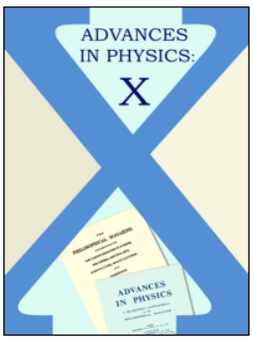


Advances in Physics: X

ISSN: (Print) 2374-6149 (Online) Journal homepage: http://www.tandfonline.com/loi/tapx20

Molecular recognition in olfaction

A. P. Horsfield, A. Haase & L. Turin

To cite this article: A. P. Horsfield, A. Haase & L. Turin (2017) Molecular recognition in olfaction, Advances in Physics: X, 2:3, 937-977

To link to this article: http://dx.doi.org/10.1080/23746149.2017.1378594

ADVANCES IN PHYSICS: X, 2017
VOL. 2, NO. 3, 937–977
https://doi.org/10.1080/23746149.2017.1378594

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

OPEN ACCESS Check for updates

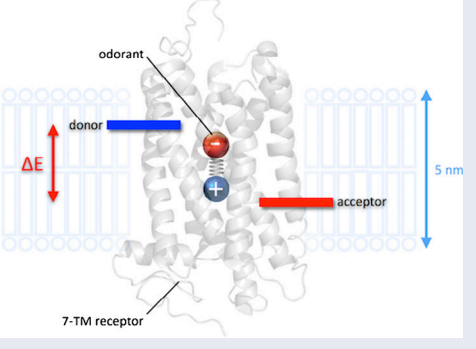
Molecular recognition in olfaction

A. P. Horsfield[a], A. Haase[b], L. Turin[c]

[a]Department of Materials and Thomas Young Centre, Imperial College London, London, UK;
[b]Department of Physics & Center for Mind/Brain Sciences, University of Trento, Trento, Italy;
[c]Biomedical Sciences Research Centre, Alexander Fleming, Vari, Greece

ABSTRACT

The mechanism by which the chemical identity of odourants is established by olfactory receptors is a matter of intense debate. Here we present an overview of recent ideas and data with a view to summarising what is known, and what has yet to be determined. We outline the competing theories, and summarise experimental results employing isotopes obtained for mammals, insects, and individual receptors that enable us to judge the relative correctness of the theories.



ARTICLE HISTORY
Received 17 May 2017
Accepted 4 September 2017

KEYWORDS
Olfaction; Turin vibrational theory; deuterium; flies; bees; humans

PACS
87.14.ep Membrane proteins; 87.15.ag Quantum calculations; 87.15.ap Molecular dynamics simulation; 87.15.ht Ultrafast dynamics; charge transfer; 87.80.Jg Patch clamping and other physiological measurements

1. Introduction and motivation

Consider the fate of a hydrogen sulphide molecule (smells of rotten eggs) which we shall name M. We join M while it is inside a small glass vessel – a stink bomb – which breaks open when dropped onto the laboratory floor. As the glass breaks, M is set free along with a great many of its companions. M then finds itself caught in the updraft produced by the inhalation of the human who dropped the glass vessel, and is carried upwards through a nostril of its liberator into a cavity behind the nose, sitting between the eyes (above) and the mouth palate (below). The ceiling of this cavity is covered by a warm damp layer of mucus,

CONTACT L. Turin turin@fleming.gr

on the other side of which are neurons projecting down from the human brain through openings in the skull. After a short while M becomes submerged in this mucus and experiences collisions with the water and proteins. M eventually completes a random walk that leads to contact with the cell membrane of an olfactory neuron. Since hydrogen sulphide is only slightly soluble in water, M spends some time in the lipid membrane of the neuron. However, the next event of interest to us is when M's journey takes it to the entrance of an olfactory receptor. M then tumbles into the receptor and is pushed and pulled by atoms in the seven transmembrane helices that form the walls. The orientation of M changes quite rapidly at first, but quickly M forms a number of weak bonds using van der Waals and electrostatic interactions. This stabilises the position and orientation of M within the receptor. The receptor is now able to recognize the chemical identity of M, and then initiate the signalling machinery.

This review is about the recognition step. How does it happen? What are the options? Two possibilities are considered: the docking theory, and the vibrational theory. First we consider an overarching theme: the structure of the receptor.

2. Olfactory receptor structure

The structure of an olfactory receptor will necessarily be strongly connected to the mechanism it employs to establish odourant chemical identity. Vertebrate olfactory receptors are g-protein coupled receptors, or GPCRs [1], and olfactory receptors constitute the largest subclass within GPCRs. However, we do not have good atomic level structural information for olfactory GPCRs, though X-ray structures have been found for other GPCRs, such as bovine rhodopsin [2]. However, the sequence of amino acids is known for many receptor proteins [3], and general structural features, and the general manner in which they operate, are well established [4].

GPCRs work by transducing the signal received on the outside of the membrane into an intracellular signal [4]. GPCRs are of enormous biological and medical importance and excellent reviews of their structure, evolutionary origin and pharmacology have been written, to which the reader is referred for background [5]. It is generally assumed that a conformational change in the receptor releases an intracellular assembly of proteins called g-proteins, though the details are an active research topic [6]. These in turn activate other enzymes, for example kinases. These kinases then phosphorylate ion channels to activate them. The multi-step system achieves gain at the expense of speed. Structurally, GPCRs appear to be remarkably conserved in the transmembrane part and highly diverse in the cytoplasmic loops, especially the extracellular ones where in many cases the ligand binding takes place [3]. The most conspicuous structural motif of GPCRs is a sequence of seven transmembrane helices spanning back and forth across the membrane, abbreviated to 7-TM.

Depending on the type of GPCR, ligands can either bind close to the extracellular face of the receptor, or in other locations on the extracellular loops which

can sometimes be extremely large, up to 500 amino-acids long (for comparison a TM helix contains about 20 amino acids) [7]. Intense crystallographic effort has given us high resolution pictures of the 7-TM part, which appears to be strikingly similar in outline in widely diverse GPCRs despite a low homology in sequence [8]. The extensive pharmacology and accurate structural data for receptors in which the binding occurs between the helices has yielded remarkably detailed models for the physics of the conformational change induced by ligands. Dynamical NMR measurements are adding a time dimension to the static X-ray picture, and the field is progressing rapidly [9,10].

It is not clear, however, whether a general mechanism for GPCR activation, applicable to all GPCR classes will emerge from these studies, which so far have been mainly focused on the β-adrenergic receptor [11]. The relationship, if any, of the larger and more distant binding sites to the intricate clockwork of the β-AR binding pocket remains to be determined. One interesting feature of the binding pocket is that it contains different structural features depending on whether the ligand is hydrophilic (neurotransmitters) or hydrophobic [12]. Hydrophobic ligands are bound under a 'lid' which shields them from the surrounding water, whereas hydrophilic ligands remain in part exposed to the outside aqueous phase.

The 7-TM arrangement of GPCRs invariably puts the amino terminal of the protein outside the cell, and the carboxylate terminal inside. By contrast, insect olfactory receptors, while also possessing a 7-TM structure (none yet determined by crystallography) have an inverse arrangement: amino inside and carboxyl outside. Furthermore, they have no sequence homology to GPCRs and – even more remarkably – a low homology to one another [13]. *Drosophila* contains 63 such receptors involved in olfaction proper, and related gustatory receptors. It should be noted that the evolutionary 'decision' to use non-GPCR receptors for olfaction is not due to a scarcity of GPCRs in insects: conventional GPCRs resembling mammalian ones are found everywhere in the fly nervous system, transducing octopamine, dopamine and acetylcholine among others [14,15].

Insect olfactory receptors appear to work as dimers made up of a constant and a variable part. The constant part is also a 7-TM receptor formerly designated as OR 83b (now ORCO for odourant receptor coreceptor). Flies in which Orco is absent are anosmic. The rapid, and rapidly extinguished response of fly receptors to odourants suggests a direct ('ionotropic') rather than a biochemically amplified ('metabotropic') mechanism, though a mixture of the two has not been ruled out [16]. From the standpoint of an electron-mediated vibrational detection mechanism this is not a crucial distinction, since the same electroreduction step (for example disulfide to dithiol) could be used both to turn on a channel directly or to effect g-protein mediated transduction. Unlike the mammalian olfactory neuroepithelium, where the exact position of neurons bearing different receptors is unknown, the position of olfactory receptor neurons and their attendant receptors in the fly is stereoptypic and has been extensively mapped [13]. This has enabled the construction of an extensive database of odourants and receptors

which respond to them, which is accessible online. The overall impression one derives from this database is that fly odourant receptors are relatively nonspecific and will often respond vigorously to several, even chemically distant odourants.

A frequently voiced objection to the notion that vertebrate olfactory receptors can sense vibrations is the fact that other GPCRs apparently do not. One editorial article put it thus: 'Why should olfactory receptors be so different?' [17]. The answer is twofold. First, it depends on one's perspective on GPCRs. For example, since rhodopsin is the ancestral GPCR, one could ask 'why don't all GPCRs sense light?' or 'why aren't they all ion pumps like bacterorhodopsin?'. The answer is, of course, that evolution frequently recycles similar structures to achieve different functions [18,19]. A related question is the apparent similarity in function between vertebrate olfactory receptors and insect ones when the sequences have no similarity and even the topology of the proteins is different. Again, evolution, this time convergent, may be the answer. If sensing vibrations confers the same advantage to animals as IR spectroscopy did to chemists, i.e. providing them with an analytical sense probing functional groups, then evolution will favour its appearance and, if necessary, rediscovery. It is remarkable in this context that insects do have other GPCRs but do not use them for olfaction.

Finally, there may be something more subtle underlying the use of electrons in olfactory receptors. The mechanism of activation of non-olfactory GPCRs may indeed be the same, but it has not been elucidated yet. In particular, it is not clear how binding causes the crucial receptor activation step. One of us has proposed that electron transfer may be an ancestral general mechanism in GPCRs, and that the additional trick of making electron transfer contingent on vibrations may be a later evolutionary advance [20].

