What is so special about smell? Olfaction as a model system in neurobiology

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Received 12 January 2015
Revised 28 August 2015
Accepted 5 October 2015

ABSTRACT

Neurobiology studies mechanisms of cell signalling. A key question is how cells recognise specific signals. In this context, olfaction has become an important experimental system over the past 25 years. The olfactory system responds to an array of structurally diverse stimuli. The discovery of the olfactory receptors (ORs), recognising these stimuli, established the olfactory pathway as part of a greater group of signalling mechanisms mediated by G-protein-coupled receptors (GPCRs). GPCRs are the largest protein family in the mammalian genome and involved in numerous fundamental physiological processes. The OR family exhibits two characteristics that make them an excellent model system to understand GPCRs: its size and the structural diversity of its members. Research on the OR binding site investigates what amino acid sequences determine the receptor-binding capacity. This promises a better understanding of how the basic genetic makeup of GPCRs relates to their diversification in ligand-binding capacities.

INTRODUCTION

The objective of this review is to discuss current interest in olfaction as a model system in neurobiology. Focus lies on the olfactory receptors (ORs) and their identification as G-protein-coupled receptors (GPCRs), which constitute the largest protein family in the mammalian genome. GPCRs regulate fundamental physiological processes, and they are one of the main targets of drug design studies. In particular, ORs exhibit genetic features that make them an excellent experimental target for studying GPCR ligand binding: while ORs exhibit high intraclass diversity of amino acid sequences in their binding domains, they also share high interclass similarity with other GPCRs. While the intraclass diversity will provide explanations of how ORs respond to a vast variety of chemical stimuli, the interclass similarity with other GPCRs may allow for the extrapolation of these explanations to other ligand-binding mechanisms. However, the details of the molecular mechanism by which ORs recognise chemical stimuli are still in debate. A pending issue is whether conformational changes in the odorant-binding process are 'induced' or 'selected' or both.

CONTEMPORARY INTEREST IN OLFACTION

Olfaction has become an important experimental system over the past 25 years. The discovery of the ORs in 1991 linked olfaction to a wide range of studies in neurobiology.[1 2] Before its integration into mainstream research, olfaction was considered a marginal, even eccentric research topic.

The sense of smell, traditionally, has received little attention either in the sciences or in the humanities. Once even called 'the most ungrateful sense of them all' by the philosopher Immanuel Kant,[3] its wider relevance to understanding human perception and cognition appeared questionable. This view still permeates popular opinion. Nonetheless, recent developments in the biological and social sciences are starting to change this marginalisation,[4 5 6 7 8] and olfaction is taking a more central role in sensory research and emerging as an important topic in neuroscience.

One of the key reasons for its marginalisation was the difficulty of conducting research into olfaction. For almost the entire 20th century any hypothesis about the molecular basis of odours remained speculative. A large number of structural hypotheses were put forward, involving steric, electrophilic and nucleophilic characteristics, peripheral functional groups and even infrared frequencies.[9] The odour of a molecule, unlike its geometric or electronic properties, is not an intrinsic property but relates to a particular mechanism of primary odour recognition. It is a sensory response that takes place when volatile molecules stimulate the appropriate receptors in the nasal epithelium. Thus, the importance of the OR discovery for research on olfactory processing cannot be overstated.[i]

Olfactory receptors are G-protein-coupled receptors

When Linda Buck and Richard Axel discovered the mammalian OR genes, olfactory research became affiliated with a wide range of cell signalling studies. In fact, the identification of ORs as GPCRs catapulted olfaction from obscurity into core neurobiological research. GPCRs are the largest protein family in the mammalian genome; they are cell surface receptors that pass the membrane seven times (thus also called 7-transmembrane receptors). The best understood GPCRs today are rhodopsin and β-adrenergic receptors.[12]

The most intriguing feature of GPCRs is their capacity to bind an astonishingly diverse array of stimuli while sharing significant genetic similarities. GPCRs bind diverse ligands such as neurotransmitters, hormones, amino acids, lipids, sugars, airborne molecules, proteins and photons. They also share a strikingly large amount of conserved amino acid sequences throughout the evolution of different taxa. The overall genetic similarity, for instance,

[i]Buck and Axel received the 2004 Nobel Prize in Physiology or Medicine for their discovery.[10 11]

To cite: Barwich A-S. *Postgrad Med J* Published Online First: [*please include* Day Month Year] doi:10.1136/postgradmedj-2015-133249

between rhodopsin and ORs is up to 40%.[13] The strong genetic family ties of GPCRs allow for the development of general models to investigate their evolutionary development, folding behaviour and ligand-binding mechanism. The latter is of particular relevance to modelling signalling proteins: if the binding capacity of a receptor is determined by the amino acid sequences of its binding site, how has this genetically closely related family of signalling proteins evolved to recognise so many different ligands?

