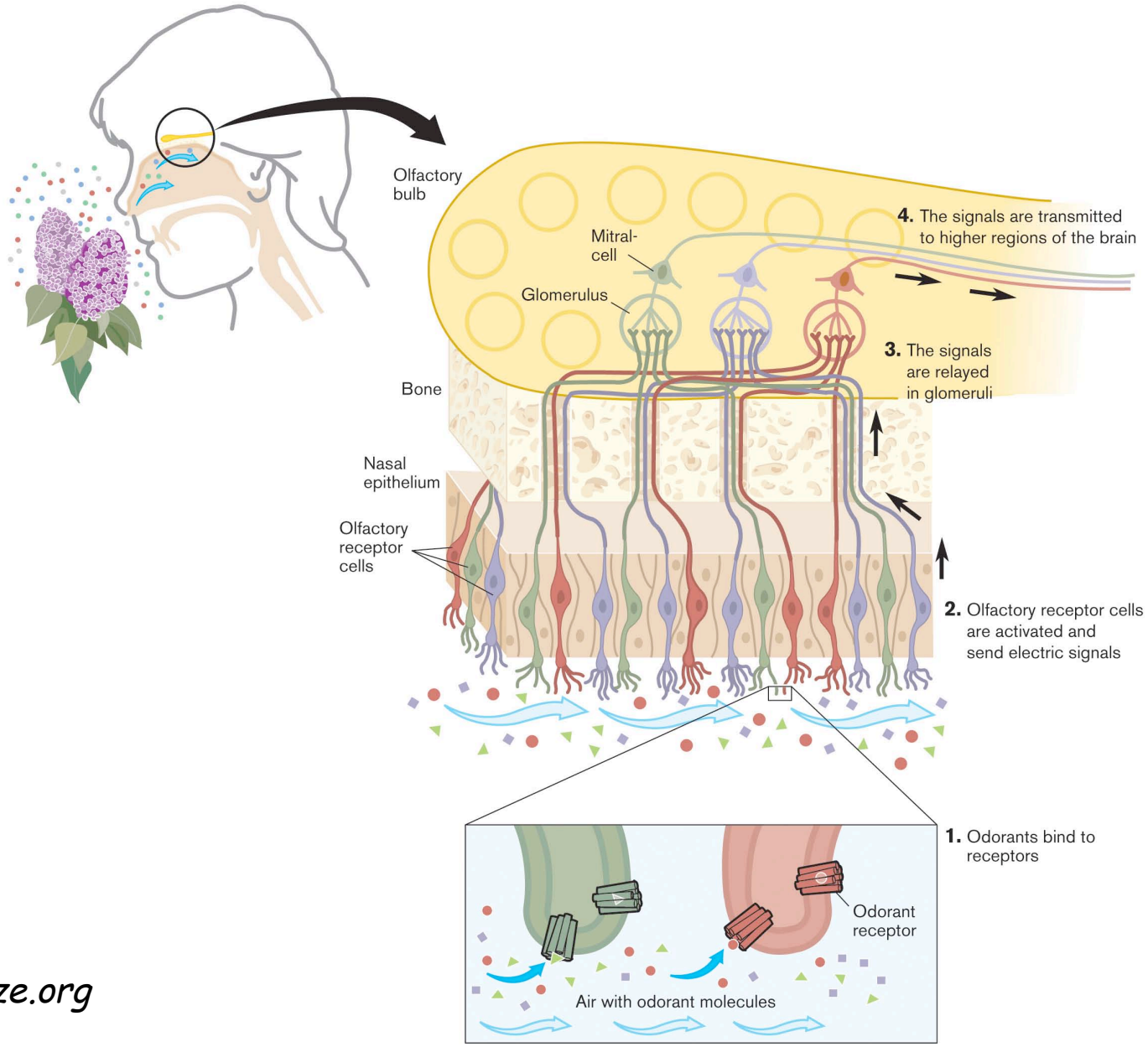
Olfaction (the sense of smell)

- Mammals can detect upwards of 1000-10000 different odors
  - Wide spectrum of chemical structures
  - Discrimination of structurally similar compounds
- How??

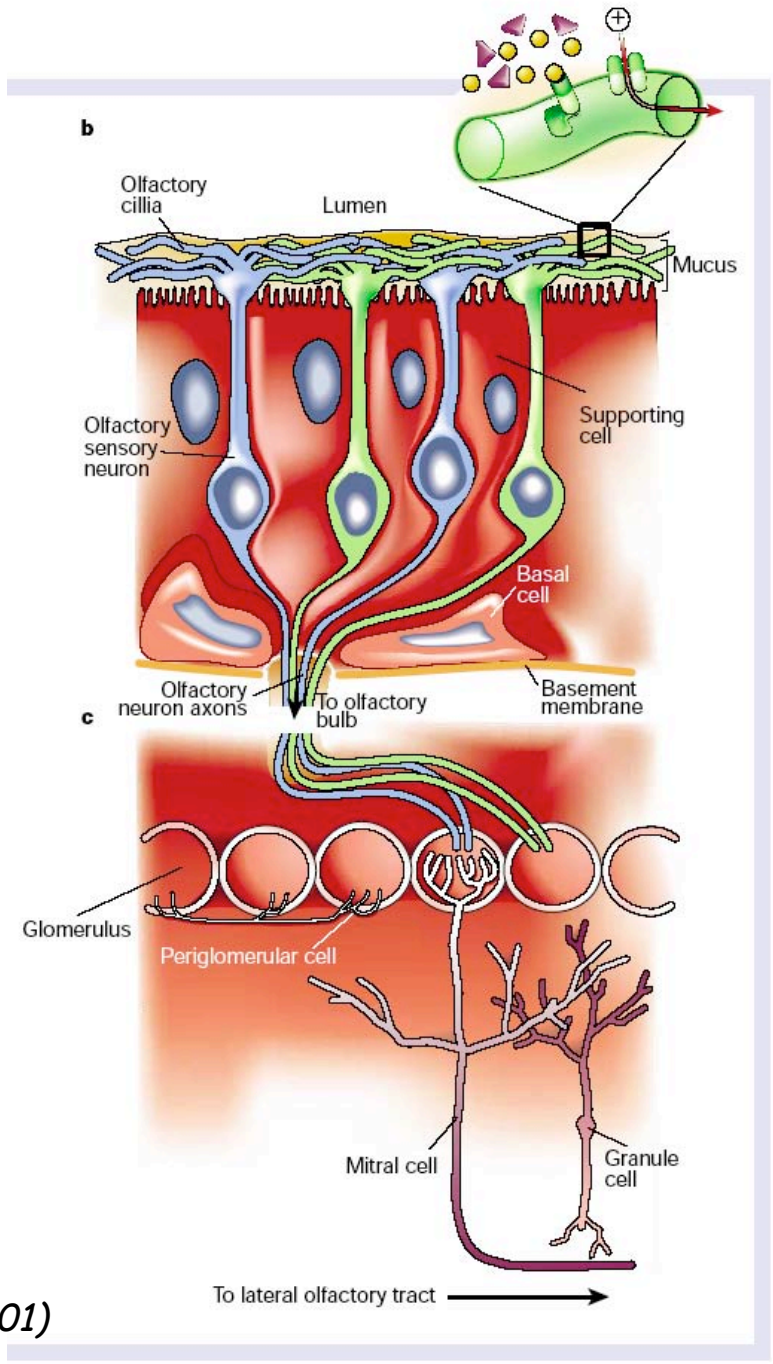
General Concepts

- Basic anatomy of the peripheral olfactory system
- Principles of signal transduction
- "Coding" of information at three levels:
  - Receptors - #'s and combinatorials
  - Neuronal specificity - 1 receptor type/sensory neuron
  - Spatial maps - how the brain keeps track of which sensory neurons (and therefore which receptors) have been activated

Odorant Receptors and the Organization of the Olfactory System



Nobelprize.org



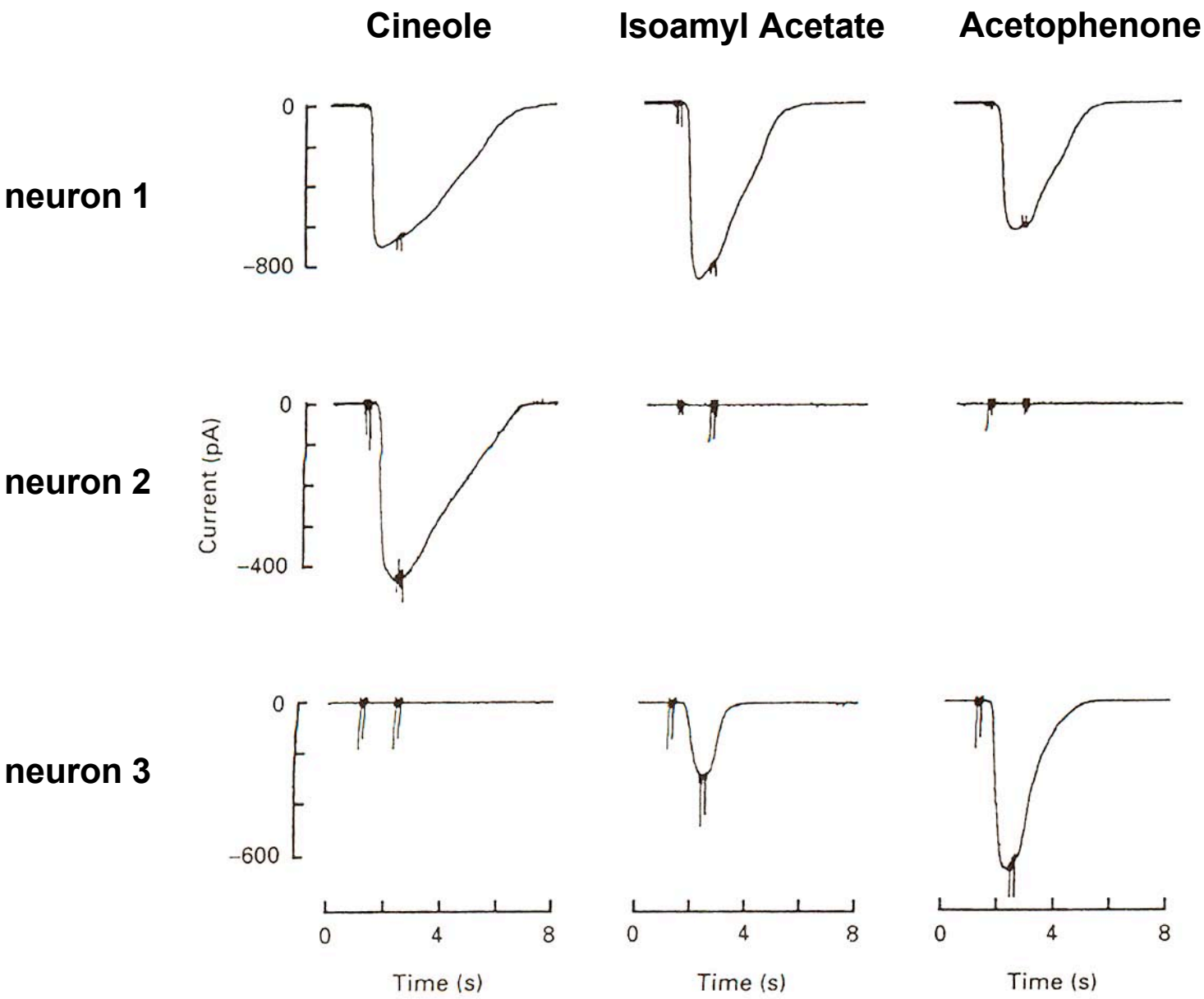
Olfactory cilium

Olfactory epithelium

Olfactory bulb

Firestein, Nature 413: 211 (2001)