3. Mechanisms of molecular recognition

Having considered the receptor structure, we now ask the question: how do olfactory receptors work? There are two proposed mechanisms for the initial molecular recognition step: one based purely on shape and weak bonding interactions between the odourant and the receptor (which we shall refer to as the docking theory); the other asserts receptors can also identify vibrational frequencies (which we shall refer to as the vibrational theory). Both theories acknowledge that a recognition event requires some level of match between the odourant and the receptor. This involves both some shape compatibility (to avoid high energy repulsive interactions), and alignment of atomic groups to support weak bonding interactions (to ensure the time the odourant resides in the receptor is sufficient to trigger recognition). However, they differ in how this contributes to the final recognition. The docking theory proposes that a change in configuration of the receptor follows the arrival of a molecule that binds sufficiently strongly, which then initiates a signal (the binding and release of a trimeric G protein in mammals). The vibrational theory proposes that the signal

can only occur once the receptor recognizes a part of the vibrational spectrum of the odourant [21].

We now analyze these mechanisms in more detail. We note that the question of mechanism plagued the vibrational theory for many years. In 1996 Luca Turin published a paper [21] in which he proposed that inelastic tunneling spectroscopy is a possible mechanism. This is the primary vibrational mechanism considered here.

3.1. Docking theory

Here we give a very brief overview of the main ideas for the docking theory of olfaction, which has evolved steadily over many decades, and continues up to the present time [22]. One early view from 1944 due to Moncrieff [23] and Pauling in 1946 [24] was that the odor of a molecule is defined by its selective adsorption. Every contemporary theory accepts this in some way: receptors have a shape and bonding landscape, so odourants will inevitably bind selectively to different receptors. In 1963 Amoore produced a more prescriptive view [25], based on the interesting idea that there might be smell 'primaries', as there are for color. Based on the seven most prevalent odor descriptors, he saw odor as defined by a set of rigid shapes. While steric effects are clearly relevant, strict shape matching is no longer held onto as individual receptors are known to accommodate multiple odourants [26–28].

While we do not know the secondary structure of any olfactory receptors, we do know that vertebrate olfactory receptors are GPCRs [1]. As the structures of some other GPCRs are known, it has been possible, by a combination of experiment and computer simulation, to work out a number of aspects of how they operate [29]. It is natural to assume that olfactory receptors share characteristics with these other receptors.

What is also clear, however, is that the typical ligands of odourant receptors are quite different from the typical ligands of other GPCRs, both in respect of affinity and specificity. Figure 1 shows three ligands, in decreasing order of affinity and specificity from left to right. These diagrams are generated by Poseview [30]. At left is norbiotin (pdb 1LDO), a ligand with a near-covalent binding energy to the protein avidin. Norbiotin makes seven hydrogen bonds with the protein, each contributing between 2 and 5 kcal/mole. At the center is an emblematic GPCR ligand, noradrenalin, depicted with its interactions with the β-adrenergic receptor. Five hydrogen bonds are listed. At right is an insect pheromone, tetradecadien-1-ol, attached to its odourant-binding protein. It is safe to assume that this protein has evolved for high affinity, since the insect can perceive the odourant at low concentrations. The pattern of interactions is quite different: most of the odourant interacts by dispersion forces, and only the terminal hydroxyl forms a single hydrogen bond with a nearby carboxylate.

The ability to form hydrogen bonds influences bulk physicochemical properties as well as binding to receptors. Biotin and noradrenalin make hydrogen

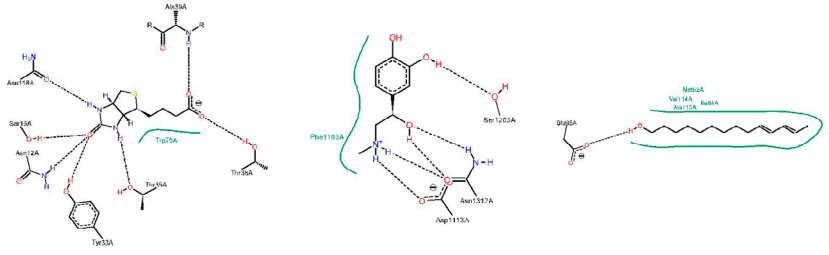


Figure 1. This figure shows three ligands in decreasing order of affinity and specificity from left to right. These diagrams are generated by Poseview [30].

bonds to themselves and the substrate and are nonvolatile solids, while tetradecenol is a volatile liquid. Accordingly, most odourants – volatile by definition – will have few molecular features available for molecular recognition. We do not have a picture of an odourant in a receptor, but human odourant-binding proteins serve as second best. Remarkably, most of the Poseview analysis of odourants bound to OBPs reveal few interactions, sometimes no interactions at all aside from generic dispersion forces. For example different odorants interacting with a bovine odorant binding protein (pdb ID 1GT1 to 1GT5) show no specific interactions, while the LUSH odorant protein of drosophila [31] interacts with odorants via one hydrogen bond.

What the above implies is that odourant receptor specificity and affinity are expected to be generally low [26], as indeed seems to be the case both in insects and vertebrates. An interesting illustration of this relates to the odor character of enantiomers. While some enantiomer pairs have distinct odours [32], many enantiomer pairs smell identical [33], which is not what would be expected from a shape-based model involving chiral receptors. Interestingly, there is some evidence that people can learn to distinguish enantiomers that previously smelled identical to them [34]. Extensive data on interactions between chiral drugs and receptors reveals in general large changes in affinity and efficacy of enantiomers [35]. Some data exists on the pattern of olfactory bulb activation by enantiomers in rat preparations. Two patterns are shown in Figure 2. What is striking about these patterns is how similar enantiomer responses are to one another. This is especially striking when compared to responses to different odourants, as can be seen in Ref. [36]. This suggests that chirality of the receptor binding pocket is weak. It is also worth noting that the limonene enantiomers, long held up as an example of odor character difference, actually smell identical to humans when sufficiently pure [37], and that the small differences in rat olfactory bulb activation may be due to impurities.

This does not mean that odourants are somehow unable to activate GPCRs by conventional means. The dynamics of a GPCR is very important to how it operates [39]. The role of the odourant may be to perturb the dynamics

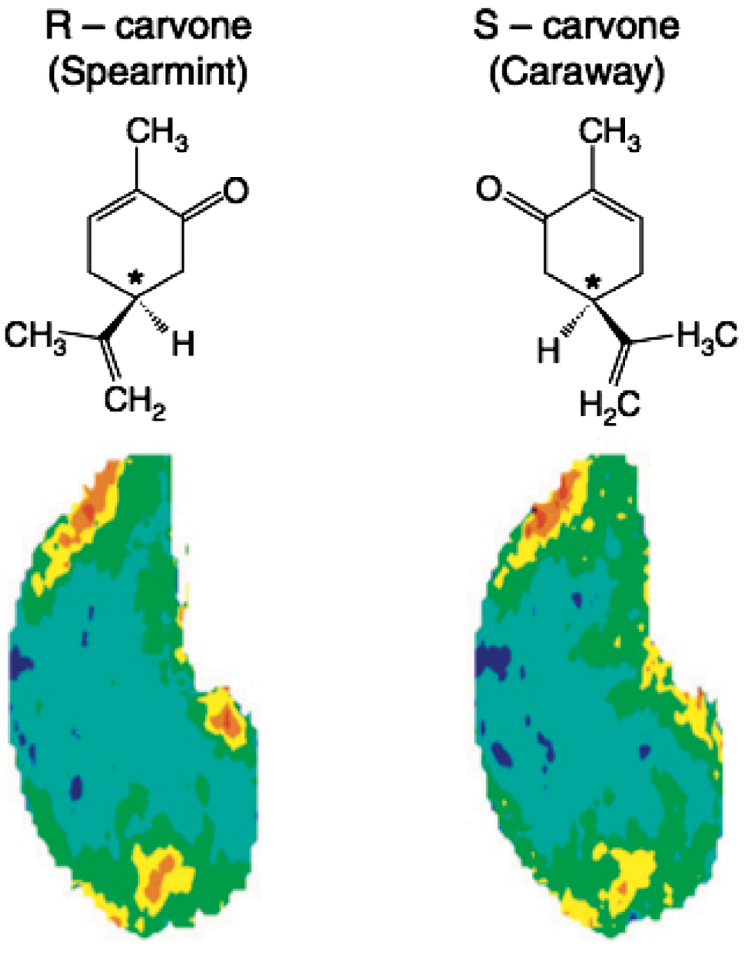


Figure 2. This figure shows olfactory bulb activation by enantiomers in rat preparations. Note how similar the activation is in both cases. Reproduced with permission from Ref. [38].

through its interactions with the residues of the receptor, causing it to explore configurations with probabilities that differ from those of the empty receptor, and which depend on the features of the odourant. Recognition is then thought to occur when configurations that support the binding and activation of a G-protein are entered sufficiently often that activation occurs with high probability [6]. Once activation occurs, there is thought to be an exchange of GDP for GTP by the G-protein, and at some later stage the release of its α subunit, which feeds into the signal amplification.

To date, docking theories have had limited success in predicting odour [40,41]. In addition, given the new understanding of the highly dynamic nature of the recognition process, it is quite hard to imagine a strong link between the structure of the odourant, the structure of the receptor, and recognition. However, as that we are still at an early stage in the research into the consequences of this dynamic behaviour, new insights may be forthcoming.

3.2. Vibrational theory

3.2.1. History

The idea that olfaction probes the molecular vibrations of odourants has a long history. If one neglects earlier, rather vague proposals [42] inspired by *Naturphilosophie* and a hoped-for unity of 'spectral' senses (hearing, vision and smell), the first clear proposal for a vibrational mechanism in olfaction is due to Dyson [43,44] soon after after the discovery of Raman spectroscopy. The theory was revived in the late 1950s by Robert Wright [45,46], found remarkable but largely overlooked experimental support in the early 1980s with the work of Clifton Meloan [47,48], and thereafter died a lingering death until revived in 1996 [21].

Its current lack of popularity is in part due to its history. The reasons for the original demise of vibrational theories in general were threefold. In increasing order of seriousness:

(1) Wright had proposed [49] that only the energy range below a few kT, that is to say $0-1000\,\mathrm{cm}^{-1}$ was being probed. The rationale for this was sensible, since a mechanical system like a protein, operating around 300 K, could not be expected to sense vibrations which were not thermally excited. This meant that the microwave spectrum of molecules was being read, but the correlations between microwave peaks and odour were rather unconvincing.
(2) It was becoming clear [50] that enantiomers sometimes possessed different odour characters, and this was taken as definitive evidence against the theory since enantiomers have identical solution spectra. In time this turned out to be a fairly weak objection.
(3) Most importantly, no mechanism was ever proposed by which cells could sense vibrations. This last objection became more acute as our understanding of olfaction progressed. The discovery of olfactory receptors in 1991 [1], the realisation that they were part of a much broader class of G-protein coupled receptors (GPCR) involved in sensing neurotransmitters [51], and the rising tide of consensus about molecular recognition, made it increasingly unlikely that olfactory receptors detected vibrations when all other GPCR receptors did not.

