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Review

Odour Transduction in Olfactory Receptor Neurons

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Abstract

The molecular mechanisms that control the binding of odorant to olfactory receptors and transduce this signal into membrane depolarization are reviewed. They are compared in vertebrates and insects for interspecific (allelochemicals) and intraspecific (pheromones) olfactory signals. Attempts to develop quantitative models of these multistage signalling networks are presented. Computational analysis of olfactory transduction is still in its infancy and appears as a promising area for future developments.

Key Words: olfactory receptors, ionic channels, OBPs, pheromones, computer models

Introduction

Olfactory receptor neurons (ORNs) are specialized cells which can transform the presence of odour molecules in their environment in trains of action potentials sent to the brain. They provide the brain with information about the chemical composition of the outside world, from which animals can locate food and preys, avoid predators and communicate with others. In this review we will focus on the molecular mechanisms of olfactory transduction and their computational modelling in terrestrial animals, vertebrates and insects.

Natural odours are usually complex mixtures of many volatile compounds, or odorants. Odorants are classically divided in two main classes, pheromones and allelochemicals. Pheromones are emitted and received by individuals of the same species (44), a typical example being sexual pheromones, like the pheromone present in the urine of the female Asian elephant at oestrus which triggers the male mounting behaviour (80). Allelochemicals are emitted by a species and received by another one, a typical example being flowers whose scents attract pollinators; they are often called "general odorants". Note, however, that some pheromones are not volatile (10); they are detected by the gustatory system (3) and in some cases by the olfactory system (56).

In both vertebrates and insects, different ORNs transduce allelochemicals and pheromones (35). ORNs sensitive to pheromones are generally more specific than those sensitive to allelochemicals and, at least in mammals, their transduction mechanisms are not identical. However, whatever the odorants and the animal group, the central event of olfactory transduction in ORNs is the interaction of odorant molecules with membrane proteins—the olfactory receptors (ORs). Linda Buck and Richard Axel (5) discovered the ORs in 1991 and received the Nobel Prize in 2004 for that. ORs form the interface between external reactions taking place in the aqueous environment of ORNs and internal reactions involving various membrane proteins, cytoplasmic modulators and ions whose main function is to amplify the weak initial signal provided by odorant binding to ORs.

The aim of the present article is to review olfactory transduction in a few groups of terrestrial animals. The sequence of extra- and intra-cellular stages giving rise to the neuron electrical response is described.

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For each stage, the experimental data and corresponding modelling investigations are summarized. For experimental aspects, because of space limitation, we will provide mostly references to review papers and recent original work, in which the reader will find more complete references. Several important topics will not be covered in this review: the transport of odorant molecules in the atmosphere and in the immediate vicinity of olfactory organs (nasal cavity of vertebrates (106); antennae of insects (21, 42)), the molecular modelling of ORs (66), the diversity of ORs (64) and ORNs (18, 96, 119), the generation of action potentials (72), the adaptation to long stimulation (118), the development and hormonal regulation of ORNs. We will restrict our attention mostly to intensity coding, *i.e.* how short odorant pulses, typically of ~1s duration, at different concentrations in the air, are translated in a sequence of graded biochemical and electrical events. The qualitative discrimination of odours (15, 27, 65) will not be treated and their temporal discrimination, which is important especially in insects (107), will be only briefly discussed.

Olfactory Organs

ORNs are associated with accessory cells to form epithelia located in the nasal cavity of vertebrates and in the antennae of insects. A significant difference is that these epithelia are continuous in vertebrates whereas they are compartimentalized in discrete sensilla in insects. Odorants enter sensilla through microscopic pores in the waxy cuticle surrounding them. Another difference is the continuous renewal of ORNs in vertebrates.

Each ORN is a typical bipolar neuron (46). One process is a dendrite ("inner dendrite" in insects) which ramifies in fine cilia ("outer dendrites" in insects) bearing identical ORs. The second process is an axon which terminates in the olfactory centre of the brain, olfactory bulb of vertebrates (119) and antennal lobe of insects (29). There, ORNs synapse with central neurons within dense spherical region of neuropil, the glomeruli.

Allelochemicals and sexual pheromones are detected by different ORNs. In reptiles and mammals, allelochemicals are detected by ORNs in the main olfactory epithelium (MOE) in the posterior dorsal part of the nasal cavity. Their axons project to the main olfactory bulb (MOB) in the brain. Pheromones are detected in the vomeronasal organ (VNO) which is located within the bone at the base of the nasal cavity (33). The axons of the vomeronasal sensory neurons project to the accessory olfactory bulb (AOB), close to the MOB. However, this separation of functions is not absolute; some pheromones can be detected by the MOE and some allelochemicals by the VNO (89).