In this context, the OR family exhibits two characteristics that make them an excellent model system for understanding the complexities of ligand binding in GPCRs: its size and the structural diversity of its members.

ORs detect a vast amount of structurally diverse odorous molecules (odorants). A recent study estimated the number of discriminable stimuli to be up to one trillion,[14] though this estimation remains in dispute.[15] Agreement exists that the olfactory system exhibits a high degree of sensitivity towards a wide range of structurally diverse stimuli, and so it was assumed that there must be an appropriate range of different receptor types corresponding to this diversity. Nonetheless, their numbers exceeded the estimated 10–15 ORs by a factor of 100, and there are currently >1000 known ORs (in comparison, we only have 3 receptors for colour and 15 known serotonin receptors). Members of the OR family represent 3–4% of the mammalian genome (an even greater amount than the undoubtedly important immune system).[16]

Another finding was the structural diversity of amino acid sequences of the transmembrane-binding domains of ORs. Such diversity allows for wide-ranging responses of ORs to chemical stimuli, signifying ORs as an excellent model system for GPCR studies: "The conserved and variable regions in the ORs make them a particularly attractive experimental system for understanding GPCR ligand binding. Receptors range from 40%–98% amino acid identity. It is as if evolution has done the perfect mutagenesis experiment and selected the mutations that work."[2] A better in-depth understanding of structure–function relations in ligand binding is of particular relevance to studies of drug design, given the lack of regularities between ligand structure and its effects on the targeted receptor site.

Experimental difficulties in receptor modelling

GPCRs are known to be difficult to study,[ii] and ORs are no different. In particular, ORs present a difficult case for protein modelling. Standard methods, such as heterologous expression and X-ray crystallography, are not easily applied to ORs. Models of odour receptor sites thus were commonly based on other GPCRs through homology modelling. Nonetheless, recent studies obtained first successes in analysing OR binding through standard methods such as heterologous expression.[18]

Despite such difficulties, a number of insights into the ligand-binding range of ORs have been made. These include studies on the combinatorial nature of odorant detection.[19 20] (Combinatorial means that an odorant is recognised by multiple receptor sites, and one receptor responds to several odorants.) Other studies tested the binding range of a single mammalian OR to a range of stimuli.[21]

[ii]Indeed, only very few breakthroughs in elucidating *active* (ligand-bound) conformations of GPCR-binding sites have been made. One recent example is Brian Kobilka's work on rhodopsin, obtaining the first crystal structure of an activated tertiary complex.[17]

Many of the insights into OR behaviour were through the use of functional equivalency. While the binding site of the ORs remains largely experimentally inaccessible, the 'functionally equivalent' olfactory sensory nerves (OSNs) allow the activity of ORs to be investigated and act experimentally as ORs. As OSNs only express one type of OR gene,[19 22] the functional equivalence of ORs and OSNs makes it possible to trace how detection signals are further represented as activation patterns in the brain, for example, by using transgenetic markers.[23]

Contemporary olfactory research thus focuses on two main questions: first, how do the receptors recognise odorous molecules? And, second, how is this information processed at various stages in the brain? In the following sections, developments in the first of these two research questions will be examined in more detail. Concerning studies on higher-level brain processing in olfaction, which exceeds the scope of this review, see refs.[10 24]

RECEPTORS AND LIGANDS: THE MECHANISM OF PRIMARY ODOUR RECOGNITION

The olfactory system is based on a three-level pathway. Olfactory perception first starts with the interaction of odorants with the appropriate ORs in the nasal epithelium (figure 1). This initiates a cascade of reactions underlying the second messenger pathway.[1 4] These impulses are transmitted through the OSNs to the brain. OSNs expressing one receptor gene join in a spherical neural structure (glomerulus) in the olfactory bulb at the frontal lobe, forming topographic odour activation patterns.[25 26] Signals from the bulb are projected to the olfactory cortex, whose organisation principles are still a matter of considerable debate.[24 27]

How odorants interact with ORs is known in principle but not in detail. As GPCRs ORs belong to an evolutionary closely related protein family that is assumed to share a common ligand-binding mechanism. Primary odour recognition, like other ligand-binding processes, works according to a shape-sensitive mechanism. Odorants bind to receptors based on complementary features, where the complementary fit of a molecule with a receptor involves various parameters. It is primarily determined by the stereochemical features of different molecular groups of an odorant. In addition to steric interactions, complementary fit is determined by various other parameters such as hydrophobicity, molecular weight, polarity, acidity or basicity.[28] Nonetheless, how the molecule and receptor change shape within these interactions is less well understood.