Olfactory neurons can respond to multiple (and different) odors



Firestein et al., J Physiol. 1993

Olfactory Signal Transduction

- Conversion of a chemical signal (odor binding) to an electrical signal (change in neuron's V_m)
- What is the nature of the odorant receptor?
  - Ligand (odor) - gated ion channel?
  - G protein-coupled receptor (GPCR)?
- Delay in olfactory neuron's response to odor (~500 ms) and odor-dependent generation of cAMP by olfactory cilia in vitro (dependent on GTP) => GPCRs
- Diversity of odorants detected suggests a large number of receptors
- 1991: identification of large family of GPCRs expressed in olfactory sensory neurons by Buck and Axel (2004 Nobel Prize in Physiology or Medicine)

Olfactory Signal Transduction:

How the chemical signal is converted to an electrical signal…

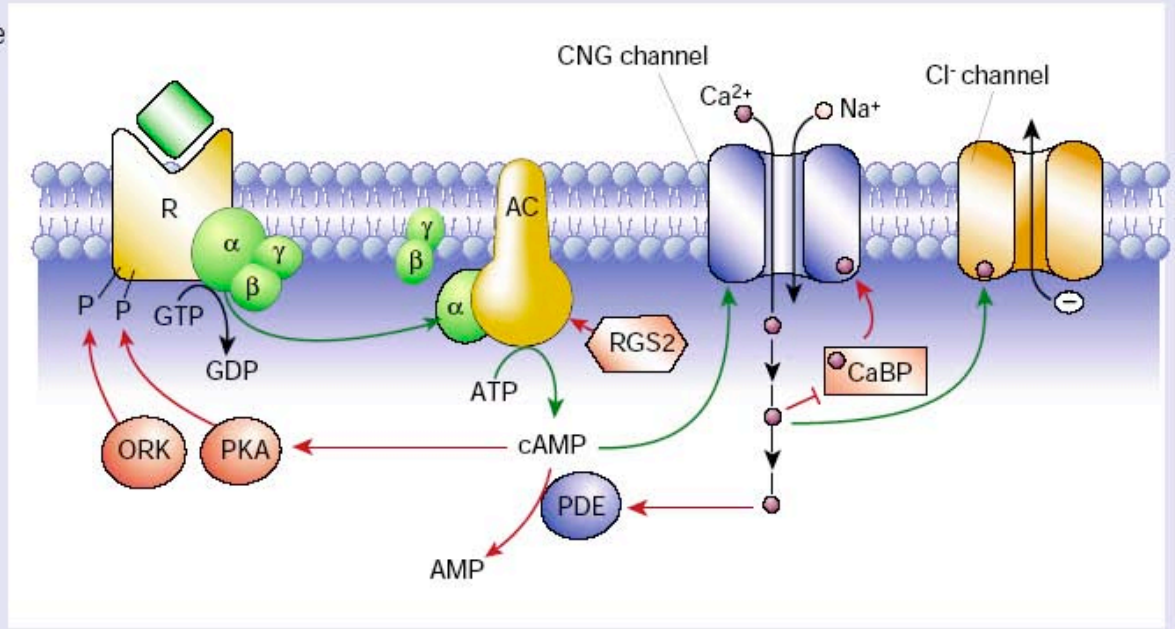


Figure 3 Sensory transduction. Within the compact cilia of the OSNs a cascade of enzymatic activity transduces the binding of an odorant molecule to a receptor into an electrical signal that can be transmitted to the brain. As described in detail in the text, this is a classic cyclic nucleotide transduction pathway in which all of the proteins involved have been identified, cloned, expressed and characterized. Additionally, many of them have been genetically deleted from strains of mice, making this one of the most investigated and best understood second-messenger pathways in the brain. AC, adenylyl cyclase; CNG channel, cyclic nucleotide-gated channel; PDE, phosphodiesterase; PKA, protein kinase A; ORK, olfactory receptor kinase; RGS, regulator of G proteins (but here acts on the AC); CaBP, calmodulin-binding protein. Green arrows indicate stimulatory pathways; red indicates inhibitory (feedback).

Firestein, Nature 413: 211 (2001)

Odorant Receptors

- Belong to the superfamily of GPCRs
- >1000 in rodents, ~350 in humans (other distantly related families of GPCRs expressed in the vomeronasal system)
- Large number of receptors suggests a model for the detection and discrimination of an even larger number of perceived odors
  - Each receptor binds to more than one odorant
  - Each odorant binds to a subset of receptors
  - The identity of the chemical being detected is determined by the combination of receptors that are activated

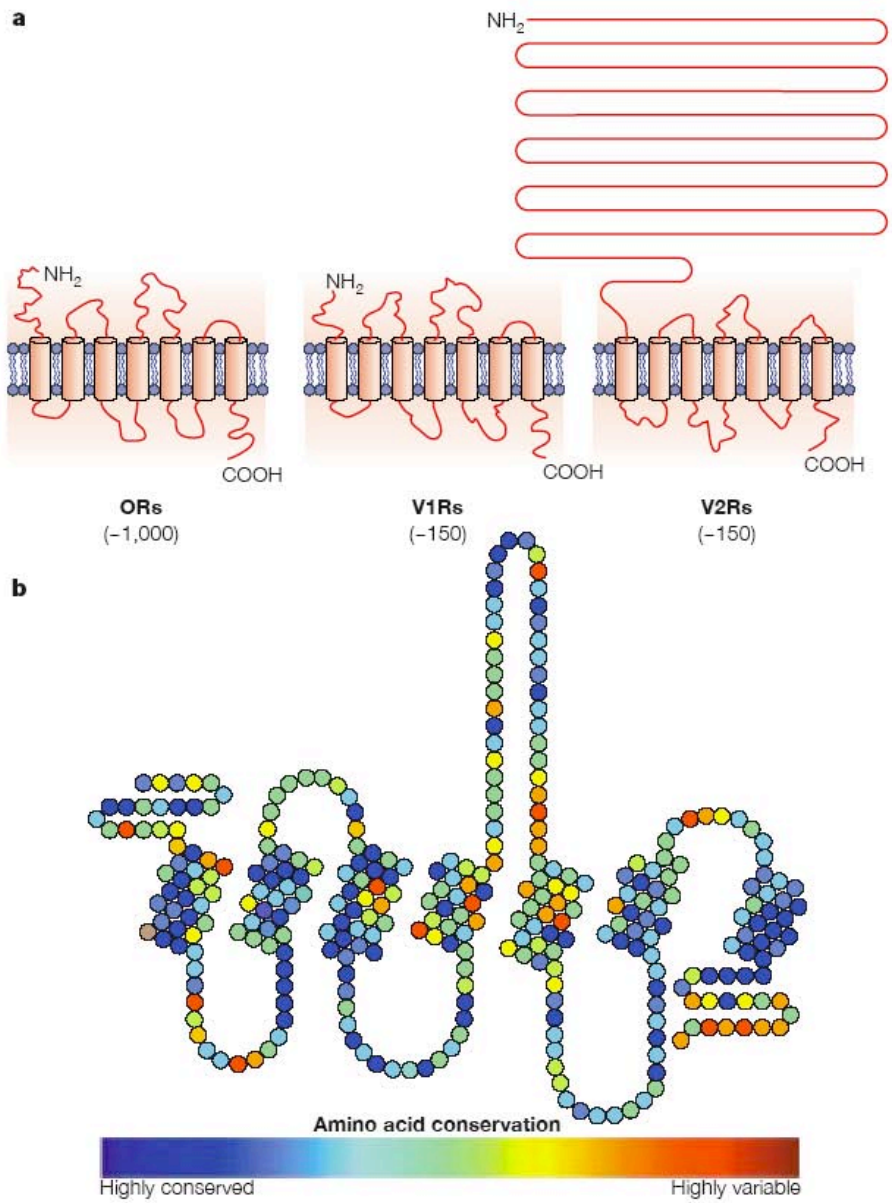


Figure 3 | **Odorant and vomeronasal receptors. a** | Odorant receptors and V1R vomeronasal receptors have short N-terminal extracellular domains, in contrast to V2Rs. The number of genes in each family is indicated in brackets. **b** | The degree of amino acid conservation in the consensus sequence of an odorant receptor is represented as a colour in the rainbow spectrum, with blue being highly conserved and red highly variable. Modified, with permission, from REF. 46 © (2003) Academic Press.