In insects, allelochemicals and pheromones are detected by ORNs located in different types of antennal sensilla which project to different parts of the antennal lobes (29). In various insect orders, the pheromone-sensitive ORNs project to a group of large-sized glomeruli, the macroglomerular complex (MGC) whereas the other ORNs project to the more numerous ordinary glomeruli (29).

Extracellular Events: Perireception and Reception

Odorant-Binding Proteins

An aqueous medium, the mucus of vertebrates and the sensillum lymph of insects, surrounds the most distal parts of ORNs. It is secreted by the auxiliary cells and must be crossed by odorants to reach the ORN membrane. The crossing of hydrophobic odorants is facilitated by small water-soluble proteins, the odorant-binding proteins (OBPs; (75)) and chemosensory proteins (CSPs; (76)), present there in high concentration. Although they share common functions in both groups, they belong to unrelated families in amino acid sequence and 3D structure. Mammalian OBPs belong to the lipocalin family of proteins and insect OBPs are divided in two subfamilies, the pheromone-binding proteins (PBPs) and general odorant binding proteins (GOBPs), both with 6 α-helices stabilized by 3 disulfide bridges. In the silkmoth *Bombyx mori* it has been suggested that the PBP conformation depends on the pH. At high pH, the PBP binds the pheromone which remains bound during transfer through the lymph. At low pH, close to the ORN membrane, the conformation changes and the pheromone is released. When bound to the PBP, the pheromone is protected from the action of degrading enzymes (114).

Degrading Enzymes

Enzymes are another important component of the extracellular medium. In both groups enzymes found in the mucus (75) and lymph (109) degrade odorants. Other enzymes are present in the epithelium, notably the cytochrome-P450 mono-oxygenases of the endoplasmic reticulum (73); these enzymes, which are involved in the degradation of xenobiotics, are as active as in the liver.

Pheromone degrading enzymes (PDEs) were described only in moths. They belong to several categories, notably esterases (16, 110). Some pheromonal ORNs can follow repetitive pulses up to 10 Hz which implies the fast inactivation of the phero-

mone (67). Whether PDEs can explain this inactivation is still controversial: *in vitro* (32, 111) but not *in vivo* (45) measurements are consistent with this hypothesis.

Olfactory Receptors

Finally, the odorant molecules interact with olfactory receptors (ORs) borne by the ORN membrane. They were first discovered in the rat MOE (5), where they represent the largest receptor family in the genome, then in *Drosophila* (9, 22, 112) using genomic analyses. In both groups ORs belong to the family of receptors with seven transmembrane domains, although they are not related as they do not present homologous sequences. The number of different ORs is about 60 in *Drosophila* (14) and 1200 in the mouse (24). Most ORNs express a single OR gene (20) and most ORNs expressing the same gene converge to a single glomerulus (or pair of glomeruli in mammals) (23, 69) on the ipsilateral olfactory bulb or antennal lobe. The expression of ORs *in situ* and in heterologous systems have shown that some ORs bind to a narrow range of odorants but most are broadly tuned and respond to specific features (functional group, carbon chain length, shape, *etc*.) called odotopes or odour determinants (87, 99).

The pheromone receptors in the VNO are also GPCRs and they belong to two families, V1R and V2R, both with about 100 functional genes in the mouse (30). V1Rs are expressed in the most superficial (apical) ORNs of the VNO, and project to the anterior region of the AOB. V2R are expressed in the basal ORNs and project to the posterior region of the AOB (120). These two families are unrelated to each other and to ORs. In moths, ORs for volatile pheromones were first identified in *Heliothis virescens* (51) and *B. mori* (95). These receptors are co-expressed with an atypical OR, highly conserved across insect orders (50); it is named OR83b in *Drosophila*. In this species it is involved in addressing ORs to the ORN plasma membrane (54) and it forms a complex with them (74).

Modelling the Extracellular Processes

The significance of extracellular processes has been first emphasized by Kaissling (39) in insect sensilla. When exposed to a constant stimulus concentration, the odorant molecules accumulate in the sensillum lymph in which they are trapped. The odorant concentration in the lymph increases until the constant influx is balanced by enzymatic removal. The odorant concentration acting on ORs is different from that in the air. It is noteworthy that such a "flux detector" cannot work without enzymes.