The cornerstone of the vibrational theory, however, remains an old observation made by chemists such as Dyson, Wright, and Meloan. Much of the organic chemist's trade consists in identifying molecules, and until NMR became widespread in the late sixties, chemists used IR spectroscopy (and frequently smell) to figure out what they had made. Much of the language of olfaction comes from chemistry: odourants smell ethereal, sulfuraceous, nitrilic, etc. Indeed it was another chemist, Hein Klopping, who pointed out that this recognition of functional groups was, from the standpoint of molecular recognition, rather a

mystery. In a seminal article titled *The odour of small molecules* [52], he pointed out that a molecular recognition system based on what is commonly known as lock-and-key needs proper keys to work, and that small molecules should in principle be able to either pick every lock, or none. Instead, they have very specific odours. An extension of Klopping's observation is the fact that the presence of functional groups can be detected whatever the molecular context. Any molecule containing an -SH group, provided it is volatile and sufficiently small (<20 carbons is the usual limit) to fit into the receptors is certain to smell sulfuraceous, and the same applies to the odor character imparted by nitriles, nitro groups, oximes, ethers *etc*. It should be emphasized that individual descriptors such as sulfuraceous are only part of the olfactory identikit of the molecule: pinanethiol (7,7-dimethyl-2-bicyclo[3.1.1]heptanyl methanethiol), for example, smells both of pine needles and 'rotten egg', and is a grapefruit material in fragrances and flavors.

Pinanethiol illustrates an important point in olfactory recognition, i.e. that the smell of a molecule is in a sense the sum of the smell of its parts. If the thiol in pinanethiol were replaced by a nitrile group, the resulting pinanenitrile would smell like nitrilic pine needles. An easily accessible illustration of this phenomenon is citronellyl nitrile. Citronellal is a well-known lemony material. Replacing the aldehyde with a nitrile yields a material which smells of both lemon and oily-metallic, a familiar smell in stovetop cleaners combination. To an IR spectroscopist, this makes good sense: many functional groups like -SH and -CN vibrate in the upper half of the range of molecular vibrations ($0-4000\,\mathrm{cm}^{-1}$, i.e. 0–0.5 eV) whereas the rest of the molecule vibrates in the fingerprint region, so named because the pattern of vibrations is determined by the topology of the bonds, and therefore unique. In many cases, changing functional group has relatively little effect on the fingerprint region.

We stress that in the vibrational theory, receptors are assumed to use shape as well as vibrational frequency to identify odourants. The multitude of receptors (63 in flies, upwards of 500 in humans, a thousand in mice, etc) reflects this important point. The 'cuvette' of the spectrometer (the receptor binding pocket) is the size of the odourant and so cannot be wholly non-selective. One must therefore have dozens of receptors in order to make sure to catch an odourant and analyse its spectrum. However, the theory asserts that shape is not enough. Indeed, the purpose of the system is revealed by the fact that most receptors, even at the individual level, appear to be relatively nonspecific.

3.2.2. Turin theory

Here we summarize the one known possible mechanism for vibrational recognition of odourants, namely that proposed by Turin [21]. The central idea is that an electron can travel from one site to another of different energy, but only if total energy is conserved. The change in energy of the electron must thus be compensated by an equal gain in vibrational energy of the odourant within the

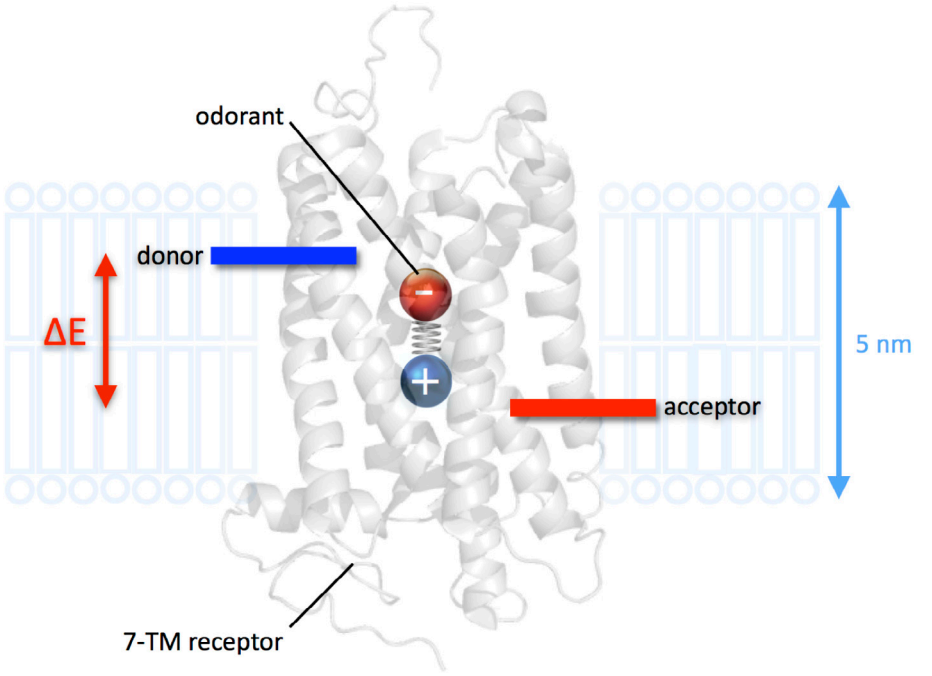


Figure 3. This cartoon illustrates the mechanism of the Turin theory of olfaction. The odourant molecule (balls and spring) is resident in a receptor (helices embedded in a membrane). If an electron hops from one electronic level (donor, D) to another (acceptor, A), then a signal is generated (activation of a G protein). In the initial state, the electron is on D which has an energy ΔE higher than the final electronic state when the electron is on A. Initially the odourant molecule is in its lowest vibrational state, but in the final state (the electron on A) the odourant vibrational mode has a higher energy, with the energy coming from the electron. To conserve energy overall, the difference in energy between the two vibrational states must also be ΔE. Suppose now that the splitting between the electronic states was increased by 50%. It would now be impossible to conserve energy and have the electron jump between D and A. Since energy must be conserved, the hop becomes forbidden, and the receptor cannot signal the presence of the odourant. Thus the presence of a signal indicates that a molecule is present that has a vibrational mode of frequency defined by the receptor (the energy splitting between D and A), as required by the vibrational theory.

receptor. The equality of these two energies means that the electron transfer can only happen if there is an odourant present with the right vibrational frequency, and hence we have a method for recognizing the presence of an odourant by its vibrational modes. The mechanism is explained further in the caption to Figure 3.

There are a number of features that need to be in place for the mechanism to operate [53].

(1) First, the itinerant electron's initial state (donor), and final state (acceptor) must exist. These are termed D and A respectively in Figure 3. Note that there is an important assumption here, namely that the electrons move by a hopping mechanism so that they have significant residence times in localized states. The other extreme would be that the electrons move in

a metallic like fashion, as occurred in the work of Lambe and Jaklevic [54]. Because electrons in proteins are at body temperature, and proteins are flexible molecules, we expect large coupling between the electrons and the atomic vibrations, and hence hopping transport to be the relevant mechanism.

(2) Second, D must have a higher energy than A. The energy ordering follows from the low occupancy of the excited vibrational state in equilibrium at the temperature found in a mammal. That is, if the electron were to travel in the reverse direction (from A to D) it would need to acquire energy from the vibrations of the odourant, which in turn would require the odourant to be in an excited state. As this excitation is improbable (occupancy of 0.0003 for a vibrational quantum of 0.2 eV at 300 K), response of the receptor would be suppressed. Of course, at equilibrium the same argument holds for the electrons; however, there are electrochemical processes that operate in living cells that can generate electrons of increased energy. We note that immediately following the transition, the molecule is in a vibrationally excited state, so the reverse process is then rapid; however, this can be suppressed by interactions of the molecule with its environment [55].

(3) Third, D and A and must not be coupled strongly to their environment: coupling to both electronic and vibrational states must be weak. Coupling to other electronic states results in a projected density of states on D or A being broadened. While this allows electrons to move on and off easily, it also means that electrons on these sites can easily be thermally excited to higher energy levels, removing a sharp separation between filled and empty states. Thus there is a loss of resolution with the broadening being about $5.4\,k_BT$ (this follows from the form of the Fermi–Dirac distribution function), where k_B is the Boltzmann constant and T the temperature. At body temperature the broadening is about 0.1 eV, which is of the same order as the vibrations we seek to measure. Ensuring the electronic coupling is weak is not a problem in practice for proteins. The broadening due to coupling to vibrations in the environment can be estimated from Marcus theory [56], and is given by $\sqrt{4\lambda k_BT}$ where λ is the reorganization energy, a measure of how strongly the electron couples electrostatically to fluctuations in the environment. A typical value for λ in proteins is of order 1 eV, giving a broadening of order 0.3 eV. It has been recognized since 2007 [53], and reiterated since then [57,58], that the reorganization energy needs to be much smaller than this for the mechanism to operate. In particular we note that once the broadening is of the order 0.1 eV, there can be a high probability that the electron can tunnel in the absence of any odourant, thus negating the value of the receptor.

(4) Fourth, the site D must be able to be charged by an electron; that is, it must be coupled to a source of some kind. In addition, site A must be able to pass on the electron in such a way as to initiate the release of the G protein;

it has been speculated that this is through the reduction of a disulfide bond [21].

(5) Fifth, the electron must couple sufficiently strongly to the vibrations in the odourant that there is a high enough probability that it can successfully exchange energy with them when jumping from D to A.

(6) Sixth, the hopping of an electron from D to A must occur on a timescale that is compatible with the upstream amplification (starting with the release of the G protein) and processing stages, namely microseconds to milliseconds. If the processes occur much faster than this it is not possible to distinguish signals from molecules with a compatible vibrational spectrum from those without.