A shape-sensitive mechanism: models of conformational changes

Neither odorants nor ORs are rigid compounds. In the event of binding, odorants can abandon their preferred minimum energy conformation to adopt a different spatial configuration. Receptors, too, change their configurations. The crucial question is whether they adopt different configurations mainly before or during the event of ligand binding, and to which extent.

Two schools of thought currently attempt to describe the general nature of the conformational changes in ligand binding. The difference determines the primary causal agent in ligand-binding mechanisms such as primary odour detection. Do ligands cause changes in the configuration of a receptor? Or do receptors adopt different states in the absence of a ligand, with the ligand binding to and stabilising a preferred active state of the receptor?

These two different interactions of ligand binding are known as the 'induced fit' model by David Koshland, George Nemethy, and David Filmer (1958)[29] and the 'selected fit' model by

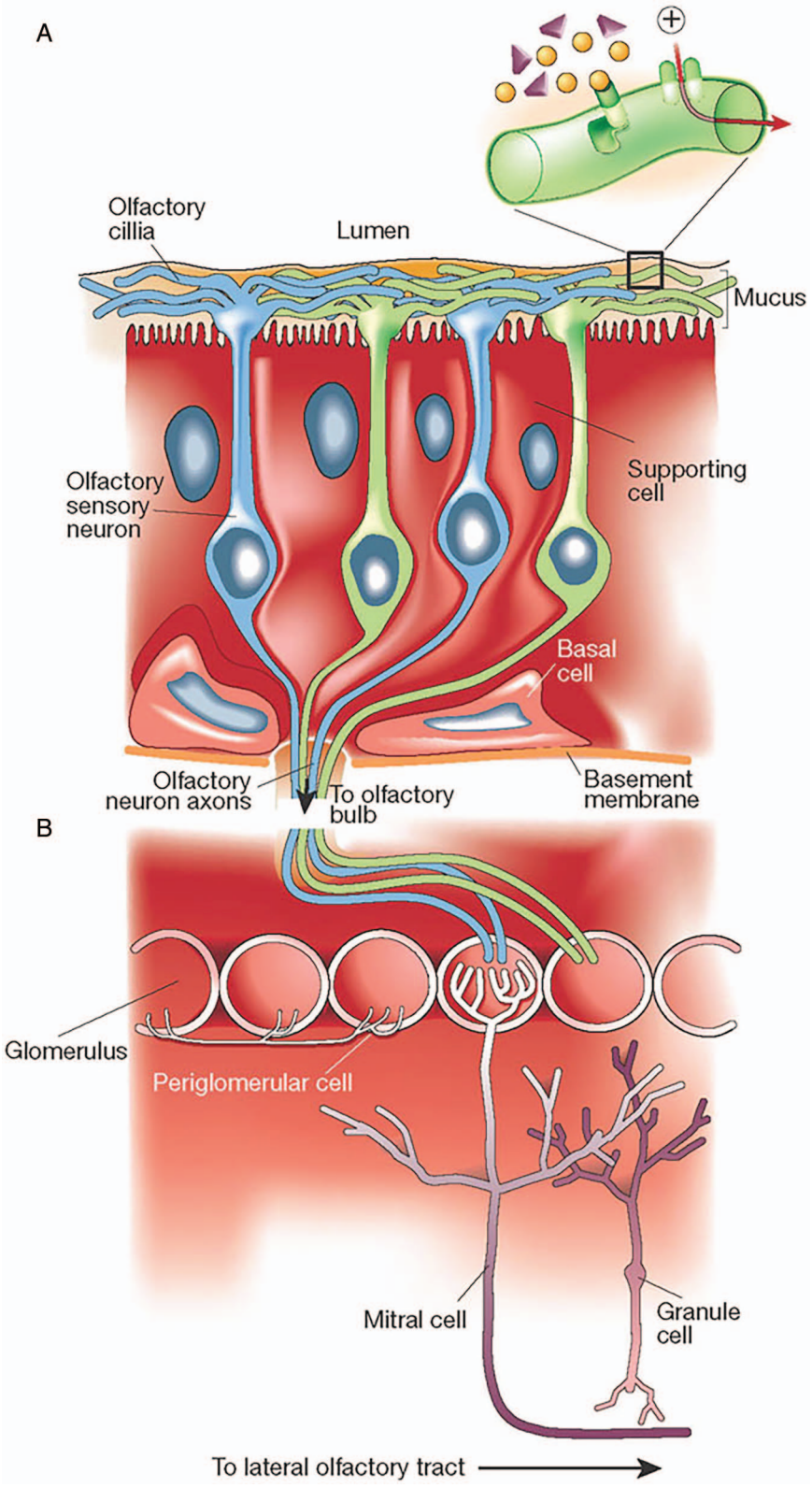

Figure 1 The olfactory pathway (image from[4]). Odorants are recognised by receptor proteins at the cilia (endings of the olfactory sensory nerves in the nasal epithelium). The signal is transmitted to the olfactory bulb, where olfactory neurons expressing the same receptor genes converge in glomeruli. From the bulb, the signal is further projected to the olfactory cortex.

Jacques Monod, Francois Jacob, and Jean-Pierre Changeux (1965).[30] Both models emphasise the dynamic nature of proteins, yet differ in temporal and causal sequences by which the changes in receptor conformations are initiated in the course of ligand binding (figure 2).[31 32]

The induced fit model assumes that the receptor adopts, or is forced to adopt, a confirmation that is best suited to a particular ligand. The selected fit model assumes that receptors pass through several conformations and ligands have a specific affinity for a particular active receptor state.

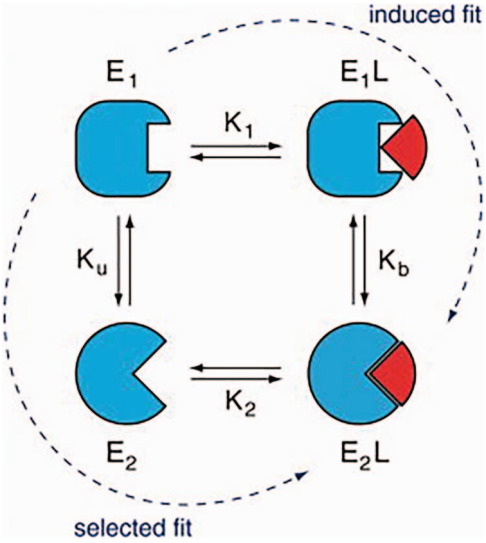

Figure 2 Models of induced fit and selected (conformational) fit (image from[32]). Induced fit interactions force the receptor to adopt different conformations during ligand binding. Depending on ligand and receptor rigidity, this interaction can be reciprocal (ligands may also undergo conformational changes). In selected fit interactions, receptors change states independently of the presence of a ligand. When a ligand (with an affinity for a particular receptor state) binds, it stabilises a particular active receptor state and changes the frequency with which a protein adopts its different energy states.