A complete description of the extracellular processes requires knowledge of all reactants and their initial concentrations, and of all reactions and their rate constants. Then, in principle, the evolution in time of the system when it is stimulated by a given odorant waveform can be computed. To our knowledge such a modelling of extracellular processes has never been attempted in vertebrates. The latest model available (41) applies to moth pheromone sensilla based mainly on experimental data taken from studies of *B. mori* and *A. polyphemus*. First, entering the hair lumen the pheromone binds to the A-form of the PBP (at neutral pH) forming the B-form which diffuses to the ORN membrane. There, at low pH, the B-form converts back to the A-form. The complex pheromone-PBP_A can dissociate and release the free pheromone, or bind to the pheromone receptor. A ternary complex is formed (pheromone-PBP_A-receptor) which can change the conformation of the receptor into an activated state. Second, the free pheromone is rapidly degraded by the PDE to a metabolite which cannot interact with the receptor. However, this degradation is not sufficient to account for the experimental results, so two other hypothetical deactivations are considered: an enzyme N (model N) or the receptor itself (model R) might deactivate the pheromone-PBP_B complex to an inactive form. Model R is supported by the fact that the kinetic properties of the receptor potential are conserved when OR genes are expressed in "empty" ORN (12, 27, 104). However, model N is favoured by the author because it is in better agreement with the fact that a blocker of the receptor modifies the amplitude of the receptor potential but not its falling time and the measurement of the elementary receptor potentials (68). The two-step activation of receptors, for which the latter study provides detailed data on reaction rates, is also needed to account for dose-response curves of vertebrates (90).

A simplified model with similar kinetic properties has been studied (43, 92). It can account for the ability of pheromonal ORNs to follow repetitive pulses up to about 2 Hz. At present, faster ORNs (up to 10 Hz) are not accounted for. This model has also been shown to be in agreement with the efficient coding principle (100) which states that the sensory neurons are adapted to the statistical properties of their natural stimuli. In the present case, the characteristics of the pheromone plume (concentrations, spectral densities, intermittency) which are optimally detected by the system were determined and shown to be similar to those measured in the field (49). The pheromonal ORN is the only ORN for which both the odorant stimulus and the neuron are known well enough to perform the comparison.

Intracellular Events: Generation of Second Messengers

G-Proteins and Effector Enzymes

The initial steps of the transduction pathway in MOE (36) and VNO (120) neurons are now well established in vertebrates, although other pathways have been described in relatively small groups of ORNs (18). In MOE neurons, the activated OR binds to a G-protein, G_{olf}, a guanosine triphosphate (GTP)-binding protein (98). Then G_{olf} activates a type III adenylyl cyclase (AC) which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). The ciliary production of cAMP has been measured (105).

In VNO neurons two different types of G-proteins were found: $G\alpha_{i2}$ in apical neurons and $G\alpha_0$ in basal neurons (119). Both G-proteins activate phospholipase C (PLC) (31, 62) which catalyzes the cleavage of phosphatidylinositol 4,5-biphosphate (PIP_2) in inositol 1,4,5-biphosphate (IP_3) and 1,2-diacyl glycerol (DAG).

In insect ORNs, until recently it was believed that PLC was necessary for the transduction of both, for both allelochemical and pheromonal odorants, based on stop-flow experiments (4) and the immunodetection of PLC_β in pheromone-sensitive sensilla of a moth (63). G_q proteins are present (34) and functional (55) in the outer dendrite of moth pheromonal ORNs. *Xenopus* oocytes transfected with *B. mori* pheromone receptors and without OR83b do not respond to pheromone stimuli unless they are co-transfected with G_q proteins (95). Also, genetic evidence supports a role for a G_q- and PLC_β-mediated signaling cascade in non-pheromonal olfactory transduction in *Drosophila* (37).

However, this classical metabotropic pathway has been challenged. The membrane topology of at least some *Drosophila* ORs differs from other GPCRs with an intracellular N-terminus and an extracellular C-terminus (2). This inverted membrane topology puts into question the coupling of insect ORs to G-proteins (1, 70). Recently, two studies indicated that odorants can activate cells co-expressing ORs (from fruitfly, silkmoth or mosquitoe) and OR83b, and generate sensory currents independently of known G-protein-coupled second messenger pathways, through a so-called ionotropic pathway (97, 113). OR83b alone forms a cationic channel opened without mediation by a G-protein (113) but it is also activated by cyclic nucleotides. The results at hand suggest the coexistence of two signaling pathways in both pheromonal and non-pheromonal insect ORNs, one ionotropic, the other metabotropic (113).