The heart of the mathematical theory is a rate equation [53,57,58]; a brief summary of its derivation, generalised from previous work, is given in the Appendix 1. In particular, if the vibrational modes have energies that match the difference in energy between D and states on the odourant M, then the itinerant electron can remain long enough on the odourant to exert a significant force on the vibrations. This limit is considered numerically by Bittner et al. [59], and shows strong resonant behaviour. It is not possible to say at this stage whether this resonant mechanism or the original one is correct, though each have advantages: having the electron avoid M means the signal is driven just by the vibrational modes of the odourant, and not by its ability to transfer an electron; having the electron reside on the molecule means the response to the odourant modes (as opposed to the environment) is maximized.

3.3. Receptor features

Earlier we considered general features of olfactory receptors: here we consider some finer details that are important to specific transduction mechanisms. The following is somewhat speculative as we do not have an X-ray structure available for an olfactory receptor, and so must depend on other known structures [60,61].

For any mechanism, the receptor must be able to provide sites at which molecules can reside. As olfactory receptors have 7-TM helices that can form a pocket accessible from outside a cell, and they belong to the family of GPCR receptors, this can be taken as given [62]. What is not known is how the receptor responds mechanically to the arrival of an odourant. This is an area of active study, but at a very early stage [6]. The full three dimensional structure would enable reliable molecular dynamics simulations to be performed, which should transform our understanding of this step [39,63].

Less obvious is whether the structural features required to make a tunneling receptor work are present. They can conveniently be split up in three parts:

(1) electron transfer across the odourant binding site
(2) resupply of electrons to the electron donor and
(3) electrochemical transduction of the current.

As we need to discuss small scale features, we require a model to work from. As our model GPCR we consider rhodopsin, because it is an ancestor to all GPCRs, it contains a hydrophobic ligand (retinal), and its structure is known at the highest resolution.

3.3.1. Electron transfer

It seems safe to assume that the donor and acceptor must be part of the protein, and therefore must be amino acid side chains. What is required therefore is an electron donor with the highest possible HOMO energy, and an acceptor with a LUMO below that energy. Tryptophan has the highest HOMO of all amino acids, and a highly conserved tryptophan is present in all GPCR binding pockets [64]. On the face of it, the requirement for a LUMO below the tryptophan HOMO appears impossible, since all amino acid side chain HOMOs lie several eV below the LUMOs. There is, however, a way to shift a LUMO downwards by several electron volts: a metal ion bound to the protein.[1]

Zinc has been proposed as a candidate, is known to be involved in olfaction (including in dogs [65]), and only exists in the +2 oxidation state, which may ensure that it passes the electron along. Remarkably, there is in fact a zinc ion in the rhodopsin core, i.e. in the middle of the membrane, clearly resolved in the 1u19 structure. It is bound in an unusual geometry to a second tryptophan, a histidine and a glutamic acid carboxylate. What makes the geometry unusual is that, while the plane of the tryptophan bisects the zinc ion as expected, the histidine which would be expected to do the same does not. Instead, the plane of the his ring is almost tangent to the zinc. This suggests that the histidine is protonated. We therefore have at least two, possibly three, positive charges around an aromatic amino acid, and the effect on the LUMO is to lower it by several electron volts. In between the 'donor' tryptophan and the 'acceptor' Zn-tryptophan-histidine complex lies the ionone core of the retinal. What prevents these residues from acting as a donor and acceptor in rhodopsin (apart from the availability of a supply of electrons) is the presence of a negatively charged carboxylate which partly cancels the positive charges and cancels the gradient favorable to electron transfer. If the glu were absent – a single-point mutation – one would indeed have a key component of an electron transfer device (see Figure 4).

3.3.2. Resupply of electrons

We now address the question of how electrons might travel to and from the donor and acceptor sites. It is generally considered established that unmodified, native proteins without prosthetic groups are insulators. This fits with the HOMO-LUMO gap described above, with the facts that proteins do not absorb in the visible range, and that pellets of dry protein exhibit a very high electrical resistant. This, however, does *not* fit with the fact that STM imaging of single proteins involves currents of 1 nA or so flowing under a fraction of a volt from substrate

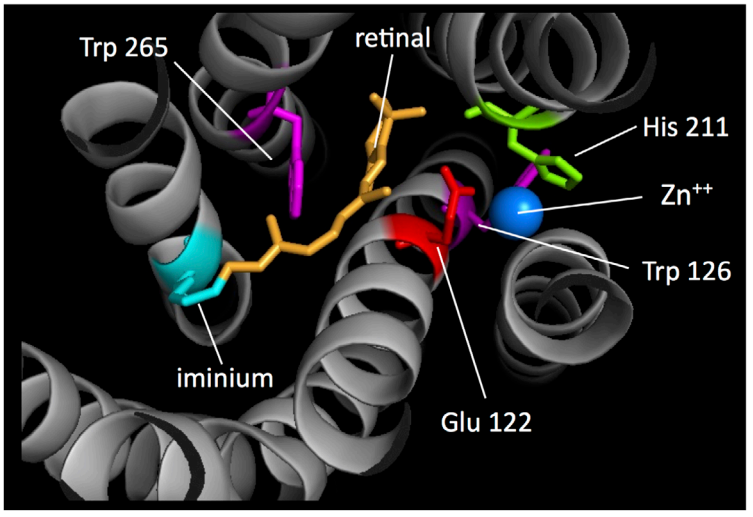


Figure 4. The structural features of bovine rhodopsin (2.2 Å resolution, pdb 1u19) illustrating possible components of an electron spectroscope. From left to right, in the direction of hypothesized electron flow: the electron donor (trp265 in purple), the ligand through which electrons tunnel (retinal, orange) and the zinc (blue)-tryptophan 265-histidine 211 complex. The negatively charged Glu 122 would likely prevent electron transfer in the desired direction in rhodopsin itself.

to tip *via* a protein [66]. Neither does it account for the existence of many electron transport enzymes, the most impressive of which being the surface membrane oxidoreductases, which transport electrons across the width of the bilayer, or with bacterial nanowires of very low electrical resistance [67]. The electron conduction mechanism in STM and membrane oxidoreductases mechanism is not known, but it is there. An interesting possibility is the existence of electron acceptors strong enough to create holes in the protein backbone, thereby allowing electron mobility. Szent-Györgyi and his collaborators had shown that proteins reacted with ketones and aldehydes capable of forming imines (and iminiums) with amine containing side chains of lysine and arginine turned brown, developed an ESR signal and conducted electrons [68].

Since then it has become clear that the imines they obtained by reacting caseine with methylglyoxal (pyruvic aldehyde) do in fact occur in vivo, among many 'glycation' end products [69]. It is not beyond the realm of possibility that some such chemical modifications might be introduced into proteins in order to render them conducting. Indeed, something resembling one of these modifications is present in the rhodopsin itself, in the form of the Schiff's base between the retinal aldehyde and a lysine side-chain. This Schiff's base is protonated to form an iminium, one of the strongest electron acceptors in biology. Indeed, were it not once again for the presence of a glutamate carboxylate next to the iminium, electron transfer would probably occur spontaneously within rhodopsin, provided the screening of the positive charge by local polarisation was small at the receiving end.

3.3.3. Transduction

Evolution has found several ways of transducing electronic transfer into structural changes. The most prominent one is the electroreduction of disulfide bridges between cysteines [70] (two joined-up cysteines are sometimes called cystines) to dithiols:

$$R - S - S - R \xrightarrow[2e^-]{2H^+} R - SH + HS - R \quad (1)$$

Disulfide bridges in the extracellular medium tend to remain oxidised, i.e. joined up, for lack of ambient reducing power. One such disulphide bridge is conserved in *every* GPCR and stitches together the extracellular structure of GPCR receptors. There is no evidence to date that this disulphide bridge is deliberately reduced to affect receptor function, but its chemical reduction unquestionably affects receptor function. An interesting parallel in this context is the disulphide bridge in the conserved extracellular 'cis-loop' of pentameric inotropic receptors (GABA-A and nicotinic acetylcholine) which, when reduced by intracellular electrons, modulates receptor function [71]. Another possible target of electron currents might be disulphide bridges in the G-proteins themselves. A recently published structure of a GPCR bound to the G-protein trimer shows two S–S bridges adjacent to one another in the receptor itself, and two more in the Gs protein, which is the nucleotide-binding subunit that activates cyclases [72].

While structural information on olfactory receptors in humans and fruit flies is currently unavailable, it is nevertheless interesting to ask whether their primary sequences contain conserved amino acids capable of functioning as electron donors and acceptors. As a first approximation based on the features of the putative electron circuit in rhodopsin, a search for conserved histidines and tryptophans in alignments of fly and human olfactory receptors yields Figure 5. It shows a random (in order of appearance in the Uniprot database) selection of 31 *Drosophila* and 31 human olfactory receptors. In human receptors the conserved tryptophan typical of GPCRs is sometimes replaced by a pheylalanine or tyrosine. It should be noted that in addition to the conserved tryptophan, human olfactory receptors also contain four highly conserved tyrosines in the transmembrane. These would also be good electron donors, particularly if deprotonated. There is also a highly conserved histidine in the 6th transmembrane domain. This histidine was suggested as a metal binding site by Turin (1996) and confirmed to be so by Block (2016). Remarkably, in the fruit fly, there are also an almost perfectly conserved tryptophan and a highly conserved histidine. When the histidine is missing, with one exception in this sample it appears to be replaced by glutamine (Q), asparagine (N) or tyrosine (Y), all amino acids capable of binding a metal ion. The missing tryptophan is replaced by phenylalanine, the second-best electron donor among amino acids.

