended the cloning to mice, humans, dogs and fish, producing sequences for over 150 different receptors. Despite concerted efforts to demonstrate function of the receptor proteins by expressing them in host cells, only one member of the family has shown any odor-elicited activity. This member was a rat receptor protein expressed in an insect cell line; membranes prepared from transfected cells and stimulated with a mixture of lyral and lilial showed a 1.5- to twofold increase in \( \text{Ins}1,4,5P_3 \) levels with a response threshold between 10 and 100 mmol l\(^{-1}\) (Raming et al. 1993). Whether a similar response is mediated by this protein in vivo is unknown.

Although we still do not know whether any, some or all of these receptor proteins are involved directly in odor transduction, antibodies to BARK-2 (but not BARK-1), a kinase that specifically phosphorylates the \( \beta \)-adrenergic G-protein-coupled receptor, prevent the rapid desensitization of odor-induced signals in preparations of rat olfactory cilia (Schleicher et al. 1993).

(6) **Non-linear summation of odorant and cyclic AMP responses.** Non-linear additivity of an odor response and the effect of exogenous second messenger has been observed in tiger salamander ORNs with an odor mixture (cineole, isoamyl acetate and 2-hexylpyridine or \( \beta \)-ionone, \( \Delta^2 \)-methone, amyl salicylate, isoamyl acetate and 2-hexylpyridine) applied focally when cyclic AMP was released intracellularly by photolysis (Lowe and Gold, 1993a,b). The results resemble the non-linear responses induced when two identical odor applications are made in rapid succession (Firestein et al. 1991a). This is consistent with the hypothesis that the odor response is mediated by the second messenger, but it is important to note that non-linear summation would be expected regardless of how the adenylyl cyclase were activated, either by an olfactory transduction pathway or otherwise by a non-transduction mechanism possibly involving high concentrations of lipophilic odorant (see above).

**Physiological implications of multiple transduction pathways**

The data reviewed here support the hypothesis that odors are detected and discriminated when they activate one or more different transduction pathways in parallel in ORNs. Similar features can be seen in the visual (DeVries and Baylor, 1993), gustatory (Kinnamon and Cummings, 1992) and somatosensory systems (French, 1992) also, suggesting that parallel but different transduction pathways provide an efficient solution for processing complex sensory information. The following conclusions can be drawn about odor transduction. (1) Odors are transduced in ORNs by modulating several different conductances in different cells from the same animal. In all species studied, single odorants have been shown to affect several components of the membrane conductance. (2) More than one odor-sensitive conductance can be found in some ORNs, and possibly in all. These conductances mediate excitatory as well as inhibitory responses. (3) A second messenger can modulate more than one type of ion channel in ORNs. (4) Some odorants can modulate more than one second-messenger pathway in ORNs, whereas others may affect just a single pathway. In either case, a single odorant
can have different effects on different cells by activating a pathway that diverges prior to the effector ion channels. (5) Even among ORNs that are sensitive to a particular stimulus, not all cells respond with an increase in the concentration of a particular second messenger or by modulation of a particular type of ion channel. There are functional subtypes of ORNs.

The variety of conductances that odors modulate allows a varied and graded effect on neuronal excitability; if the effect were simply to increase or decrease excitability, the variety of transduction pathways would be redundant. Increases as well as decreases in excitability are caused by hyperpolarizing and depolarizing receptor potentials, by increases and decreases in the resting conductance and, possibly, by modulation of the kinetics of voltage-activated ion channels. Odors are discriminated by extraction of certain relevant parameters from the pattern of odor-elicited activity in peripheral ORNs (Gesteland et al. 1965; Giradot and Derby, 1990; Shirley, 1992). Since odor recognition probably requires a comparison of responses across the population of ORNs, increasing the potential diversity of output from individual cells by allowing graded excitatory and inhibitory responses could sharpen and enhance pattern differences. The pattern of activity elicited by a single odor should change when it is presented as one component of a mixture, since parallel signalling pathways in individual ORNs and cross-talk between the pathways can occur. Determining how the olfactory system discriminates individual odors under these conditions will require an understanding of the critical parameters extracted by higher-order neurons. Together with an emerging appreciation of the overlap of neuronal responses in ORNs in terms of specific odor receptors and converging transduction pathways, we may finally account for the chemosensitive abilities of the olfactory system.

References


Transduction diversity in olfaction


Transduction diversity in olfaction


Transduction diversity in olfaction


Transduction diversity in olfaction