Mombaerts, Nature Reviews Neuroscience 5: 263 (2004)

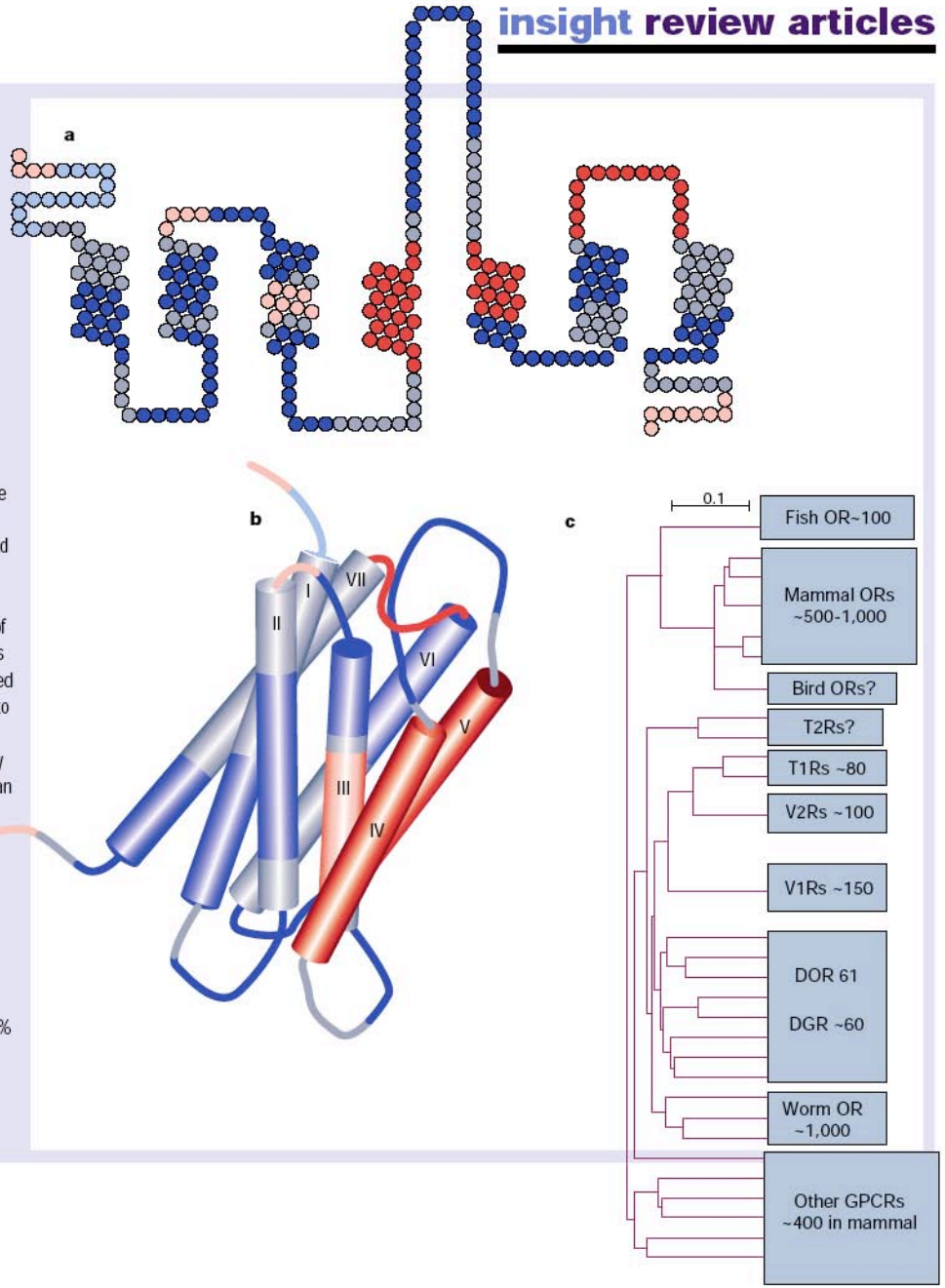


Figure 2 Odorant receptors are the jewel of olfactory research in the past 10 years. The odorant receptors comprise the largest family of GPCRs. In mammals, odour receptors are represented by as many as 1,000 genes and may account for as much as 2% of the genome. Sequence comparison across the receptors has revealed many regions of conservation and variability that may be related to function. **a**, In a 'snake' diagram showing the amino acids for a particular receptor (M71), those residues that are most highly conserved are shown in shades of blue and those that are most variable are shown in shades of red. The seven α-helical regions (boxed) are connected by intracellular and extracellular loops. **b**, A schematic view of the proposed three-dimensional structure of the receptor based on the recently solved structure of rhodopsin. Each of the transmembrane regions is numbered according to that model. The conserved (blue) and variable (red) regions are sketched onto this qualitative view and suggest that a ligand-binding region may be at least partially formed by the variable regions of the receptor. **c**, Mammalian odour receptors are related phylogenetically to other chemosensory receptors. In the tree depicted here the numbers refer to the approximate number of receptors in each family. OR, Odorant receptors; T1R, T2R, taste receptors; V3R, vomeronsal receptors; DOR, DGR, *Drosophila* odour and gustatory receptors; worm refers to *C. elegans*. The scale bar is a graphical distance equal to 10% sequence divergence.

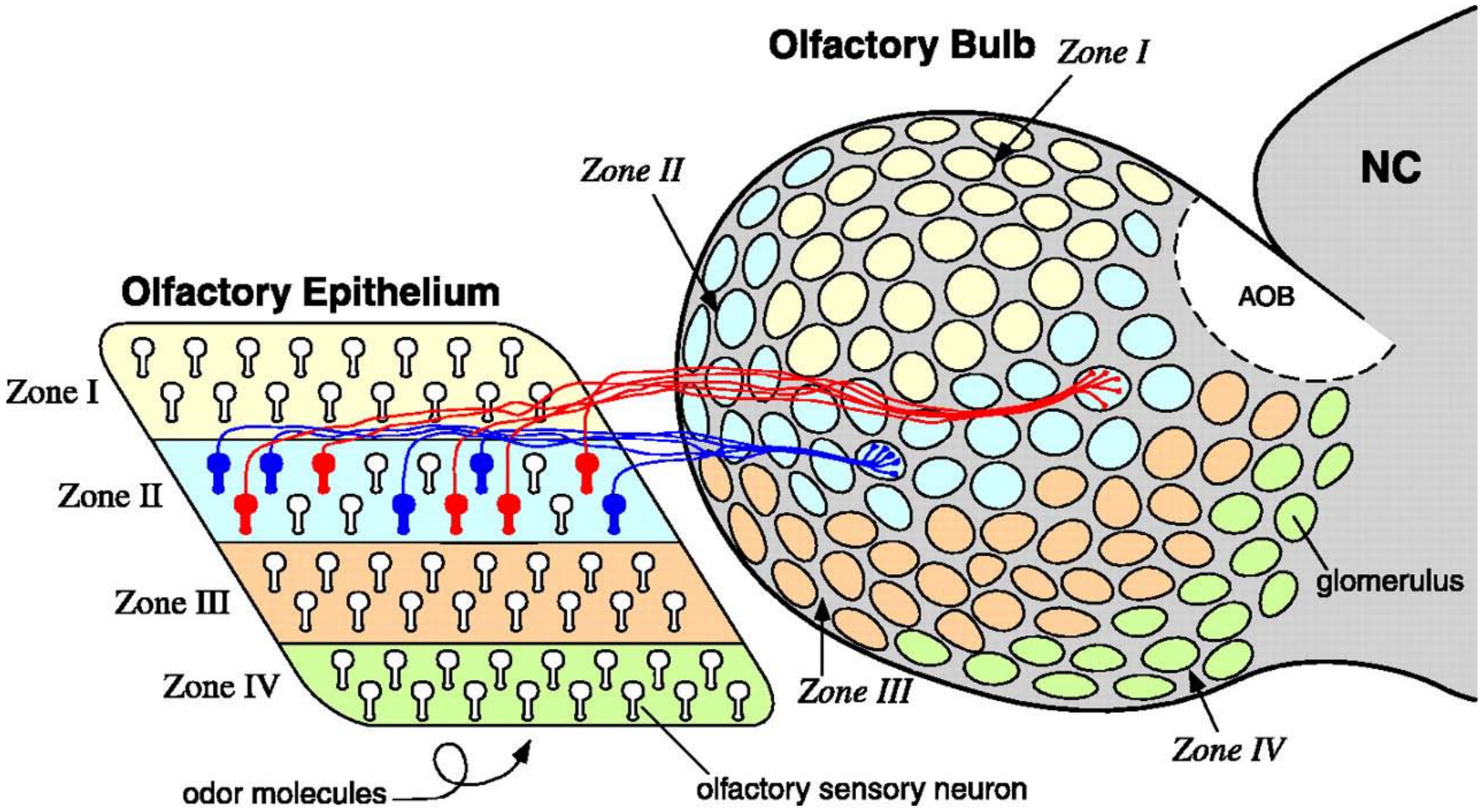
Neuronal Specificity

- If a given odor activates a subset of odorant receptors, how does the brain know which receptors are being activated (out of a possible ~1000)?
- Simplest model: each olfactory neuron expresses just one type of odorant receptor
- Problem of identifying which receptor(s) is activated is reduced to identifying which neuron(s) is activated

Spatial Maps

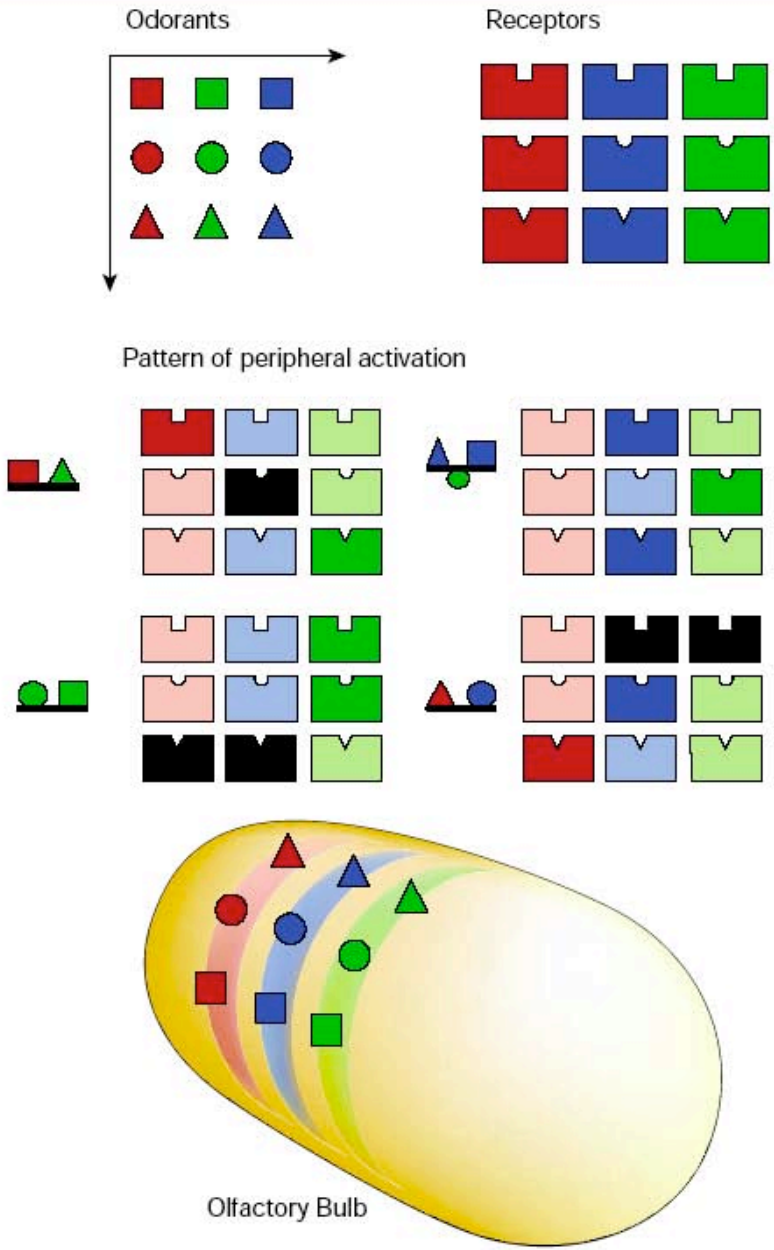
- Problem: how does the nervous system know which olfactory neurons are being activated?
- Cells expressing the same receptor type (and therefore responsive to the same odorants) converge to common glomeruli in the olfactory bulb
- Pattern of this convergence is invariant from animal to animal
- This forms the basis of a spatial map of olfactory sensory information - the pattern of glomerular activation is a "read-out" for the chemical identity of the odorant being detected
- (Big) future question: how is this "olfactory map" interpreted to form an olfactory percept?