In conclusion, it seems that many of the component parts of a putative electron transfer spectroscope are present in known structures of GPCRs. It is therefore

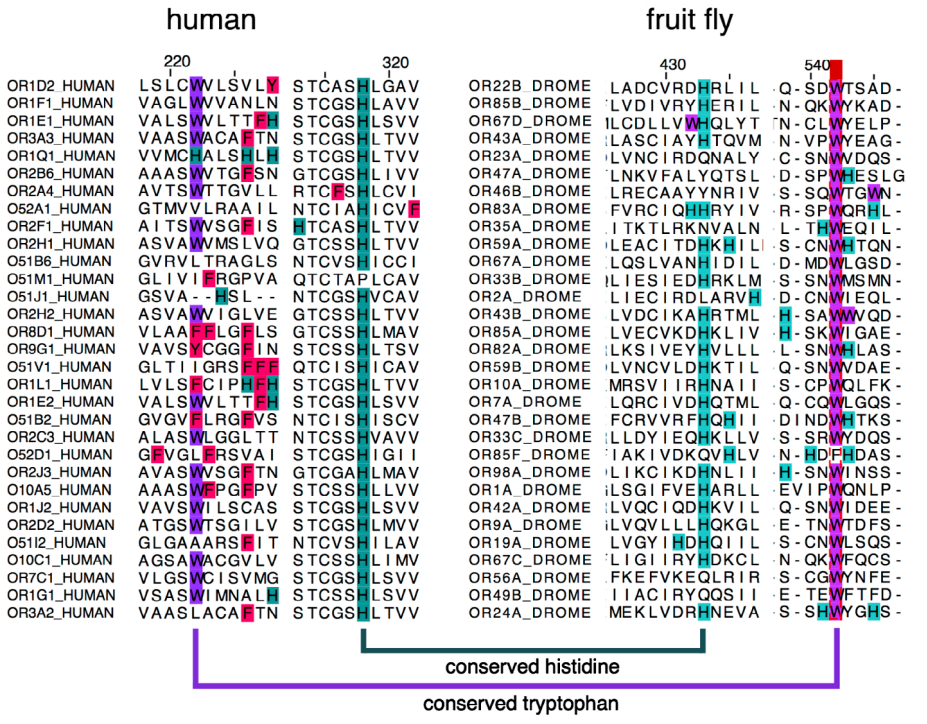


Figure 5. Excerpts from 31 aligned sequences of randomly chosen fruit fly (left) and human (right) olfactory receptors showing conserved histidines and tryptophans. Histidines are indicated in green, tryptophans in purple. Red F and Y letters in the human sequence denote respectively phenylalanine and tyrosine. See text for details.

not too far-fetched to suggest that one or more classes of GPCR have taken advantage of preexisting features by fine tuning them, for example by removing counter ions near strong electron acceptors. It goes almost without saying that possibility does not imply existence, and that direct proof of the involvement of electrons in receptor function, currently lacking, will be required.

4. Experimental evidence

Here we assess a cross-section of the available experimental evidence that sheds light on the mechanisms being used by olfactory receptors. If we see the possibilities as vibrational or docking theories, then we need to focus on experiments that clearly distinguish between them. The approach that has received most attention is isotope exchange: replacement of hydrogen (mass 1.007) with deuterium (mass 2.014) leaves molecular shape largely unchanged but significantly alters molecular vibrations. As this is a sensible approach to distinguishing between the possible theories, we focus of much of the discussion on it.

We note at the outset that small isotope effects other than vibrations have been suggested as explanations for differences in the smell of deuterated compounds [58]. To help assess the importance of these effects we make some observations.

The effect of deuteration on C–H(D) bond lengths is essentially undetectable by neutron diffraction [73], and many effects previously attributed to small differences in C–D vs. C–H bond lengths are likely instead due to differences in noncovalent interactions between H and D atoms themselves [74]. There is no question that polarisability, and thus hydrophobicity, of deuterated compounds is slightly lower: they exit a hydrophobic gas-chromatography (GC) column very slightly earlier than their hydrogen counterparts [75]. However, the magnitude of this effect should be seen in context: the GC column needs to be several tens of meters long to achieve even incomplete separation of isotopes. Large isotope effects are only seen in situations where covalent C–H(D) bonds are broken [76], which we believe is not the case in olfaction (receptors are, as far as we know, not enzymes). It could conceivably occur during odourant metabolism by catabolic enzymes: these are known as perireceptor events. Experiments, though, suggest this is not the case for insects [77]. Smaller but still potentially important effects are seen where H and D are compared in their ability to make hydrogen bonds: H_2O differs in viscosity and melting point from D_2O, as may be expected from a liquid glued together by a network of hydrogen bonds [78]. These effects are not expected to be present when none of the deuteriums in an odourant isotopolog are exchangeable and involved in hydrogen bonded interactions.

The question nevertheless remains whether a small difference in smell character between isotopologs can be unequivocally attributed to differences in molecular vibrations. Two possible sources of error arise. The first is impurities. Humans are extraordinarily good at smelling small amounts of impurities in a nominally pure compound, as attested by the fact that nominally pure odourants sourced from different suppliers frequently smell slightly different. This can be dealt with only by taking adequate precautions during synthesis and by assessing odour character in GC-pure compounds under conditions where GC peaks can be safely considered to be monodisperse, i.e. to contain only one molecule. The second, more serious potential confusion arises from the fact that the resolution of olfaction considered as a multi-receptor system is unknown. Specifically, how small a change in binding (caused by deuteration) of a pure odourant to one of the hundreds of human olfactory receptors would be sufficient to cause a detectable change in the pattern of receptor activation and therefore in odour character? Nobody knows, and it could be very small indeed. This possibility is made drastically less likely if only one or a few receptors are involved, as will be seen below.

Experimental tests of the vibrational theory were so far performed in two biological classes: insects and mammals. Olfactory sensing and processing in these classes have several common features as well as fundamental differences. The olfactory receptors (ORs) are proteins expressed in the cell membrane of olfactory receptor neurons (ORNs). While in mammals they are located in the nasal cavity, in insects they are sitting within sensilla pores on the antennas. Insect odour receptors are genetically unrelated to those of vertebrates [79].

While the receptor protein structure shows some similarities, only in mammals are they coupled to a G-protein [1] which switches neuronal activation. In insects receptors consist in a heteromeric complex that serves both as a receptor for the odourant and the ion channel that is gated by the odourant detection [80]. ORN axons project into a first olfactory neuropil, called antennal lobe (AL) in insects and olfactory bulb (OB) in mammals, which share remarkable properties. Single types of ORN synapse with other neurons within spherical structures called glomeruli, the functional units of these primary olfactory networks. There are 62 glomeruli in the fruitfly, 165 in the honeybee, 1800 in mice, and between 1100 and 1200 in humans. Each glomerulus is invaded by a single type of OR only [81]. Glomeruli are interconnected by local neurons (LNs) and project into higher brain centers where odour information is further processed.

A natural question to ask is: given the differences, how can we be sure the same mechanism is used by the receptors in both mammals and insects? The answer is, we cannot until we have very reliable atomic level structures for the different types of receptors. However, the fundamental problem of identifying a disparate collection of molecules is shared, and so the optimum solution might well be the same.

4.1. Insects

4.1.1. Insect olfactory system

In insects, the olfactory code produced in the antennal lobe was deciphered by fluorescence imaging techniques, which allowed us to resolve the neuronal activity in single glomeruli in response to odour stimuli [82]. In this way, it was found that single ORs were not activated by single specific components, but responded to several odourants with varying sensitivity. Equally, single odour molecules excite not only single receptors, but a whole set with varying affinity. Electrophysiological recordings revealed odour-specific temporal features of these responses [83]. Together this represents a spatio-temporal odour code [84] which is highly conserved across individual animals. From the antennal lobe this information is then forwarded to the mushroom body and the lateral horn where odourants are identified, evaluated, and memorized [85] and will trigger a behavioural response. The advantage of insect models in studying olfaction is the availability of various methods to trace back a behavioural signal via the underlying information pathway in the brain to its origin at the level of the olfactory receptors [86]. Several of these complementary methods were recently applied to test the vibrational theory of olfaction in insects at different neural processing levels and the behavioural outcome.

4.1.2. Behavioral experiments

Initial studies looked for differences in behavioral action in response to odourant stimuli, from which it was determined whether odours were distinguished by the animals or not. Those paradigms were then used to implement the proposal

of isotope replacement suggested in Turin's original work [21]. The distinguishability of different isotopomers was evaluated by quantifying differences in behavioural responses.

Franco et al. [87] used T-maze shaped olfactometers where groups of fruitflies (*Drosophila melanogaster*) entered via an input arm and were exposed to two odourant stimuli from symmetric transversal output arms. After a fixed experimental time, preference for one or other odour was evaluated by counting the number of insects in the two output arms. Odourant purities were all >99%.

Experiments using acetophenone (ACP, C_8H_8O) diluted in nonvolatile, odourless isopropyl myristate (IPM, $C_{17}H_{34}O_2$) showed a clear natural preference for ACP with >15% excess flies in the responding arm. When the natural ACP was deuterated by replacing 3, 5, and finally all 8 hydrogen atoms by deuterium, the preference was first found to be reduced and then inverted, such that for the fully deuterated isotopomer d_8-ACP (C_8D_8O) a significant number of flies preferred IPM instead.

They then tested whether flies could spontaneously discriminate between different isotopomers of the same odourant in the two arms, which was found to be the case for natural ACP against d_8-ACP, and for natural 1-octanol (OCT, $C_8H_{18}O$) against a highly deuterated isotopomer d_{17}-OCT ($C_8D_{17}OH$). In mutant flies lacking the function of the olfactory receptor Or83b the discrimination of ACP against d_8-ACP and OCT against d_{17}-OCT was eliminated, which shows that the discrimination must be due to odour perception.

To amplify or invert these natural preferences, Franco and colleagues then used mild electric shocks applied to animals within one of the T-maze output arms. In this way, flies were conditioned to avoid the respective odour. Again, direct comparisons between two isotopomer pairs of ACP, OCT, and BZA were performed. In a first experiment, flies were conditioned to avoid one isotopomer; they were then tested by another experiment in which they confronted two isotopomers of the same compound without punishment. Conditioned flies showed in all tests a significant avoidance of the isotopomer associated with the punishment. This proved distinguishability between isotopomers in all 3 odourant pairs.

To exclude that these results were caused by different impurities in the odour samples, generalization to the presence of deuterium was tested. Flies were conditioned and tested using distinct odourants. If then flies were conditioned to avoid a deuterated odourant, they avoided also the unrelated deuterated test isotopomer, and equivalently for natural hydrogenated odourants. This suggests that a common feature such as the vibrations of C–H and C–D bonds might be involved in the odour reception.