Interestingly, current data remain inconclusive on which model to prefer or whether ligand binding may involve a combination of both, plus potentially other, mechanisms throughout the subsequent stages of the binding process.[33 34 35]

Overall, current models of conformational changes differ from previous, rigid understanding of lock-and-key interactions in ligand binding. In comparison to lock-and-key, receptor behaviour in conformational change models is seen as explanatorily more central than explanations derived from ligand structure. ORs can detect more rigid odorants through stereochemical features in an odorant's minimum energy state, but they can also detect more flexible odorants by 'deforming' them within the receptor-binding site (eg, by 'bending' odorants with a chain structure into ring conformations).[36]

The strong evolutionary bond of GPCRs heightened scientific interest in olfaction as a modern model system. In addition to modelling practices, understanding the type of mechanism involved in ligand binding also has interesting evolutionary implications. Some studies "suggest that, in the course of evolution, some mechanisms of conformational change have been selected that are shared by entire groups of proteins and that these occur independently of the diversity of regulatory ligands involved."[35] Basically, to what extent have certain recognition mechanisms evolved (in)dependently of present ligand structures? So-called "orphan receptors", that is, receptors to which no ligand is currently known to bind and to some of which no known ligand potentially binds, provide an obvious example for the relevance and research perspectives of such questions.[37 38]

A vibration-sensitive mechanism and its criticism

In contrast to this evolutionary understanding of signalling proteins and their mechanism, an alternative model for odour detection was proposed by Luca Turin in 1996.[39] This alternative mechanism refers to intramolecular vibrations in the infrared range as the key feature underlying odour detection (figure 3). In this model, electrons travel through an odorant across the OR-binding site by means of inelastic electron tunnelling spectroscopy (IETS), meaning electrons jump small gaps between a donor (nicotinamide adenine dinucleotide phosphate) and an acceptor site (zinc).