A Spatial Map Encodes Sensory Information in the Olfactory System



Mori et al., Science, 286, 711-715, 1999

A code in the nose



Although there are some 1,000 ORs, detecting the enormous repertoire of odours requires a combinatorial strategy. Most odour molecules are recognized by more than one receptor (perhaps by dozens) and most receptors recognize several odours, probably related by chemical property. The scheme in the figure represents a current consensus model. There are numerous molecular features, two of which are represented here by colour and shape. Receptors are able to recognize different features of molecules, and a particular odour compound may also consist of a number of these 'epitopes' or 'determinants' that possess some of these features. Thus the recognition of an odorant molecule depends on which receptors are activated and to what extent, as shown by the shade of colour (black represents no colour or shape match and thus no activation). Four odour compounds are depicted with the specific array of receptors each would activate. Note that there are best receptors (for example, red square), but also other receptors that are able to recognize some feature of the molecule (for example, any square) and would participate in the discrimination of that compound. In the olfactory bulb there seem to be wide areas of sensitivity to different features (for example, functional group or molecular length). This model is based on current experimental evidence, but is likely to undergo considerable revision as more data become available.

Firestein, Nature 413: 211 (2001)

Additional assigned reading:

- Firestein, S. (2001). How the olfactory system makes sense of scents. *Nature* 413, 211-218.

How the olfactory system makes sense of scents

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The human nose is often considered something of a luxury, but in the rest of the animal world, from bacteria to mammals, detecting chemicals in the environment has been critical to the successful organism. An indication of the importance of olfactory systems is the significant proportion — as much as 4% — of the genomes of many higher eukaryotes that is devoted to encoding the proteins of smell. Growing interest in the detection of diverse compounds at single-molecule levels has made the olfactory system an important system for biological modelling.

The sensitivity and range of olfactory systems is remarkable, enabling organisms to detect and discriminate between thousands of low molecular mass, mostly organic compounds, which we commonly call odours. Represented in the olfactory repertoire are aliphatic and aromatic molecules with varied carbon backbones and diverse functional groups, including aldehydes, esters, ketones, alcohols, alkenes, carboxylic acids, amines, imines, thiols, halides, nitriles, sulphides and ethers. This remarkable chemical-detecting system, developed over eons of evolutionary time, has received considerable attention in the past decade, revealing sensing and signalling mechanisms common to other areas of the brain, but developed here to unusual sophistication.

How does the olfactory system manage this sophisticated discriminatory task? Beginning with the identification of a large family of G-protein-coupled receptors (GPCRs) in the nose, the foundations of a comprehensive understanding have emerged in surprisingly short order. The advent of advanced molecular and physiological techniques, as well as the publication of eukaryotic genomes from *Caenorhabditis elegans* to *Homo sapiens*, has provided the critical tools for unveiling some of the secrets. We now possess a detailed description of the transduction mechanism responsible for generating the stimulus-induced signal in primary sensory neurons, and also an explicit picture of the neural wiring, at least in the early parts of the system. From this body of work a view of molecular coding in the olfactory system has arisen that is surely incomplete, but nonetheless compelling in its simplicity and power.

Among higher eukaryotes, from flies through to mammals, there is a striking evolutionary convergence towards a conserved organization of signalling pathways in olfactory systems[1]. Two olfactory systems have developed in most animals. The common or main olfactory system is the sensor of the environment, the primary sense used by animals to find food, detect predators and prey, and mark territory. It is noteworthy for its breadth and discriminatory power. Like the immune complex, it is an open system built on the condition that it is not possible to predict, *a priori*, what molecules it (that is, you) might run into. Therefore, it is necessary to maintain an indeterminate but nonetheless precise sensory array. A second, or accessory, olfactory system has developed for the specific task of finding a receptive mate — a task of sufficient complexity that evolution has recognized the need for an independent and dedicated system. Known as the vomeronasal system, it specializes in recognizing species-specific olfactory signals produced by one sex and perceived by the other, and which contain information not only about location but also reproductive state and availability. In addition to its role in sexual behaviours, it is important in influencing other social behaviours such as territoriality, aggression and suckling. This review will describe the recent advances that have emerged from molecular, physiological, imaging and genetic studies, and will highlight many of the remaining questions, especially as concerns the primary tasks of olfactory function: detecting, discriminating and signalling. Critical issues in areas such as development, gene regulation and higher processing, which are beyond the scope of this article, can be found in other recent reviews[2,3].

Anatomical organization

The sensory neuron

In vertebrates, the olfactory sensory neurons (OSNs) in the periphery are the primary sensing cell (Fig. 1b). Some 6–10 million of them form a neuroepithelium that lines a series of cartilaginous outcroppings, called turbinates, in the upper reaches of the nasal cavity in mammals; other vertebrates have similar specialized structures containing OSNs. The OSNs are bipolar neurons with a single dendrite that reaches up to the surface of the tissue and ends in a knob-like swelling from which project some 20–30 very fine cilia. These cilia, which actually lie in the thin layer of mucus covering the tissue, are the site of the sensory transduction apparatus. A thin axon from the proximal pole of the cell projects directly to higher brain regions (Fig. 1a,c). Invertebrates, particularly the arthropods, use a similar plan in which polarized neurons are specialized at one end for chemical detection and at the other for signalling.

Central pathways

OSNs send their axons into a region of the forebrain known as the olfactory bulb. Recent molecular-genetic studies using transgenic mice have shown that all the neurons expressing a particular receptor, no matter where they are found on the epithelial sheet, converge to a single 'target' in the olfactory bulb[4]. These targets are the glomeruli, spherical conglomerates of neuropil some 50–100 μm in diameter that consist of the incoming axons of OSNs and the dendrites of the main projection cell in the bulb, the mitral cell (Fig. 1c). It is beyond the scope of this review to consider the complex wiring of the olfactory bulb, but at its simplest

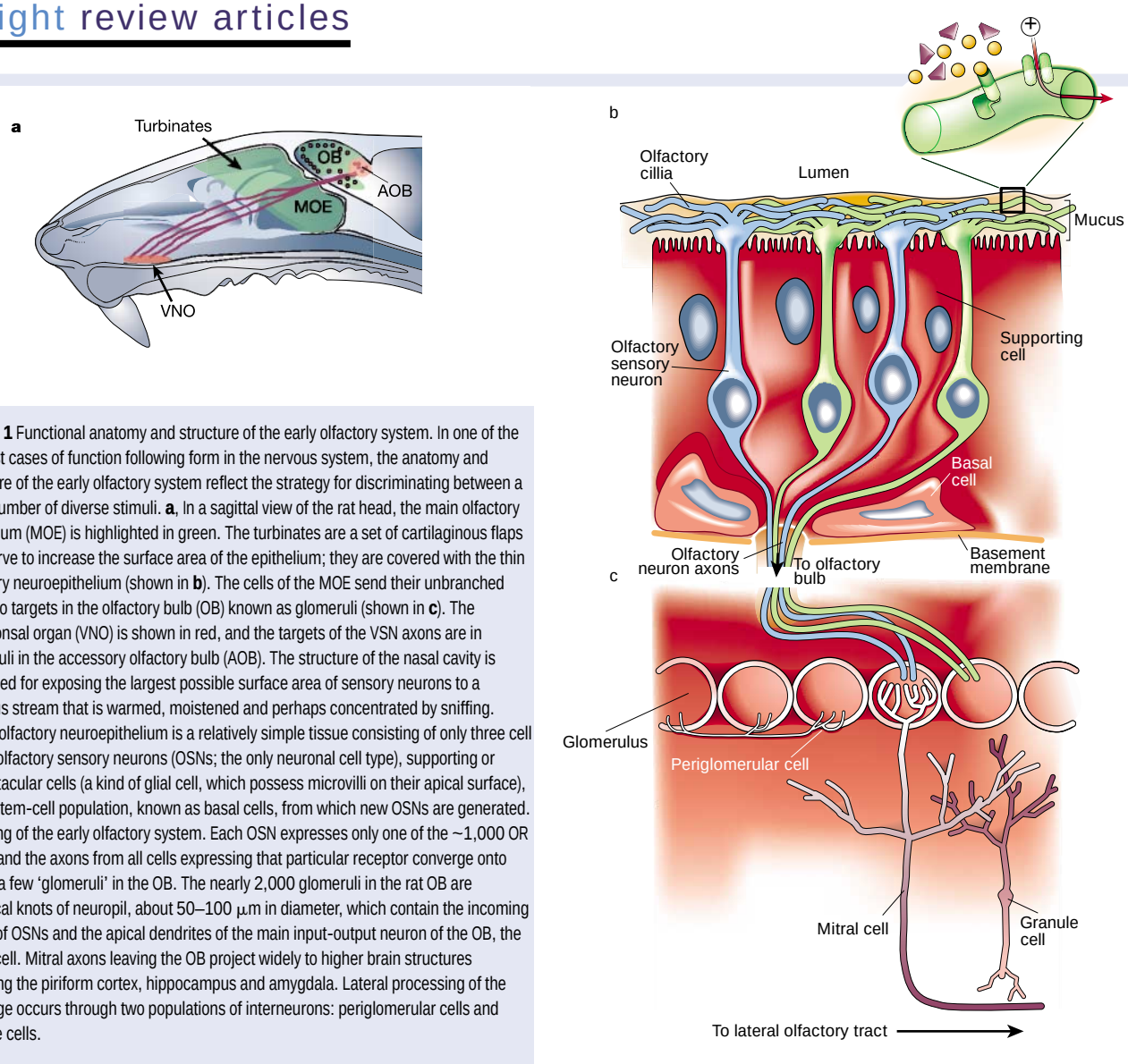


Figure 1 Functional anatomy and structure of the early olfactory system. In one of the clearest cases of function following form in the nervous system, the anatomy and structure of the early olfactory system reflect the strategy for discriminating between a large number of diverse stimuli. **a**, In a sagittal view of the rat head, the main olfactory epithelium (MOE) is highlighted in green. The turbinates are a set of cartilaginous flaps that serve to increase the surface area of the epithelium; they are covered with the thin olfactory neuroepithelium (shown in **b**). The cells of the MOE send their unbranched axons to targets in the olfactory bulb (OB) known as glomeruli (shown in **c**). The vomeronsal organ (VNO) is shown in red, and the targets of the VSN axons are in glomeruli in the accessory olfactory bulb (AOB). The structure of the nasal cavity is optimized for exposing the largest possible surface area of sensory neurons to a stimulus stream that is warmed, moistened and perhaps concentrated by sniffing. **b**, The olfactory neuroepithelium is a relatively simple tissue consisting of only three cell types: olfactory sensory neurons (OSNs; the only neuronal cell type), supporting or sustentacular cells (a kind of glial cell, which possess microvilli on their apical surface), and a stem-cell population, known as basal cells, from which new OSNs are generated. **c**, Wiring of the early olfactory system. Each OSN expresses only one of the ~1,000 OR genes and the axons from all cells expressing that particular receptor converge onto one or a few 'glomeruli' in the OB. The nearly 2,000 glomeruli in the rat OB are spherical knots of neuropil, about 50–100 μm in diameter, which contain the incoming axons of OSNs and the apical dendrites of the main input-output neuron of the OB, the mitral cell. Mitral axons leaving the OB project widely to higher brain structures including the piriform cortex, hippocampus and amygdala. Lateral processing of the message occurs through two populations of interneurons: periglomerular cells and granule cells.