Modelling the Generation of Second Messengers

Following the quantitative description of G-protein cascades (52), a model of the interactions between receptor, G-protein and PLC molecules has been proposed for moth pheromonal ORNs (94). In the model studied, the G-protein cascade obeys the concept of the random walk amplifier (57) in which the proteins freely diffuse on the two-dimensional dendritic membrane, so that each activated OR (R*) can sequentially encounter and activate several molecules of G-protein. Then, each of the active G-protein molecules (G*) can in turn bind and activate an effector enzyme (E*). The response is turned off by deactivation reactions acting on the receptor, the G-protein and the effector. Numerical constants, based either on direct experimental measurements in moth pheromone ORNs or derived from models integrating such measurements, were used when available. For the G-protein and the effector in the ORN, the diffusion-limited rate for the G-protein transducin and the phosphodiesterase effector in photoreceptors (17, 52) were used, based on the expectation that their diffusion properties are similar in both the photoreceptor neuron and the ORN. To agree with the experimental dose-response curves, two rate constants were modified: the effector deactivation constant was increased and the G-protein activation constant (limited by diffusion) was decreased. As a result, the amplification factor of the cascade measured by the ratio E*/R* fell from 330 with the photoreceptor values to 7.5 and the kinetics of G* and E* became very similar to that of R*.

This insect model was extended one step further to include cAMP production and adapted to vertebrates (28). Parameter values were determined by comparing the predicted cAMP levels to those measured *in vitro* from experimental recordings of the receptor current in the amphibian ORN (105). A sensitivity analysis of the model showed that the cAMP production rate depends mainly on the initial density of G-proteins and the catalytic constant for cAMP production. In the model the cAMP concentrations obtained are of the same order of magnitude as the odorant (cineole) concentration applied. This result is in agreement with the conclusion that the molar amplification is extremely low in ORN cilia in comparison to that of the rod photoreceptor, where the cAMP production is circa 250,000 molecules per photon.

Intracellular Events: Ionic Channels

Second Messenger-Gated Channels

In canonical vertebrate ORNs of the MOE the increased concentration of cAMP resulting from AC activation gates a cyclic nucleotide-gated (CNG) cationic channel present in the ciliary membrane

which is permeable to Ca^{2+} (71). The resulting cationic current is depolarizing. Its intensity is a sigmoid (Hill) function of the cAMP concentration. Cytoplasmic Ca^{2+}, *via* Ca^{2+}-calmodulin (CaCaM), reduces the sensitivity of the CNG channel: it modulates its open probability and shifts its dose-response curve to higher cAMP concentrations (47, 82).

In rodent VNO neurons, no CNG channels were found. Instead a member, named TRPC2, of the transient receptor potential (TRP) family of ion channels was involved in pheromone transduction and cloned (59). In the mouse, TRPC2 is a Ca^{2+} permeable cationic channel directly activated by DAG (62).

In moth pheromonal ORNs, DAG activates also a non-specific cationic channel (117). A possible interpretation of these results is that the cationic current could be triggered by two pathways: the ionotropic pathway, rapid and transient, but not amplified, would be the most visible at high concentration, whereas the metabotropic pathway, slower, prolonged and more sensitive, would be more important at low concentration (113). Both pathways gate cationic channels, although OR83b is apparently different from the DAG-gated cationic channel (7).

Calcium-Gated Channels

In vertebrates the increased intracellular concentration of Ca^{2+} resulting from the opening of CNG channels gates a second depolarizing current. In the MOE, this is a Cl^- current (19). The unit conductance of the Cl^- channel is small (1.6 pA or less; (47)). The density of the Cl^- channels is the same as the density of CNG channels in frog (53), but is 8 times greater in rat (87). The Cl^- current is a Hill function of the intracellular Ca^{2+} concentration. It has no known modulator (47).

In VNO neurons, a similar Ca^{2+}-mediated amplification may involve cationic channels (58, 101) and/or Cl^- channels (115).

In moth pheromonal ORNs a Ca^{2+}-gated cationic (102) and Cl^- currents (77) have been found. It is not yet known whether the Cl^- current is depolarizing or repolarising; no modulation of this current is known.

Termination of the Receptor Current

In vertebrate ORNs, inactivation of the odour response requires an influx of Ca^{2+}. At least ten mechanisms can contribute to this inactivation (47). They include the feedback regulation of CNG channels by CaCaM, of AC by CaCaM-dependent protein kinase II, and of OR by GPCR-kinase-3 and β-arrestin-2; they include also membrane repolarization by a ciliary Ca^{2+}-gated K^+ current (11). The restoration of resting ionic concentrations involves various pumps. For example, Ca^{2+} is removed by two mechanisms, the Na^+-Ca^{2+} exchanger (NCX) (83) and the plasma membrane calcium ATPase (PMCA) (6), with PMCA accounting for only a small fraction of the Ca^{2+} expulsion (48).