Turin's account is a revival of earlier 'vibration theories of odours' proposed by Malcolm Dyson in the 1920s–1930s[40] and Robert Wright in the 1960s–1970s.[41] It has received wider attention in popular science and media outlets in recent years.[42 43] The highly speculative nature of the vibration model has not found greater resonance in mainstream research.[44] Its popularised portrayal has incurred discontent among some researchers who found the depiction of standard olfactory research as still sticking to the old 'lock-and-key model' misleading.[45]

Criticism directed at the vibration mechanism is twofold. First, suggested evidence in its favour is considered far too anecdotal. Turin's model largely draws support from selected examples of structure–odour relations (presenting molecules of similar smell with a similar vibrational spectrum) and sensory performance studies (testing the detection of odour differences between isotopic variants).[46 47] One inference from the IETS model is that odorants identical in stereochemical composition that differ in molecular vibrations (such as isotopic variants) should smell different. While studies on insect behaviour, using drosophila, indeed have shown a higher sensitivity towards different isotopic versions,[48] human sensory performance tests are more difficult and have yielded conflicting or ambiguous results at best.[49 50]

Second, the vibration model yet lacks experimental links to receptor studies. Nothing comparable to the proposed IETS mechanism has been encountered in any other protein interaction. Support for a vibrational detection mechanism needs either proof from general GPCR studies or sufficient experimental explanation as to why ORs should act fundamentally

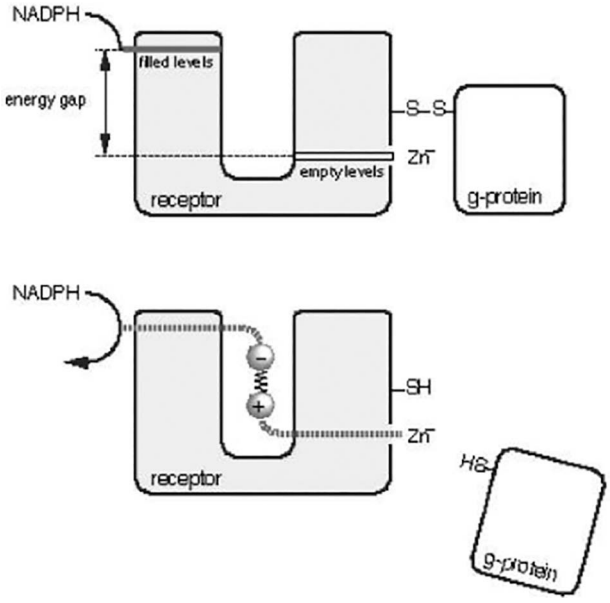

Figure 3 Controversial model of a vibration-sensing mechanism (image from[39]). Electrons travel through an odorant across the OR-binding site by inelastic electron tunnelling; nicotinamide adenine dinucleotide phosphate (NADPH) acts as electron donor and Zink as electron acceptor site.

different to other GPCRs.[51] A very recent multilab and multi-method study set out to challenge the vibrational hypothesis about the detection of isotopic versions on the receptor level. Block *et al*[52] tested the response of OR5AN1, a receptor responsive to cyclopentadecanone (a sensory performance study on humans with positive results for the IETS mechanism was conducted with cyclopentadecanone[50]). OR5AN1 responded equally well to cyclopentadecanone and other musk variants, providing a negative result for the vibrational hypothesis about the differential detection of isotopic versions. Nine additional receptors were tested for other isotopic isomers with the same negative outcome. These results were criticised by Turin *et al*[53] for applying to an in vitro not in vivo environment.

In commenting on Block *et al*'s study, Leslie Vosshall[51] made an important point: while negative results may indeed not lead to conclusive disproof of a model, they nonetheless prompt the need for the delivery of stronger positive evidence in its favour.[iii]

SUMMARY AND OUTLOOK: FROM OLFACTORY RESEARCH TO BIOMEDICAL PRACTICE

Scientific interest in the sense of smell is fairly recent and closely linked to advances in genetics.[55] The significance of olfaction for neurobiological research was made clear through the discovery of the OR multigene family and its identification as part of the superfamily of GPCRs.