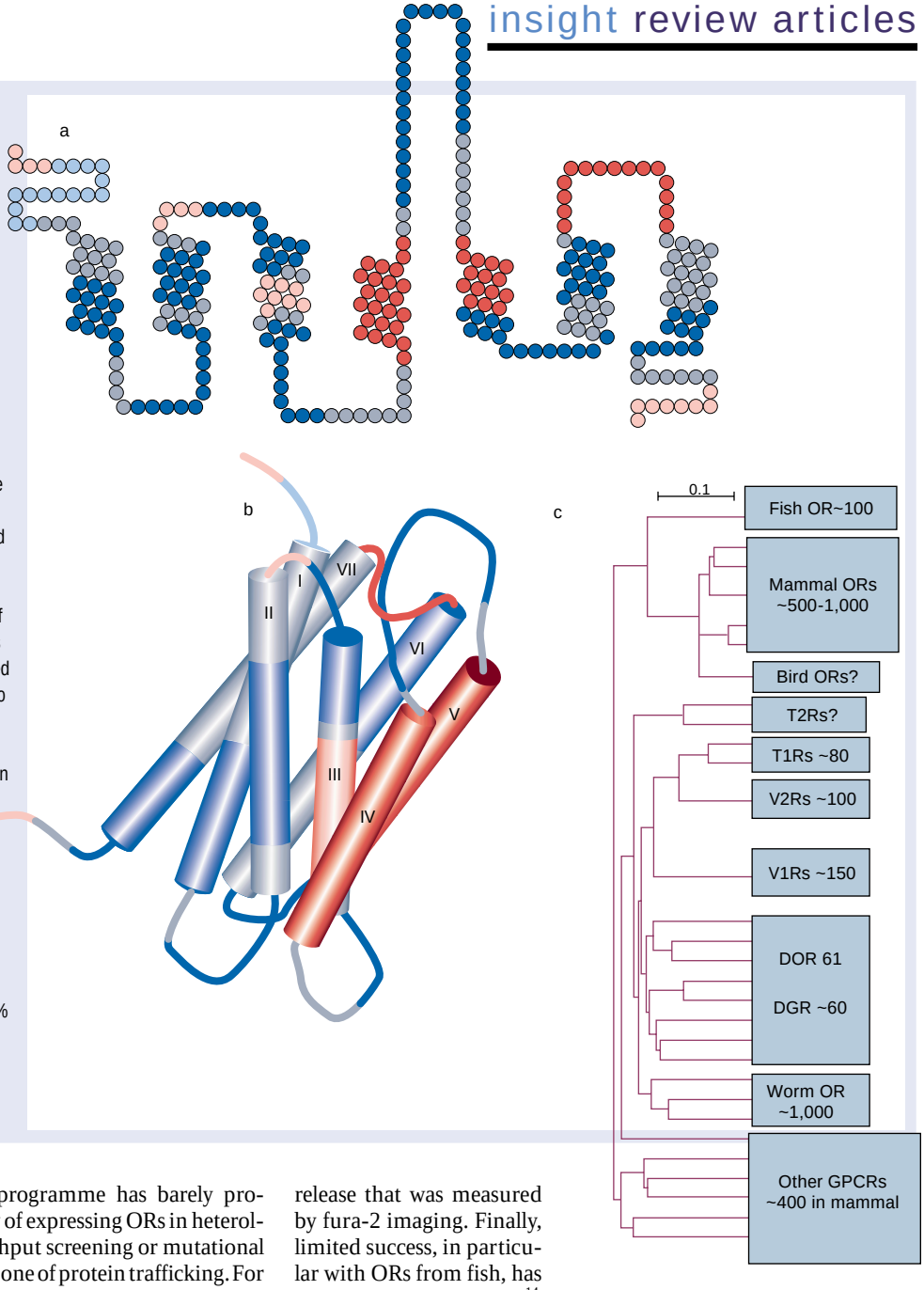
the mitral cells receive information from the OSNs, and their axons in turn project to various higher brain regions. In one of the most extreme cases of convergence in the nervous system, several thousand OSN axons synapse on to the dendrites of only 5–25 mitral cells in each glomerulus.

Odour receptors in vertebrates and mammals

The discovery and publication a decade ago of the mammalian family of odour receptors[5] produced one anticipated and one surprising result. Expected was that olfactory receptors (ORs) are GPCRs similar to those known to be important in neurotransmission, photoreception (rhodopsin is a GPCR) and many other cellular processes. Unanticipated was the astonishing finding that there are as many as 1,000 genes for ORs in the mammalian genome, making it by far the largest family of GPCRs, and probably the largest gene family in the entire genome (Fig. 2). Fish and amphibians are less well endowed with about 100 ORs, and in the human genome an unexplained 60% of OR genes seem to be pseudogenes[6].

Vertebrate odour receptors share many features with other GPCRs, including a coding region that lacks introns, a structure that predicts seven α-helical membrane-spanning domains connected by intracellular and extracellular loops of variable lengths, and numerous conserved short sequences. But there are certain characteristics specific to ORs, such as an unusually long second extracellular loop, an extra pair of conserved cysteines in that loop, and other short sequences (see a recent review by Mombaerts[3] for a detailed analysis of the receptor gene). Within the family of ORs there is a range of similarity, from less than 40% to over 90% identity. Perhaps most interesting is that there is a region of hypervariability, where the sequences show particularly strong divergence, in the third, fourth and fifth transmembrane regions. In three-dimensional models of the GPCRs, these three α-helical barrels are thought to face each other and form a pocket about one-third of the way into the membrane[7] (Fig. 2). Based on studies from other GPCRs of this class (for example adrenergic receptors[8]), and in common with the binding site of retinal in rhodopsin[9], this pocket is the probable binding site for ligands. The variability observed among the ORs in this region provides the first molecular basis for understanding the range, diversity and large number of olfactory ligands that can be detected and discriminated.

Such features make these receptors excellent models for structure–function studies in this class of receptors. In the case of a receptor known as I7, for example, the mouse and rat orthologues showed a differential response with one being more sensitive to octanal and the other to heptanal. Among the 15 amino acids that are different in the two genes, a single residue in transmembrane domain 5 (valine or isoleucine) was found to be sufficient to confer this alternate ligand sensitivity[10]. These sorts of results provide the impetus for developing a pharmacology of odour receptors that would produce activity matrices of large numbers of receptors tested against equally large chemical libraries.



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But this ambitious experimental programme has barely progressed, owing to the puzzling difficulty of expressing ORs in heterologous systems suitable for high-throughput screening or mutational analysis. The main difficulty seems to be one of protein trafficking. For unknown reasons, the OR protein, although produced in transfected cells, seems to be trapped in endoplasmic reticulum, Golgi and endosomal compartments, with little or no receptor finding its way to the membrane[11]. In one solution to this problem, a recombinant receptor was engineered into an adenovirus, which was then used to infect OSNs in rat olfactory epithelia[12]. The infected OSNs were driven to express the recombinant receptor (the I7 receptor was used in this case) so that infected epithelia produced larger physiological responses to the ligands for that receptor than to other odours. In this way the first positive identification of a family of ligands was determined for a single OR. The ability of OSNs to express cloned receptors while other cells could not is further evidence for the involvement of some olfactory-specific chaperone or co-factor necessary for functional receptor expression. What that co-factor might be remains the focus of significant research efforts.

A few other ORs have been expressed in heterologous cell systems by including additional amino acids on the amino terminal. Fusing 20 amino acids from rhodopsin[10] or a short piece of serotonin receptor[13] to the N terminal of several ORs has enabled low levels of membrane expression in HEK 293 cells. Paired with expression of the promiscuous G protein ($G_{\alpha 15}$), odours induced intracellular calcium release that was measured by fura-2 imaging. Finally, limited success, in particular with ORs from fish, has been achieved in oocytes[14]. But in general there remains no robust, reliable, efficient system for expressing and assaying ORs. The eventual solution of this problem will surely initiate a research bonanza in GPCR receptor–ligand and structure–activity research. Molecular sensing by GPCRs, so well developed in the olfactory sense, is biologically ubiquitous, and over 50% of the pharmaceuticals currently on the market or in development are targeted at these receptors.

Invertebrate odour receptors

In what is something of a reversal of the standard strategy, mammalian molecular studies have often preceded those in invertebrates. The first invertebrate family of ORs were identified in *C. elegans*, but they are unrelated to those of any other species investigated[15]. They comprise an independent super family of 500+ ORs. The nematode olfactory system seems to be built on a strategy more comparable to vertebrate taste than olfaction, with multiple receptors expressed in a few sensory neurons. Nonetheless, many important accessory molecules have been discovered in this system and future genomic and structural research seems promising.