In insect ORNs, repolarization depends on extracellular Ca^{2+} and likely involves a Ca^{2+}- and voltage-dependent K^+ current (78). Ca^{2+}-dependent feedback mechanisms acting on cationic channels *via* CaCaM (61) and on PLCβ *via* protein kinase C (8, 63) are known. The mechanisms of Ca^{2+} extrusion have not yet been elucidated.

Modelling the Ionic Receptor Current

The electrotonic structure of salamander ORN, *in situ* and isolated from the epithelium, was determined (79). It provides useful geometrical and electrical data on this ORN, notably the ciliary membrane resistance.

A phenomenological, essentially electrical, model of ORN in steady state (*i.e.* under constant stimulation) was developed (91), extending previous theoretical results (38, 93, 108). It is based on 3 equations and 13 known parameters describing the lumped ciliary membrane conductance as a function of odorant concentration, the receptor potential at the base of cilia and the axon initial segment as a function of conductance, and the firing frequency as a function of the receptor potential. It was applied to *in vivo* recordings of frog ORNs in response to four odorants at various concentrations. The characteristics of the Hill dose-conductance curves (maximum conductance, dose a half-maximum conductance and Hill coefficient) were derived for the population of ORNs, showing the diversity of individual ORNs (see also (25)). This important variability is a major difficulty for quantitative modelling of ORNs.

Larsson *et al*. (53) developed a method to analyse channel noise in a voltage-clamped isolated cilium. They used it to determine the unit conductance and density of CNG-gated and Ca^{2+}-gated channels. Lindemann (60) studied the change in ion concentrations occurring during transduction within a single cilium in the same conditions. He showed that the long and slender ciliary geometry had large effects on ion distributions and transduction currents. Cl^- and K^+ were depleted at the tip of the cilium, whereas Ca^{2+} was in excess at its base.

The model presented in (103), based on recordings from rainbow trout ORNs in response to stimulation with amino acid mixtures, aimed at explaining the damped oscillations elicited by single pulses of stimuli of various durations. The model involves 12 differential equations with 44 parameters.

It shows that the oscillations are mainly due to the oscillatory properties of intracellular cAMP and Ca^{2+} concentrations. The inhibition of AC by Ca^{2+} is necessary for the apparition of oscillations.

The model developed by Dougherty *et al*. (13) is based on experiments by Reisert and Matthews (84, 85) in frog. These experiments recorded the receptor current in three conditions: a single 1s-pulse of odorant at various concentrations, a 1s-pulse at a single concentration following a 4s conditioning stimulation, and a prolonged (30-60s) stimulation which induces oscillations of the receptor current. The basic model comprises 7 differential equations and 32 parameters. Three sets of parameters were fitted using a genetic algorithm, one to each condition.

Ca^{2+} oscillations were also investigated by Reidl *et al*. (81) based on various experimental data, notably (86). Their model, very different from (13), is based on direct negative regulation of cyclic nucleotide-gated channels by CaCaM. It includes 4 differential equations and 13 parameters. It is in quantitative agreement with available experimental data, both with respect to oscillations and to fast adaptation. It gives predictions for the ranges of parameters in which oscillations should be observable.

In insects, a model of the male moth pheromonal ORN (26) has been proposed. It is based on the pre-effector model (94) summarized above and focuses on the post-effector cascade. The main assumptions of the model are that Cl^- channels are depolarizing, as in vertebrates, and that K^+ channels located in the inner dendritic segment are repolarizing. It includes 23 differential equations and 55 parameters. The model was fitted to experimental data, principally those giving the amplitude, half-rise time and half-fall time of the receptor potential in response to 2-s pulse stimulation at different pheromone doses in the moth *A. polyphemus* (40, 116). It gives an interpretation of the wide dynamic range of the pheromonal ORN in which the DAG-gated cationic channel plays the major role at low concentrations and the Ca^{2+}-gated Cl^- at middle and high concentrations. It shows also that most (~80% ± 20% depending on the dose) of the short rising time and long falling time of the receptor potential results from the extracellular reactions.

Conclusion

Although much progress has been done in recent years in the understanding of olfactory transduction, much remains to be known (47, 87). At the present time, in vertebrates and much more in insects, quantitative modelling is essentially limited by an insufficient qualitative knowledge of the components (proteins and modulators) involved in olfactory transduction and their location along the neuron. Built on firmer qualitative foundations, modelling methods could draw more information from available measurements, for testing hypotheses, estimating the values of unknown parameters and analyzing the properties of this intricate network of reactions.

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