A final experiment evaluated whether indeed vibrational resonances might be a feature that is probed by odour reception. Two molecules were used which possess similar odour characteristics (to the human nose), namely citronellal (ALD, $C_{10}H_{18}O$) and citronellyl nitrile (NIT, $C_{10}H_{17}N$) both having a lemongrass-like

smell. Naïve fruitflies showed no preference for aldehyde over nitrile. If, however, they were conditioned to avoid the unrelated deuterated d_{17}-OCT with respect to the natural OCT, this behaviour changed drastically, and flies avoided the nitrile (vs. aldehyde) (Figure 6(c)). Comparing the molecular vibration spectra of the pairs OCT/d_{17}-OCT and NIT/ALD, one finds that there is a single feature that is overlapping between d17-OCT and NIT and is not there in OCT or ALD. This is the resonance of the C–D stretch vibration at 2150 cm^{-1} in d_{17}-OCT which happens to coincide with the stretch vibration of the triple bond C≡N in NIT (Figure 6(a),(b)). Being conditioned to avoid deuterium, flies seem to be repelled also by the nitrile functional group. The control experiment where flies are conditioned to avoid hydrogenated OCT showed no effect between NIT and ALD, since both contain C–H bonds. If instead flies are conditioned to avoid NIT with respect to ALD, again they showed significantly stronger avoidance of d_{17}-OCT against OCT (Figure 6(d)).

Summarizing, this fundamental work showed distinguishability between isotopomers by spontaneous preference; differential conditioning of one isotopomer against the other enhanced the effect; a generalization of avoidance of deuterium or hydrogen was found in otherwise unrelated odours. Finally, an odour preference between an aldehyde-nitrile pair could be induced by conditioning to avoid deuterium which has a common vibrational resonance with the nitrile, but not the aldehyde.

These results were verified by Bittner and colleagues [59], who in a comparable T-maze setup first checked single isotopomers of acetophenone for avoidance in naïve flies. They found significant differences between two groups: ACP/d_3-ACP on one hand and d_5-ACP/d_8-ACP on the other, but not within these groups.

The distinguishability within these groups was then checked by negatively enforced learning experiments, where single isotopomers were again connected to mild electric shocks. In a following test experiment, learned and new isotopomers were directly confronted in the T-maze arms. This again amplified effects and showed clear distinguishability also between ACP and d_3-ACP, as well as between d_5-ACP and d_8-ACP.

A different paradigm was used to test isotope discrimination in the honeybee *Apis mellifera* [88]. Positive odour conditioning was tested via the proboscis extension reflex (PER) [89]. Bees were mounted in front of an odourant stimulus generator. For the training, animals were exposed to odourant pulses loosely followed by a reward in the form of a drop of sucrose solution. The animals responded to this reward by extending their proboscis to ingest the sugar. Repeating this procedure, one observes a proboscis extension already in response to the odourant stimulus. The learning curve reaches a maximum success probability usually after 5 repetitions. Memory tests can then be applied at arbitrary time delays by applying the positively conditioned odourant stimulus, and then counting the proboscis extensions.

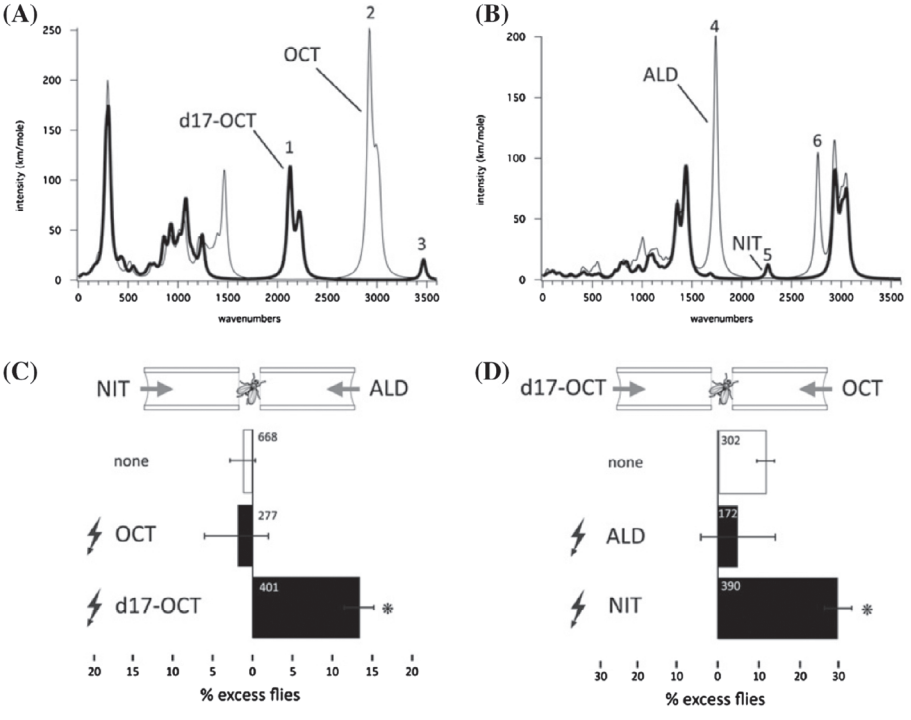


Figure 6. *Drosophila* can be conditioned to selectively avoid a vibrational frequency. Computed vibrational spectra of 1-octanol isotopomers: OCT vs. d_{17}-OCT (a) and citronellal (ALD) vs. citronellyl nitrile (NIT) (b). In NIT the aldehyde carbonyl stretch around 1750 cm^{-1} is absent, replaced by a nitrile stretch around 2150 cm^{-1}. The low-lying aldehyde C.H stretch vibration is also absent. The vibration band centered at 2150 cm^{-1} is the only one common to d_{17}-octanol and NIT but not present in OCT or ALD. (c) *Drosophila* selectively avoid the molecular vibrations of deuterium. Flies conditioned to selectively avoid d_{17}-OCT exhibited strong preferential avoidance of NIT ($p < 0.001$ vs. naïve), but flies punished to OCT did not selectively avoid ALD ($p = 0.691$ vs. naïve). The only common element potentially recognizable in the test odour pair to aid in selective avoidance is the overlap in the vibrational spectrum of the C–D bonds in d_{17}-OCT and the C≡N triple bond in NIT as illustrated in ((a),(b)). In contrast, they were not selective toward a novel odour without any recognizable molecular features. (d) In the converse experiment, flies conditioned to selectively avoid NIT exhibited highly significant avoidance of d_{17}-OCT as a testing odour ($p < 0.001$ vs. naïve), but flies punished to ALD did not selectively avoid OCT ($p = 0.999$ vs. naïve). Figure from [87].

Gronenberg and colleagues [88] used the PER paradigm to test whether honeybees could distinguish between isotopomer pairs of the same odourant. Therefore, the paradigm was extended to test differential conditioning, where positively conditioned odour stimuli were alternated pseudo-randomly with non-rewarded stimuli with a second odourant. Counting PER responses to both odourants allowed evaluation of whether the unrewarded odour could be distinguished from the rewarded one.

Testing differential learning between isotopomer pairs, they showed clear distinction between normal and fully deuterated acetophenone in both cases, with ACP or d_8-ACP rewarded. The same result was achieved between BZA

and d_7-BZA and between OCT and d_{17}-OCT. Sample purity was measured by gas-chromatography to be $\geq$ 99.1%. To further test whether impurities could be the basis for the distinguishability of the single odour stimuli, differential learning was tested with artificially impure samples. When ACP was rewarded and alternated with unrewarded stimuli of ACP mixed with 0.5% BZA, bees did not recognize the difference and responded identically with proboscis extension also to the unrewarded stimuli. The same effect was observed when rewarded BZA was alternated with unrewarded stimuli of BZA with 0.5% ACP.

4.1.3. Neuroimaging

Behaviour is a complex readout and gives no information on how single olfactory receptors (ORs) might respond to isotopomer pairs. To assess a potential isotopomer distinction at the level of the ORs, Paoli and colleagues studied via in vivo brain imaging the neural representation of isotopomers in the primary coding and processing centres of the olfactory pathway in honeybees, the antennal lobes [90]. They recorded and confronted spatio-temporal response patterns of 4 natural odours with isotopomers of varying degree of deuteration, from complete (ACP vs. d_8-ACP), strong (OCT vs. d_{17}-OCT), medium (BZA vs. d_5-BZA), to weak (isoamyl acetate, ISO: $C_7H_{14}O_2$ vs. d_3-ISO: $C_6H_{11}CD_3$).

To record brain activity, they used two-photon excited fluorescence microscopy with calcium-sensitive fluorescent markers [91]. Those markers exhibit a shift in the absorption spectrum if calcium binds to the molecules. Injecting these markers via the axonal tracts of the antennal lobe output neurons, one achieves a selective staining of the antennal lobe glomeruli. When single glomeruli are then activated by the corresponding OR type, neuronal action potentials cause an influx of calcium ions, which induces abrupt fluorescence changes. Calcium imaging via two-photon microscopy allows recording of glomerular response patterns with high spatio-temporal resolution and minimal photodamage, which is ideally suited for a minimally invasive recording of even subtle changes in the odour code of the insect brain. Bees were exposed to a sequence of odour pulses of all 4 isotopomer pairs produced by an multichannel olfactometer, synchronized with the image acquisition [92]. In a sample of 11 bees the odour response code was recorded in 19 glomeruli.

Between normal and deuterated isotopomers drastic changes in glomerular responses could be observed, where glomerular activity between isotopes in some cases even switched from activation to inhibition. Averaging response differences over all bees gave for OCT vs. d_{17}-OCT significant differences in 6 out of 19 glomerular responses, for BZA vs. d_5-BZA 6 significant differences as well, for ACP vs. d_8-ACP 2 differences, and for ISO vs. d_3-ISO one glomerulus responded differently (Figure 7(a),(b)). An interesting aspect is that the glomeruli that responded differentially were not the same for the different odourant pairs. This speaks for a general mechanism underlying this effect, which is not limited to single OR types.

To quantify the influence of these single receptor effects on the overall odour code which drives behavioural responses, a Euclidean distance was calculated in the glomerular coding space. It showed an equidistant distribution of chemically unrelated odourants. Similar odourants like benzaldehyde and acetophenone, sharing a benzene ring, showed a reduced distance. If the distance is calculated between isotopomer pairs of the same odourant, it turns out to be close to those of chemically similar but distinguishable odourants for the full to medium deuteration, where OCT and d_{17}-OCT show the strongest separation in coding space (Figure 7(d)). Only the weakly deuterated d_3-ISO was apparently indistinguishable from the natural isotopomer, which is demonstrated by fully overlapping activation curves in a coding space that was reduced to 3 dimensions via principal component analysis (Figure 7(c)).

A further analysis involved the molecular vibration spectra of the applied odourants, which was directly measured from the olfactometer via FT-IR spectroscopy. A quantitative analysis was performed on the spectral window containing C–H and C–D stretch vibrations (2000−3300 cm^{-1}). As a basic measure for the distinguishability of the spectra of isotopomers, absorption spectra were subtracted and the area below this difference spectrum was calculated. Plotting distinguishability of the odour code against these spectral differences, Paoli and colleagues found a clear correlation between both.