Research on the olfactory system is key to advancing understanding in a number of different areas. Not only will it facilitate a better understanding of a variety of complex biological processes such as ligand binding and signal transduction, but also lead to insights into higher brain organisation. In parallel with protein studies, contemporary research on olfaction focuses on how information detected at the receptor level is processed at various stages in the brain.[10]

The emerging data on how olfactory information are processed in the brain (olfactory bulb and cortex projections) start to debunk earlier assumptions that the olfactory pathway works like the visual system, forming and maintaining topographic activation patterns over various brain processing stages. Studies of the olfactory bulb (a multilayered neural structure situated at the frontal lobe of the brain) have successfully established a clear activation pattern.[25 26] Contrary to expectations, however, this topographic pattern of the olfactory bulb is not maintained through further projections to the olfactory cortex.[24 27 56 57] The implications following from such apparent idiosyncrasies of the olfactory cortex for our understanding of higher brain processing and olfaction need to be seen.

Findings from studies of receptor behaviour and higher brain organisation in olfaction will eventually contribute to better medical understanding and diagnosis of many disorders that are associated with olfactory decline and loss. In addition to nose and sinus diseases,[58] better understanding of the olfactory pathway will enhance biomedical research on major neurodegenerative disorders such as Alzheimer's and Parkinson's, which are more and more studied in relation to their accompanying decline in smell perception.[59] Olfactory performance tests such as the 'Sniffin Sticks' and the 'University of Pennsylvania Smell Identification Kit' have become prominent in their potential as a preclinical tool for the diagnosis of major neurodegenerative disorders.[60 61]

[iii]For Block *et al*'s reply to Turin, see ref.[54]

These and other pending enquiries about the nature of the olfactory system promise more exciting findings to follow in the next years. Olfaction might be the oldest sensory system in an evolutionary sense, but its scientific history has only begun.

Main messages

- The identification of the olfactory receptors (ORs) as G-protein-coupled receptors (GPCRs) by Buck and Axel in 1991 linked research on olfaction to a wide range of neurobiological studies on cell signalling mechanisms. The genetic intraclass diversity of ORs promises to facilitate a better understanding of ligand-binding mechanisms in other GPCRs.
- GPCRs are involved in a huge number of significant physiological processes and respond to a diverse array of stimuli (eg, neurotransmitters, peptide hormones, amino acids, lipids, sugars, airborne molecules, proteins and photons). They are the largest protein family in the mammalian genome and exhibit a large amount of conserved amino acid sequences throughout evolution.
- The *mechanism of primary odour recognition* is known in principle but unknown in its details. Current opinion adopts a shape-selective mechanism involving conformational changes in the odorant-binding process. A pending issue is the question whether these changes are 'induced' or 'selected' or both.

Current research questions

- Structure–function relations in OR binding: which amino acid sequences of an OR-binding site determine its odorant-binding capacity?
- OR activation patterns: what (range of) odorants bind to particular ORs?
- GPCR family relations: How does the basic genetic makeup of GPCRs relate to their diversification in ligand-binding capacities?

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Self-assessment questions

1. Constituents of the olfactory system:
   A. Cell surface receptors at the cilia
   B. Glomeruli in the olfactory bulb
   C. Cytoplasmic receptors in the nasal epithelium
2. ORs…
   A. Exhibit 40–98% intraclass similarity of amino acid sequences
   B. Exhibit up to 40% intraclass similarity of amino acid sequences
   C. Share up to 40% similarity of amino acid sequences with rhodopsin
3. GPCRs…
   A. Were discovered through PCR in 1991
   B. Bind neurotransmitters, hormones, odorants, photons
   C. Are the largest protein family in the mammalian genome
4. Odorants bind to receptors
   A. By forming a hydrogen bond in a shape-sensitive mechanism
   B. With ~71% success rate
   C. In a combinatorial way
5. Olfaction turned into a modern model system
   A. Because it promises better understanding of ligand binding in GPCRs
   B. Through advances in genetics
   C. Because its ligand binding mechanism was demonstrated to work different from other GPCRs

Acknowledgements The authors thank John Dupré, Isabella Sarto-Jackson, Louise Bezuidenhout, Christine Hauskeller, two reviewers and the editors for their helpful comments.

Funding Konrad Lorenz Institute for Evolution and Cognition Research.

Competing interests None declared.

Provenance and peer review Commissioned; externally peer reviewed.

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Answers

1.
A. True
B. True
C. False
2.
A. True
B. False
C. True
3.
A. False
B. True
C. True
4.
A. True
B. False
C. True
5.
A. True
B. True
C. False