The appearance of the *Drosophila* odour receptor (DOR) family was perhaps the most important advance in olfactory studies in the

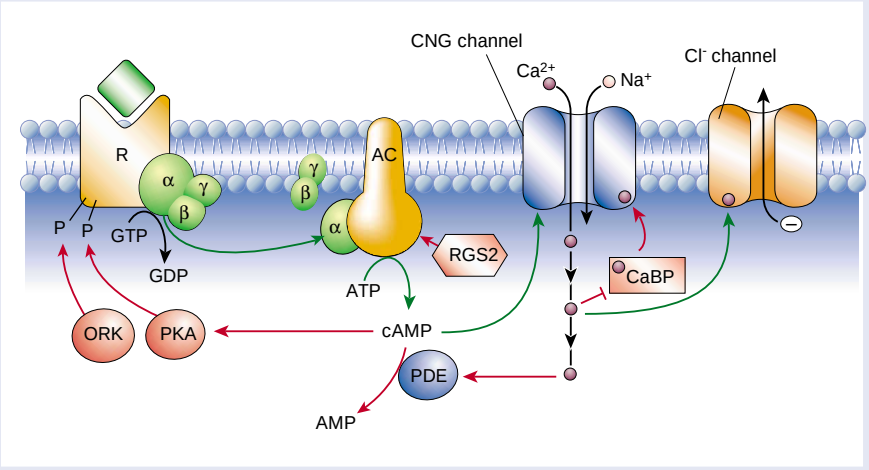


Figure 3 Sensory transduction. Within the compact cilia of the OSNs a cascade of enzymatic activity transduces the binding of an odorant molecule to a receptor into an electrical signal that can be transmitted to the brain. As described in detail in the text, this is a classic cyclic nucleotide transduction pathway in which all of the proteins involved have been identified, cloned, expressed and characterized. Additionally, many of them have been genetically deleted from strains of mice, making this one of the most investigated and best understood second-messenger pathways in the brain. AC, adenylyl cyclase; CNG channel, cyclic nucleotide-gated channel; PDE, phosphodiesterase; PKA, protein kinase A; ORK, olfactory receptor kinase; RGS, regulator of G proteins (but here acts on the AC); CaBP, calmodulin-binding protein. Green arrows indicate stimulatory pathways; red indicates inhibitory (feedback).

past five years[16,17]. These elusive receptors number at least 60 in the adult fly, with additional chemosensory receptors in the larva. They bear no homology to vertebrate ORs, and indeed have very little similarity with each other. Their classification as a family relies on a mildly conserved region in the seventh transmembrane domain and their common expression in olfactory tissues. They do share, with the vertebrate ORs, the unfortunate attribute of not expressing functionally in heterologous expression systems. Thus no DOR has been paired definitively with a cognate ligand. However, it seems that the *Drosophila* system is organized along the lines of the vertebrate system[18], with each sensory neuron expressing only a single OR (with one curious exception, a single receptor that is also expressed in nearly every OSN), and all cells expressing the same receptor contacting a single glomerulus in the antennal lobe (a structure analogous to the olfactory bulb).

Because the fly is structurally more stereotypical, it is possible to map odour sensitivity across an antenna, and there are patterns of sensitivity that suggest a tight control on how receptors are expressed. With its similarity to the vertebrate system, its numerically simpler receptor repertoire and its genetic tractability, the *Drosophila* olfactory system should be very useful for investigating crucial issues of gene regulation, axon targeting and stimulus coding. And the potential value of utilizing insect olfaction as part of an integrated pest management strategy should not be overlooked. With the fly as a model, identification of receptors in insects that have prominent roles in agriculture and public health may lead to the discovery of repellents and attractants that can alter insect behaviour, without the disagreeable side effects of neurotoxic insecticides.

Signal transduction

Once the receptor has bound an odour molecule, a cascade of events is initiated that transforms the chemical energy of binding into a neural signal (that is, a change in the membrane potential of the OSN). Although still obscure in invertebrates, this process is now generally well understood in mammals and other vertebrates (Fig. 3).

The ligand-bound receptor activates a G protein (an olfactory-specific subtype, G_{olf}), which in turn activates an adenylyl cyclase (ACIII). The cyclase converts the abundant intracellular molecule ATP into cyclic AMP, a molecule that has numerous signalling roles in cells. In the case of OSNs the cAMP binds to the intracellular face of an ion channel (a cyclic nucleotide-gated (CNG) channel closely related to that found in photoreceptors; see review in this issue by Hardie and Raghu, pages 186–193), enabling it to conduct cations such as Na^+ and Ca^{2+} (ref. 19). Inactive OSNs normally maintain a resting voltage across their plasma membrane of about –65 mV (inside with respect to outside). When the CNG channels open, the influx of Na^+ and Ca^{2+} ions causes the inside of the cell to become less negative. If enough channels are open for long enough, causing the membrane potential to become about 20 mV less negative, the cell reaches threshold and generates an action potential. The action potential is then propagated along the axon, which crosses through a thin bone known as the cribiform plate, and into the forebrain where it synapses with second-order neurons in the olfactory bulb. Genetically altered mice in which various components of this transduction cascade have been deleted (G_{olf}, ACIII and most notably the CNG channel[20–22]) indicate that the cAMP pathway is the common pathway for all OSNs; involvement of other second messengers in modulatory roles awaits conclusive proof.

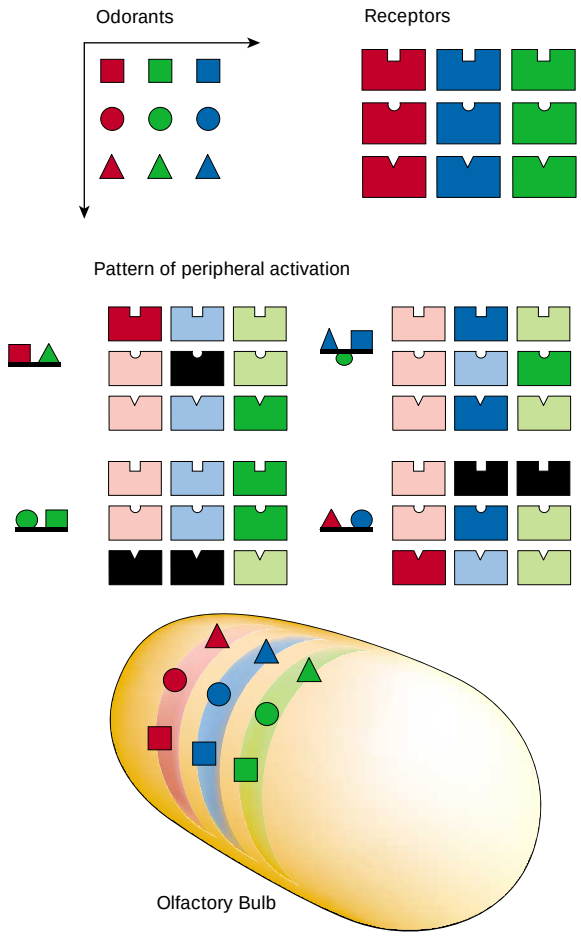
The second-messenger cascade of enzymes provides amplification and integration of odour-binding events. One membrane receptor activated by a bound odour can in turn activate tens of G proteins, each of which will activate a cyclase molecule capable of producing about a thousand molecules of cAMP per second. Three cAMP molecules are required to open a channel, but hundreds of thousands of ions can cross the membrane through a single open channel. It seems that a single odour molecule can produce a measurable electrical event in an OSN (although probably not a perceivable event in the brain) and just a few channels opening together could pass sufficient current to induce action-potential generation[23,24].

Appended to this pathway is an additional, and somewhat unique, amplification mechanism in OSNs. The calcium ions entering through the CNG channel are able to activate another ion channel that is permeable to the negatively charged chloride ion[25]. Neuronal Cl^- channels normally mediate inhibitory responses, as Cl^- ions tend to be distributed in such a way that they will enter the cell through an open channel. But OSNs maintain an unusually high intracellular Cl^- concentration (presumably by the action of a membrane pump) such that there is a Cl^- efflux when these channels are activated. Left behind is a net positive charge on the membrane that further depolarizes the cells, thus adding to the excitatory response magnitude. This is an interesting evolutionary adaptation to the fact that the olfactory cilia reside in the mucus, outside the body proper, and where the concentrations of ions are not as well regulated as they are in normal interstitial compartments[26,27]. Thus the OSN maintains its own Cl^- battery, in case the Na^+ gradient in the mucus is insufficient to support a threshold current, and uses it to boost the response.

Calcium ions entering through the CNG channels are also important in response adaptation through a negative feedback pathway involving the ion channel[28]. As intracellular calcium increases during the odour response, it acts on the channel (probably with calmodulin) to decrease its sensitivity to cAMP, thereby requiring a stronger odour stimulus to produce sufficient cAMP to open the channel[29–31]. This adaptation response is critical, as physiological recordings from OSNs indicate that they have very steep concentration–response relations (typically, responses from 10–90% of the maximum occur

Box 1

A code in the nose



Although there are some 1,000 ORs, detecting the enormous repertoire of odours requires a combinatorial strategy. Most odour molecules are recognized by more than one receptor (perhaps by dozens) and most receptors recognize several odours, probably related by chemical property. The scheme in the figure represents a current consensus model. There are numerous molecular features, two of which are represented here by colour and shape. Receptors are able to recognize different features of molecules, and a particular odour compound may also consist of a number of these 'epitopes' or 'determinants' that possess some of these features. Thus the recognition of an odorant molecule depends on which receptors are activated and to what extent, as shown by the shade of colour (black represents no colour or shape match and thus no activation). Four odour compounds are depicted with the specific array of receptors each would activate. Note that there are best receptors (for example, red square), but also other receptors that are able to recognize some feature of the molecule (for example, any square) and would participate in the discrimination of that compound. In the olfactory bulb there seem to be wide areas of sensitivity to different features (for example, functional group or molecular length). This model is based on current experimental evidence, but is likely to undergo considerable revision as more data become available.

over a stimulus concentration of about one log unit[32]). This means that the cells are particularly sensitive to small changes in concentration, but without adaptation to re-set the gain they would be able to respond only over a narrow dynamic range[33]. This is just one of several mechanisms that OSNs use for adjusting their sensitivity. Others include a recently discovered RGS (regulator of G-protein signalling) protein that apparently acts on the adenylyl cyclase to decrease its activity[34], and a kinase that phosphorylates activated receptors sending them into a desensitized state[35,36].

Signal transduction and generation in invertebrates is far less well understood, possibly because there is not a single system at work. In lobsters, a lipid pathway involving inositol phosphates seems to be dominant[37], and in *Drosophila* and the moth, there is also strong evidence for inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) as a second messenger[38]. The difficulty in these systems is that the final target of the cascade, the analogue of the CNG channel in vertebrates, remains to be identified. In contrast to vertebrates, invertebrates have both excitatory and inhibitory responses to odours, so there are likely to be multiple transduction pathways[39,40].

Tuning curves in primary sensory cells

A classical means of describing and classifying primary sensory neurons uses the concept of a 'tuning curve' — the relationship between stimulus quality and response. For photoreceptors this would be a plot showing the range of wavelengths of light at which they are activated, and for auditory receptors it would be a similar representation of frequency. But for OSNs this is a somewhat more difficult task as odours vary along multiple dimensions. To understand the rules of olfactory stimulus encoding it is useful to attempt to describe a 'molecular receptive range'[41] for OSNs.