Finally, the fast brain imaging setup made it possible to dispel the objection that distinguishability of isotopomers could be caused by perireceptor events, due to which different isotopomers might arrive at the receptors at different rates. Looking at the kinetics of single glomerular responses, in all cases, including those with differential responses, the signal onset was found to be identical for the isotopomer pairs within the experimental resolution of 20 ms.

4.1.4. Electrophysiology

To study effects at the direct output of the olfactory receptors in *Drosophila*, Drimyli and colleagues [77] use electroantennography (EAG) to measure the depolarization currents of all ORNs activated by an odour stimulus. They chose as stimuli isotopomer pairs with different functional groups: simple alcohols (1-hexanol, HEL: $C_6H_{13}OH$ and d_2-/ d_5-/ d_{13}-HEL), aromatic ketones (acetophenone, ACP and d_3-/ d_5-/ d_8-ACP), aldehydes (benzaldehyde, BNZ and d_6-BZA), simple ketone (2-hexanone, HEN: $C_6H_{12}O$ and d_5-HEN), and nitriles (benzonitrile, BNL: C_7H_5N and d_5-BNL), all at various concentrations (0.01%, 0.1%, and 1% v/v).

They found the maximum amplitudes of the depolarization currents to be consistently different between isotopomers at the lower dilutions (0.1% and 1%). Interestingly, HEL always evoked larger EAG amplitudes than d_{13}-HEL and HEN larger ones than d_5-HEN. In contrast, BNZ yielded the opposite result with respect to d_6-BNZ. BNL isotopomer pairs showed no significant differences,

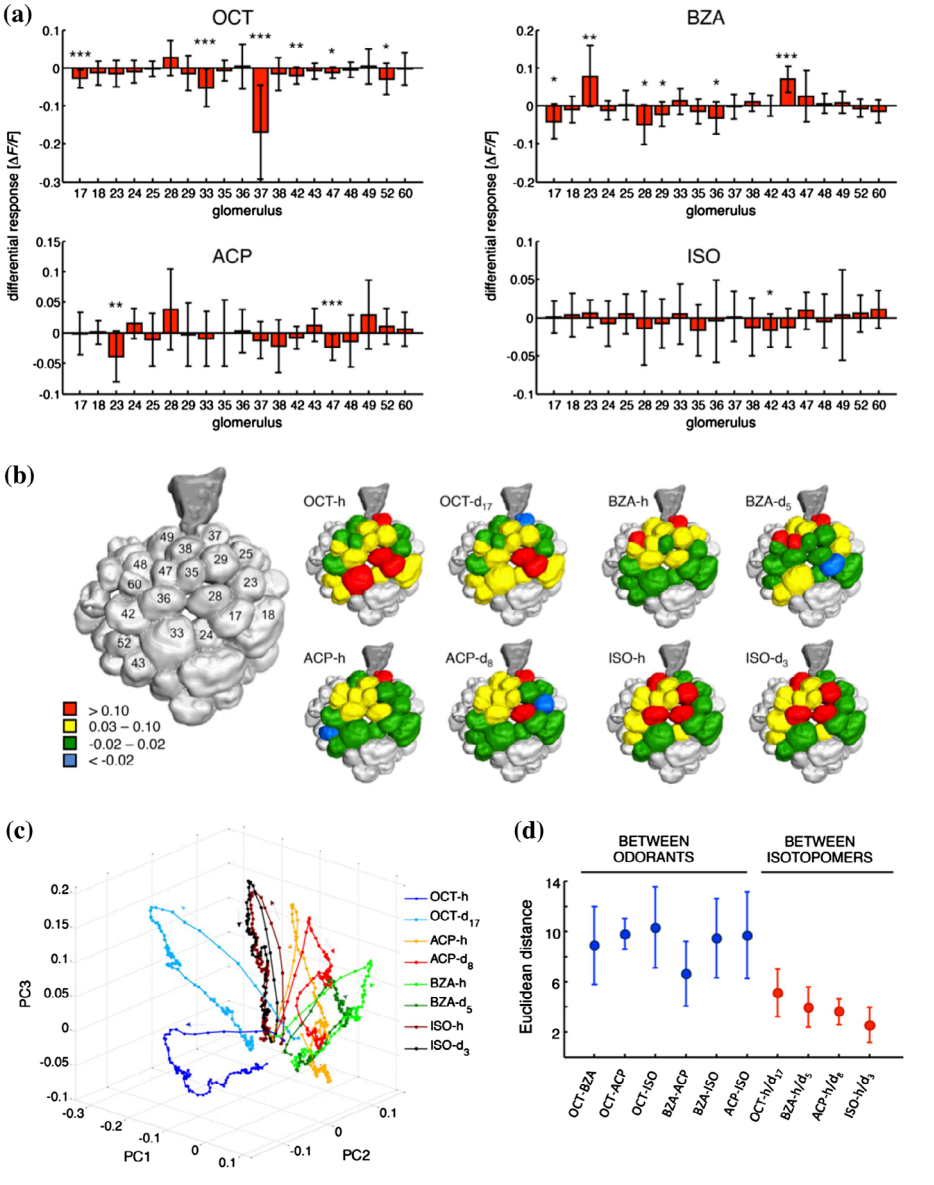


Figure 7. (a) Mean response difference across subjects between the responses of single glomeruli to H- and D-isotopomers (±s.d.) (paired-sample *t*-test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$). (b) Odourant-induced response maps classified in 4 groups: high activation: $-\Delta F/F > 0.10$ (red); low activation: $0.03 \leq -\Delta F/F \leq 0.10$ (yellow); no activity: $-0.02 \leq -\Delta F/F \leq 0.02$ (green); inhibition: $-\Delta F/F < -0.02$ (blue); glomeruli in grey were not included in the analysis. (c) PCA of AL response dynamics elicited by the odourants during 1 s stimulus exposure and 1 s post-stimulus phase. Arrows indicate the temporal order of signal build-up and decay. (d) Mean Euclidean distances ± s.d. between different pairs of odourants (blue) and within pairs of isotopomers (red). Figure from [90]

which could be explained by the fact that the C≡N resonance coincides with the C–D stretch, so this vibrational feature is present in both isotopomers.

A next important result was that the degree of deuteration did not affect the EAG amplitude: d_2-HEL and d_{13}-HEL evoked similar signals, but distinct from the normal HEL. The same was true for d_3-ACP and d_8-ACT against the normal ACP. This contradicts the assumption that a reduced volatility of heavier molecules is causing the isotopomer difference.

Also here the hypothesis was tested according to which isotopomer-specific differences could depend on perireceptor mechanisms. The main enzymes responsible for biotransformation and detoxification in the *Drosophila* antennae are of the cytochrome P450 family (CYP) [93,94]. If biotransformation is required before OR engagement, or afterwards to clear the odourant from the perireceptor space, deuterated odourants could be processed at a different rate from the normal isotopomers causing differential EAG properties and amplitudes. Therefore, CYPs were inhibited using a piperonyl butoxide (PBO) based protocol [95], but this did not alter the isotopomer-specific differential responses.

Finally, Drimyli and colleagues performed EAG studies with further members of the genus *Drosophila*: *D. simulans*, *D. pseudoobscura*, and *D. virilis*. They found that the direction of all differential EAG response was conserved. For odourants HEL and BNL, even EAG amplitude differences were identical.

4.1.5. Experimental constraints

In the above experiments, various approaches were used to evaluate a possible bias due to odour impurities. However, Paoli et al. have shown in a very recent study, that even a marginal odour contamination can alter the response of single receptors drastically [96]. They presented different isotopomers of benzaldehyde of highest commercially available purity (> 99%) to fruitflies, while imaging the antennal lobe response via calcium-sensitive fluorescence microscopy. An initial experiment using conventional stimulus presentation suggests a differential response between isotopomers in at least one type of OR. They identified Or42b as a likely candidate and produced via the GAL4/UAS system [97,98] a transgenic fly line, in which only Or42b-expressing cells are marked with a calcium-sensitive fluorophore, to optically isolate the response of Or42b. To now study a potential influence of impurities in the odourant samples, a gas-chromatography (GC) column is included into the setup, between odourant source and microscope, such that different volatile compounds arrive at the fly antennas at different times. In this way they found indeed that the differential response to benzaldehyde isotopomers in Or42b was caused by a minute impurity that was invisible by conventional GC field ionization detection. Identification and concentration calibration allowed to quantify the relative impurity concentration to be only 6 ppm. These findings are of high importance beyond the test of vibration theories. Since all experiments that study the selectivity and sensitivity of olfactory receptors should use GC-purified stimuli, to fully exclude the influence of sample impurities.

4.1.6. Discussion

The reviewed experiments study differences in isotopomer odour representations at very different processing levels. *In vivo* calcium imaging at the antennal lobe levels resolves single receptor type activities which represent the odour response code [90,96], while electroantennography compared the sum of all ORs responding directly to odourant stimuli [77]. Finally, T-maze experiments [59,87] and proboscis extension reflex paradigms [88] measure behavioural responses elicited by an odourant after it was identified and evaluated in higher order brain centers. Behavioral methods also evaluate whether isotopomers could be distinguished in differential learning paradigms with positive (PER) or negative (T-maze) conditioning.

All experiments but one [96] agree on the fact that fruitflies as well as honeybees are able to distinguish isotopomer pairs of the same odourant. While at the level of the receptor activation no dependence on the deuteration grade could be seen, at the level of the antennal lobes and the spontaneous behavioural responses, higher deuteration grade seems to amplify effects.

Besides this distinguishability between single isotopomers, the T-maze experiment of Franco and colleagues [87] uses cross-learning experiments to show that a generalization of odourants containing deuterium happens in the brain. They further showed that responses might be correlated to single spectral features, because animals associated odourants with overlapping vibrational resonances. This last finding was confirmed by the EAG experiments where the only isotopomer pair that did not show significant response differences was a nitrile, where the normal isotopomer also had a vibrational resonance at the wavelength of the C–D stretch.

These across-odour learning effects are also a strong argument against the theory that isotopomer distinguishability can be solely caused by different impurities in the samples. Since impurities cannot explain how animals generalize the presence of deuterium or the presence of single vibrational features as shown by Franco et al. [87]. In the study of Gronenberg et al. [88] artificial impurities were added to test their relevance to behavioural responses. In the differential learning experiments honeybees did not distinguish between pure and impure odourants.