Several approaches to this problem have been taken (Box 1). What has become clear is that most if not all receptors can be activated by multiple odours, and conversely most odours are able to activate more than one type of receptor. But this vast combinatorial strategy only underscores the importance of understanding how broadly tuned a particular OR may be. Limited physiological recordings from individual cells have produced conflicting data, probably because there are a range of tuning profiles, from specific to broad, and physiological experiments generally use necessarily limited sets of odours[42]. The standard means of characterizing the molecular range of a receptor is through a medicinal chemistry approach that seeks to define a pharmacophore, that is, the molecular determinants that are common to a set of agonists or antagonists for a given receptor.

Taking this approach, Araneda *et al.*[43] were able to provide such a characterization for at least one particular odour receptor in the rat. After screening an extensive panel of compounds they were able to determine three critical chemical features common to agonists at this receptor, and also determined that a related structural compound could serve as an antagonist, reducing the response to a known agonist. This indicates that standard pharmacology could indeed be applied profitably to the analysis of odour receptors, although large-scale screens for 1,000 ORs might be a task better suited to industrial rather than academic laboratories. It may also mean that blockers for specific malodours could be found or synthesized.

An alternative strategy would be intrinsic imaging in the olfactory bulb, which would rely on all of the OSNs expressing a particular receptor and converging onto a single glomerulus. Taking advantage of this feature, several groups[44–46] have recently used such optical imaging in the living olfactory bulb of rodents (see review by Dudai[47]). These experiments confirm that a given odour activates a set of glomeruli (that is, OSNs, and hence ORs) and that different odours activate overlapping but non-identical patterns of glomeruli (receptors). In one particularly striking case involving enantiomers (compounds of identical molecular composition that differ only in the three-dimensional arrangement of their atomic groupings) that can be discriminated behaviourally by rats, the pattern of glomerular activity induced by either stereoisomer differed by as little as a single glomerulus[48]. Such studies suggest that receptors that recognize similar odours tend to map in the same general area in the olfactory bulb, although at present this is merely a postulate as too few odours have been screened and only ~20% of the bulb can be visualized with this method.

Encoding other features of the olfactory stimulus

Olfactory thresholds measured behaviourally in animals, or psychophysically in humans, are often lower than what is seen in single-cell recordings. There may be two underlying causes for this. One is the convergence at the glomerular layer described above, allowing each mitral cell to sample a large population of identically tuned primary neurons, sending even weak messages through to the brain. But there is also a cellular source for the discrepancy. Sensitivity is usually measured psychophysically as a threshold level of stimulus at which a response occurs more often than chance. At the cellular level, sensitivity is measured as the EC_{50}, or midpoint, on a dose–response curve; that is, the concentration of odour that elicits a half-maximal response. To bring these two measures together it is helpful to recognize that OSNs cannot really measure concentration, which is something of an abstraction, but rather they operate as molecular counters, tallying each interaction between an odour molecule and a receptor. A measure of the stimulus that better captures this notion would be flux, or concentration over time. With the introduction of a temporal dimension the importance of the second messenger as an integrator, as well as amplifier, becomes apparent. The second-messenger system allows the OSN to sum or integrate many individual binding events over some period of time (which has been measured at ~1 s in salamanders, and is probably shorter in mammals).

What is the value of this? For many odours the dose–response curves in single cells have relatively elevated EC_{50} values — in the range 10–100 μM. This seems high in comparison to other GPCRs, in particular neurotransmitter receptors, but the task of the OR is different from that of a serotonin receptor. ORs are broadly tuned so as to be able to recognize a number of related but not identical molecules; this is what gives the system its tremendous range. But broadly tuned receptors cannot also have high affinities. By counting molecules and integrating over relatively long times, OSNs are able to include even low-probability binding events in generating their response. In effect the system gives up affinity for a broader receptive range, but recovers at least some of that lost sensitivity by giving up temporal resolution and using a long integration time. This seems a fair trade-off as the olfactory system is rarely called on to act quickly, as the visual or auditory systems might be.

How many odours can we detect? The literature is replete with numbers ranging from ~2,000 to more than 100,000. Theoretically it could be billions, based on the possible combinations of 1,000 receptors. In fact, the question is probably not relevant, just as it makes little sense to ask how many colours or hues we can see. Perfumers, chefs, *sommeliers* or highly trained animals are likely able to discriminate more odours than the rest of us, but this is not due to an inherent difference in equipment. Physical chemistry may be the primary limiting factor as odour chemicals must possess a certain volatility, solubility and stability to act on the nasal sensory tissue.

Another issue that has yet to be resolved concerns the effects of intensity on olfactory coding. It is often remarked that some odours change their perceived quality depending on the stimulus intensity. For example, thiols, which smell unbearably awful at high concentrations, have a sweet citrus aroma at lower concentrations. But this is far less remarkable than the fact that most odours remain constant in their quality over orders of magnitude of concentration. Amyl acetate, a pleasant fruity smelling substance, can be easily identified at concentrations from 0.1 μM to 10 mM. By monitoring activity in the olfactory bulb it is clear that as the concentration of an odour is increased, additional glomeruli are recruited into the pattern of activity, suggesting that new receptors are being activated as concentrations increase[45]. Precisely how the percept remains constant as new receptors are recruited into the response is not clear. One possibility is that there may be a class of broadly tuned low-affinity receptors that are simply intensity detectors. That is, they are activated by a large number of substances, but only at higher concentrations, so that their introduction into the pattern of activity signals only a higher concentration of whatever odour the rest of the pattern was signalling.

Pheromones and the vomeronasal system

In many mammals there is an accessory olfactory system located in a cigar-shaped organ (the vomeronasal organ or VNO) separate from the main olfactory epithelium (MOE). The VNO has been identified with the action of pheromones, molecules produced and emitted by other members of the same species. Pheromones have been implicated in mating, suckling, courtship and other behaviours and are believed to interact, through the VNO, with the endocrine system.

Two additional families of GPCRs, again unrelated to the family of ORs, have been identified in the VNO (Fig. 2), and they are differentially expressed in two segregated populations of vomeronasal sensory neurons (VSNs)[49–52]. Those located in the most apical portion of the epithelium express the G_i type of G protein, whereas those in the basal portion are G_o positive[53]. Although there is no evidence that these G proteins are involved in sensory transduction, the two families of receptors are distributed in precise coincidence with them[52,54]. Thus the G_i-positive neurons express receptors of the V1R family, whereas the V2R receptors are expressed in G_o-positive cells. V1R receptors number about 150 and are of the same general type of GPCR as the ORs (that is, they have short N termini). V2Rs on the other hand are similar to metabotropic glutamate receptors in that they have a long extracellular N-terminal region believed to be involved in ligand binding. There are estimated to be some 150 V2Rs in rodents, arrayed into several sub-families[55,56]. The organization of the vomeronasal system is somewhat different from the MOE as at least some VSNs may express more than one receptor[57]. VSNs project their axons to a caudal region of the olfactory bulb known as the accessory olfactory bulb (AOB; Fig. 1a), but VSN axons do not converge onto single glomeruli as in the main bulb[58,59]. In the AOB, sensory neurons expressing the same V1R converge on the same glomeruli, but as many as 10–30 glomeruli receive input from a given receptor (as opposed to the 1–3 in the main bulb).

With the exception of one pseudogene, none of the VRs seem to be expressed differentially in males or females[50,52], indicating that sexually divergent responses to pheromones are the result of higher brain function, and that both sexes can detect all pheromones. Indeed, it may be important for a female to know that another receptive female is in the area, even though her response will be quite different from that of a nearby male.

The transduction mechanism in VSNs is not yet known, but recent physiological and biochemical evidence implicates phospholipase C and a lipid pathway possibly including $Ins(1,4,5)P_3$, diacyl glycerol, calcium release and perhaps the involvement of a transient receptor potential (TRP)-like calcium channel, similar to that identified in *Drosophila* phototransduction (see refs 54, 60, 61, and review this issue by Hardie and Raghu, pages 186–193). One difficulty in

obtaining these data is the lack of well characterized stimuli. Pheromones are typically a minor component of excreted fluids such as urine or sweat, and are therefore difficult to identify, purify and obtain in quantity. In at least one recent physiological study, less than 0.5% of VSNs responded to any one of a group of six putative mouse pheromones[61]. But with over 200 receptors expressed in the VNO, other substances besides pheromones might also be stimuli. Using calcium imaging to observe responses, Buck and colleagues measured VSN responses to 18 out of a panel of 82 common odours[62]. Perhaps compounds associated with the fluids containing pheromones are also sensed by this accessory olfactory system. Structurally the V2R class of receptors are candidates for binding amino acids or small peptides, as does the related mGluR. In fish, an OR similar to the V2R family was shown to bind the amino acid arginine with a high affinity[14], although similar responses have not yet been shown in VSNs.

Unlike the MOE, responses of VSNs to pheromones have been observed at concentrations as low as 0.1 nM, and even at higher concentrations VSNs seem to remain highly specific for particular compounds[61]. This suggests that these high-affinity pathways do not use a combinatorial mechanism for sensory coding.

Are humans sensitive to pheromones? The VNO in humans is vestigial, disappearing before birth. In the human genome all putative members of the VR family are pseudogenes, with one exception. A single V1R gene was found to be intact and complementary DNA for this gene was recovered from 11 individuals of varying ethnic background. No ligand is known for this receptor nor is it known where it is expressed[63]. There are various behavioural studies that implicate putative pheromones in regulating endocrine-dependent behaviours such as menstruation, but the precise site of action is unknown. For an excellent recent review of the current data on this issue, see ref. 64.

Summary and future directions

All cells, no matter what their principal function might be, need to sense and respond to important molecules in their environment. But in the olfactory system, molecular sensing is the primary occupation, and is most highly developed. During the past decade the olfactory system has emerged from relative obscurity as an idiosyncratic sensory system to its current place of interest as a model system for molecular detection. With a now firm foundation of molecular, physiological and chemical data, we can turn to the next generation of issues.

For many questions in olfaction the tools are also the puzzles. The large receptor repertoires will tell us much about GPCR structure–function relations, but how these receptors are controlled and how their expression is regulated remains mysterious. The enormous selection of ligands provides a varied chemical catalogue of agonists and antagonists, but the variety and range are bewildering and frustrate attempts at easy pharmacological-like classification. The gene families of 'genome project species' (for example, the nematode, fly, mouse and human) provide complete sets of OR sequences, but the challenge will be to integrate this with developmental and behavioural data. The organization of the sensory neuron inputs to the olfactory bulb is perhaps the first neural circuit to be described by molecular-genetic techniques, but the 'meaning' of the stimulus will not be clear until we have a better idea of what subsequent physiological processing occurs in the bulb and in the higher cortical centres.

The olfactory system is clearly ripe with opportunities for the imaginative investigator. While a sound footing has been provided by molecular, physiological, genetic, developmental and computational work over the past decade, this has all been as a prelude to addressing some the most compelling problems in neuroscience, among them that fundamental human question: How do I smell? □

1. Hildebrand, J. G. & Shepherd, G. M. Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annu. Rev. Neurosci.* **20,** 595–631 (1997).
2. Buck, L. B. The molecular architecture of odor and pheromone sensing in mammals. *Cell* **100,** 611–618 (2000).
3. Mombaerts, P. Seven-transmembrane proteins as odorant and chemosensory receptors. *Science* **286,** 707–711 (1999).
4. Mombaerts, P. *et al.* Visualizing an olfactory sensory map. *Cell* **87,** 675–686 (1996).
5. Buck, L. & Axel, R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **65,** 175–187 (1991).
6. Sosinsky, A., Glusman, G. & Lancet, D. The genomic structure of human olfactory receptor genes. *Genomics* **70,** 49–61 (2000).
7. Pilpel, Y. & Lancet, D. The variable and conserved interfaces of modeled olfactory receptor proteins. *Protein Sci.* **8,** 969–977 (1999).
8. Kobilka, B. K. *et al.* Chimeric α2-,β2-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity. *Science* **240,** 1310–1316 (1988).
9. Palczewski, K. *et al.* Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* **289,** 739–745 (2000).
10. Krautwurst, D., Yau, K. W. & Reed, R. R. Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell* **95,** 917–926 (1998).
11. Gimelbrant, A. A., Haley, S. L. & McClintock, T. S. Olfactory receptor trafficking involves conserved regulatory steps. *J. Biol. Chem.* **1,** 1 (2000).
12. Zhao, H. *et al.* Functional expression of a mammalian odorant receptor. *Science* **279,** 237–242 (1998).
13. Wetzel, C. H. *et al.* Specificity and sensitivity of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and *Xenopus laevis* oocytes. *J. Neurosci.* **19,** 7426–7433 (1999).
14. Speca, D. J. *et al.* Functional identification of a goldfish odorant receptor. *Neuron* **23,** 487–498 (1999).
15. Sengupta, P., Chou, J. H. & Bargmann, C. I. *odr-10* encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. *Cell* **84,** 899–909 (1996).
16. Clyne, P. J. *et al.* A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in *Drosophila*. *Neuron* **22,** 327–338 (1999).
17. Vosshall, L. B., Amrein, H., Morozov, P. S., Rzhetsky, A. & Axel, R. A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96,** 725–736 (1999).
18. Vosshall, L. B., Wong, A. M. & Axel, R. An olfactory sensory map in the fly brain. *Cell* **102,** 147–159 (2000).
19. Firestein, S., Darrow, B. & Shepherd, G. M. Activation of the sensory current in salamander olfactory receptor neurons depends on a G protein-mediated cAMP second messenger system. *Neuron* **6,** 825–835 (1991).
20. Wong, S. T. *et al.* Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. *Neuron* **27,** 487–497 (2000).
21. Belluscio, L., Gold, G. H., Nemes, A. & Axel, R. Mice deficient in G_{olf} are anosmic. *Neuron* **20,** 69–81 (1998).
22. Brunet, L. J., Gold, G. H. & Ngai, J. General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron* **17,** 681–693 (1996).
23. Lynch, J. W. & Barry, P. H. Action potentials initiated by single channels opening in a small neuron (rat olfactory receptor). *Biophys J.* **55,** 755–768 (1989).
24. Menini, A., Picco, C. & Firestein, S. Quantal-like current fluctuations induced by odorants in olfactory receptor cells. *Nature* **373,** 435–437 (1995).
25. Kleene, S. J. & Gesteland, R. C. Calcium-activated chloride conductance in frog olfactory cilia. *J. Neurosci.* **11,** 3624–3629 (1991).
26. Lowe, G. & Gold, G. H. Nonlinear amplification by calcium-dependent chloride channels in olfactory receptor cells. *Nature* **366,** 283–286 (1993).
27. Kurahashi, T. & Yau, K. W. Co-existence of cationic and chloride components in odorant-induced current of vertebrate olfactory receptor cells. *Nature* **363,** 71–74 (1993).
28. Kurahashi, T. & Menini, A. Mechanism of odorant adaptation in the olfactory receptor cell. *Nature* **385,** 725–729 (1997).
29. Chen, T. Y. & Yau, K. W. Direct modulation by Ca^{2+}-calmodulin of cyclic nucleotide-activated channel of rat olfactory receptor neurons. *Nature* **368,** 545–548 (1994).
30. Liu, M., Chen, T. Y., Ahamed, B., Li, J. & Yau, K. W. Calcium-calmodulin modulation of the olfactory cyclic nucleotide-gated cation channel. *Science* **266,** 1348–1354 (1994).
31. Kramer, R. H. & Siegelbaum, S. A. Intracellular Ca^{2+} regulates the sensitivity of cyclic nucleotide-gated channels in olfactory receptor neurons. *Neuron* **9,** 897–906 (1992).
32. Firestein, S., Picco, C. & Menini, A. The relation between stimulus and response in olfactory receptor cells of the tiger salamander. *J. Physiol.* **468,** 1–10 (1993).
33. Reisert, J. & Matthews, H. R. Adaptation-induced changes in sensitivity in frog olfactory receptor cells. *Chem. Senses* **25,** 483–486 (2000).
34. Sinnarajah, S. *et al.* RGS2 regulates signal transduction in olfactory neurons by attenuating activation of adenylyl cyclase III. *Nature* **409,** 1051–1055 (2001).
35. Dawson, T. M. *et al.* Beta-adrenergic receptor kinase-2 and beta-arrestin-2 as mediators of odorant-induced desensitization. *Science* **259,** 825–829 (1993).
36. Schleicher, S., Boekhoff, I., Arriza, J., Lefkowitz, R. J. & Breer, H. A β-adrenergic receptor kinase-like enzyme is involved in olfactory signal termination. *Proc. Natl Acad. Sci. USA* **90,** 1420–1424 (1993).
37. Fadool, D. A. & Ache, B. W. Plasma membrane inositol 1,4,5-trisphosphate-activated channels mediate signal transduction in lobster olfactory receptor neurons. *Neuron* **9,** 907–918 (1992).
38. Krieger, J., Mameli, M. & Breer, H. Elements of the olfactory signaling pathways in insect antennae. *Invert. Neurosci.* **3,** 137–144 (1997).
39. Boekhoff, I., Michel, W. C., Breer, H. & Ache, B. W. Single odors differentially stimulate dual second messenger pathways in lobster olfactory receptor cells. *J. Neurosci.* **14,** 3304–3309 (1994).
40. Ache, B. W. & Zhainazarov, A. Dual second-messenger pathways in olfactory transduction. *Curr. Opin. Neurobiol.* **5,** 461–466 (1995).
41. Mori, K. & Yoshihara, Y. Molecular recognition and olfactory processing in the mammalian olfactory system. *Prog. Neurobiol.* **45,** 585–619 (1995).
42. Duchamp-Viret, P., Chaput, M. A. & Duchamp, A. Odor response properties of rat olfactory receptor neurons. *Science* **284,** 2171–2174 (1999).
43. Araneda, R. C., Kini, A. D. & Firestein, S. The molecular receptive range of an odorant receptor. *Nature Neurosci.* **3,** 1248–1255 (2000).
44. Meister, M. & Bonhoeffer, T. Tuning and topography in an odor map on the rat olfactory bulb. *J. Neurosci.* **21,** 1351–1360 (2001).
45. Rubin, B. D. & Katz, L. C. Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron* **23,** 499–511 (1999).
46. Uchida, N., Takahashi, Y. K., Tanifuji, M. & Mori, K. Odor maps in the mammalian olfactory bulb:

domain organization and odorant structural features. *Nature Neurosci.* **3,** 1035–1043 (2000).
47. Dudai, Y. The smell of representations. *Neuron* **23,** 633–635 (1999).
48. Rubin, B. D. & Katz, L. C. Spatial coding of enantiomers in the rat olfactory bulb. *Nature Neurosci.* **4,** 355–356 (2001).
49. Dulac, C. & Axel, R. A novel family of genes encoding putative pheromone receptors in mammals. *Cell* **83,** 195–206 (1995).
50. Matsunami, H. & Buck, L. B. A multigene family encoding a diverse array of putative pheromone receptors in mammals. *Cell* **90,** 775–784 (1997).
51. Ryba, N. J. & Tirindelli, R. A new multigene family of putative pheromone receptors. *Neuron* **19,** 371–379 (1997).
52. Herrada, G. & Dulac, C. A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. *Cell* **90,** 763–773 (1997).
53. Jia, C. & Halpern, M. Subclasses of vomeronasal receptor neurons: differential expression of G proteins ($G_{i\alpha 2}$ and $G_{o\alpha}$) and segregated projections to the accessory olfactory bulb. *Brain Res.* **719,** 117–128 (1996).
54. Krieger, J. *et al.* Selective activation of G protein subtypes in the vomeronasal organ upon stimulation with urine-derived compounds. *J. Biol. Chem.* **274,** 4655–4662 (1999).
55. Del Punta, K., Rothman, A., Rodriguez, I. & Mombaerts, P. Sequence diversity and genomic organization of vomeronasal receptor genes in the mouse. *Genome Res.* **10,** 1958–1967 (2000).
56. Pantages, E. & Dulac, C. A novel family of candidate pheromone receptors in mammals. *Neuron* **28,** 835–845 (2000).
57. Martini, S., Silvotti, L., Shirazi, A., Ryba, N. J. & Tirindelli, R. Co-expression of putative pheromone receptors in the sensory neurons of the vomeronasal organ. *J. Neurosci.* **21,** 843–848 (2001).
58. Rodriguez, I., Feinstein, P. & Mombaerts, P. Variable patterns of axonal projections of sensory neurons in the mouse vomeronasal system. *Cell* **97,** 199–208 (1999).
59. Belluscio, L., Koentges, G., Axel, R. & Dulac, C. A map of pheromone receptor activation in the mammalian brain. *Cell* **97,** 209–220 (1999).
60. Holy, T. E., Dulac, C. & Meister, M. Responses of vomeronasal neurons to natural stimuli. *Science* **289,** 1569–1572 (2000).
61. Leinders-Zufall, T. *et al.* Ultrasensitive pheromone detection by mammalian vomeronasal neurons. *Nature* **405,** 792–796 (2000).
62. Sam, M. *et al.* Odorants may arouse instinctive behaviours. *Nature* **412,** 142 (2001).
63. Rodriguez, I., Greer, C. A., Mok, M. Y. & Mombaerts, P. A putative pheromone receptor gene expressed in human olfactory mucosa. *Nature Genet.* **26,** 18–19 (2000).
64. Meredith, M. Human vomeronasal organ function: a critical review of best and worst cases. *Chem. Senses* **26,** 433–445 (2001).

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