Another common argument against molecular vibration dependence is that perireceptor events might depend on the different physical properties of isotopomers and therefore cause the odourants to arrive or leave the receptors at different rates. Here imaging experiments as well as electroantennography could show that there was no measurable difference in the signal kinetics [77,90]. Drimyli and colleagues [77] could further show that a blocking of enzymes most involved in perireceptor events in *Drosophila* antennae did not change the differential EAG response to isotopomers.

The neuroimaging study in the honeybee brain [90] resolved the isotopomer response pattern for single glomeruli, each receiving direct inputs from a single

type of OR. In addition, differential responses between normal and deuterated isotopes were observed. These differences were glomerulus- and odourant-dependent. The fact that most of the glomeruli showed isotopomer-dependent activation for some odourants indicates that the underlying mechanism must be a general feature of olfactory receptors. The fact that for each odourant only a fraction showed significant isotopomer differences seems to confirm the so called swipe-card model [53,57] according to which ORs are probing both chemical as well as vibrational features of an odourant. Still, the recent findings by Paoli et al. [96] on the influence of minute impurities on single OR responses suggest to repeat these experiments with gas-chromatographically purified stimuli.

4.2. Mammals

Isotope smell differences in humans have a chequered history. Turin [21] claimed to have detected a small odour difference between acetophenone isotopes which was not seen in other tests [99]. More extensive tests (Gane, personal communication) revealed a small difference which would have required hundreds of subjects, if ever, to reach possible statistical significance. Acetophenone is, in hindsight, a non-ideal odourant to test this in humans, because it causes rapid habituation and may possibly have other pharmacological effects [100]. The choice of deuterated odourants was for a long time restricted by commercial availability of deuterated compounds. They are mostly required as solvents for NMR and therefore typically cover a smallish range of molecular weights. Gane et al. decided to approach the problem differently, availing themselves of recently discovered efficient deuterium exchange reactions catalysed by metals under mild conditions [101]. The aim was to: use a commercially available, nominally pure odourant containing as many hydrogens as possible; come as close to perdeuteration as possible; and assess the smell character of GC-pure samples.

In light of the chemical selectivity of the deuterium exchange, a macrocyclic ketone was chosen, cyclopentadecanone, comprising 28 hydrogens [102]. This is a powerful odourant with a very distinctive, musky odour character. Further, it lies in the upper range of size for human odourants, which means in only binds to a small number of receptors, probably no more than 2 or 3 [103,104]. Since smelling peaks as they come out of a gas chromatograph is a difficult skill, a peak capture method invented by Christina Zelano was used, akin to preparative GC but on a microscale. The results conclusively showed an odour character difference perceptible by untrained subjects [75]. One of us (LT) was a subject and reports that the odour difference was very marked insofar as the musky odour character was absent from the nominally perdeuterated samples, replaced by a harsh (often described as waxy) burnt-candle smell. Interestingly, some subjects rejected for the test because of anosmia to cyclopentadecanone could nevertheless smell the deuterated compound, suggesting that more than one receptor is involved. The conclusion Gane et al. came to was that musk

odour character is essentially monochromatic: one of the very few receptors able to bind musks is sensing a fingerprint region band around 1400 cm^{-1}. This offers a belated explanation for the intense musk smell of nitrobenzenes bearing bulky substituents, the nitro groups providing intense vibrations around 1400 cm^{-1} and the bulky substituents narrowing the number of receptors to the musk receptor alone.

4.3. Individual receptors

The experiments on insects discussed above span a range of levels of analysis from glomerulus through antennae to behaviour, while those on mammals have all been behavioural. However, none of these experiments tells us what individual receptors do. This is problematic as it leaves ambiguity in the analysis of the observations: what is due to receptors, and what is due to other events?

A number of groups have engaged in detailed experiments on the response of individual receptors [105,106]. As we are focusing on experimental data that are produced using isotopes to distinguish between vibrational and docking mechanisms, we here focus on the recent set of experiments performed by Block et al. [58]. The team addressed several aspects of the vibrational theory. The extensive and useful critique of the theory will be discussed below as part of the general discussion of the vibrational theory.

The experiments involve the expression of a set of human and mouse olfactory receptors in kidney cells, and then measuring their response to a range of molecules, both deuterated and non-deuterated. The signal was light produced by luciferase in response to receptor activation. In every case the measured response of the cells was the same regardless of hydrogen isotope used. The natural conclusion, and the one drawn by the authors of the paper, is that receptors are blind to isotope, and hence vibrational frequency. Consequently, any perceived difference in odour that results from deuteration must be associated with other events.

Known ways that deuteration could influence the olfactory percept have been considered, and controlled for, in the more recent insect experiments described above. As we have seen, so far they have not been able to explain the differences between iosotopes. So we have an impasse that requires new experiments if it is to be overcome. The central question is: is the absence of difference in response is a genuine feature of the receptors (in which case the vibrational theory is not correct), or is it an artefact of how the experiments were performed (in which case the vibrational theory could be correct)?

We here note that several aspects of this study are open to question. First, the significance level (expressed as a *p*-value) of their observations is .05, which translates as only 3:1 chance of a real effect [107]. Second, they claim to have used deuterated musks of a higher purity than those used by Gane et. al, but did not report whether the isotopologs smell the same or different [108]. They claim this is not relevant [109]; we respectfully disagree on the grounds that if a difference

in odour had been detected, then an explanation is needed to account for the discrepancy between the two sets of observations.

5. Critique of the mechanisms

Having laid out the basic facts about both the docking theory and the vibrational theory of Turin, and having reviewed the key experimental evidence, we can now assess the relative merits of the proposed theories. We note from the outset that a fully watertight argument is not possible as there are still large gaps in the experimental landscape.

5.1. Docking theory

The docking theory of molecular recognition has two features immediately in its favour: everyone agrees that it makes at least a partial contribution (odourants still have to occupy receptors even for the vibrational mechanism to operate) [21,53]; there are huge numbers of other receptors (including GPCRs) that are accepted to operate in this way [7]. However it has difficulties explaining some observations, and possesses theoretical difficulties.

Soon after the discovery that olfactory receptors are GPCRs [1] it was recognised that multiple receptors are involved in the characterisation of one odour, and that any given receptor is involved in the identification of multiple molecules [26]. The normal understanding of receptors that operate through a docking mechanism is that there needs to be precise alignment of one molecule with one receptor, allowing a good induced fit [110]. This follows from the need for an alternative configuration of the receptor to be stabilized by the presence of the ligand to which it is tuned, but not by other ligands. Even if just one component of the ligand is key to the stability (e.g. some group that binds strongly to a metal ion [111]), the rearrangement could be sensitive to the remainder of the molecule.

It is difficult to reconcile these two observations. One attempt to find a way forward was to suggest that receptors only bind to parts of the odourant: this is the odotope theory [112]. The positive side of this theory is that odour appears to be a characteristic of chemical groups [111] (for example -SH or -NH_2 groups have odours associated with them), and thus the receptors could bind these groups. It is not clear how this operates in practice though [52]. In addition, following this purely structural approach has not been successful at predicting odour [40].

As we have seen, experiments have been performed on insects and mammals in which H is replaced in the odourant by D. The key idea is that this changes vibrational frequencies, but not structure. The ability of humans to distinguish isotopes by their odour has proven highly controversial [58,75,99], and there is no settled view. However, in insects the evidence that isotopes can be distinguished by smell is much stronger, as shown above. That isotopes can be distinguished in this way poses a significant challenge to the docking theory. It has been

noted that deuteration changes chemical reaction rates [58] (the kinetic isotope effect [113]), and increases bond strengths while reducing bond lengths. The relative importance of the various effects we discussed earlier. This could directly influence the receptors by changing the dynamics of the receptor reorganization after the arrival of the odourant, though the details of how this would proceed are not yet clear. Deuteration might influence olfaction if the odourants experience chemical reactions in the mucus layer coating the cilia of the olfactory neurons, and thus modify the products entering the receptor [58]. This is, however, rather speculative at present having no direct experimental support, and even some evidence against it, as discussed above.

Special cases need to be treated with caution: are they representative of general trends, or curiosities? However, we note that there are some individual cases that have been put forward as being problematic for the docking theory.

(1) Ferrocene and nickelocene have nearly the same structure (a pair of five membered carbon rings with a metal ion sandwiched between them), but distinct odours [21]. Because of the change in size and mass, different metal atoms give different frequencies for those vibrations that involve the metal atoms [114]. This observation is clearly compatible with the vibrational theory.
(2) Molecules with an -SH, -SeH or -TeH group share a common odour character, which is not held in general by other molecules. While not possessing structural similarity, decaborane ($B_{10}H_{14}$) does have a similar odour [21], and this correlates with their similar vibrational frequencies: H–S stretch at about 2550 cm^{-1}, and about 2600 cm^{-1} for a B-H stretch [114]. It is natural to conclude that the similarity in odour is connected to the shared vibrational frequency.

5.2. Vibrational theory

The problem may not be with the idea that component parts of molecules define odour character, but rather how these parts are characterized. Indeed, it underlies the vibrational theory [115,116], which appears promising [117]. We stress again that the vibrational theory considered here is sensitive to odourant structure as the odourant must reside in the receptor for its vibrational spectrum to be analyzed. The presence of zinc in the receptor may help bind odourants [111], but the limitations of a purely structural approach [40] can be overcome by the additional information acquired from the vibrational spectrum. Similarly, the limitations of a pure vibrational theory, such as being unable to distinguish enantiomers [50], are removed by the structural restraints imposed by the need for residency [118].

The strongest argument in favour of the vibrational theory is that it accounts for observations not obviously explained by the alternatives. The original observation that odour relates to chemical groups, even when attached to various

other chemical components, is one case. As discussed above, another clear case is the ability for flies, and possibly humans, to distinguish deuterated from hydrogenated odourants. As discussed earlier, the large study by Block et al. [58] of the response of receptors grown in kidney cells to deuterated odourants failed to find any ability of the receptors to recognize deuteration. While we acknowledge that receptor level experiments are precisely what is needed at this stage, and these results need a proper response, at this time we are unclear as to their meaning. The uniformly negative result does not provide enough information to distinguish between receptors unable to identify deuteration from an experiment that prevents the receptors from operating properly. Maybe less clear, but still evidence in favour, is the practical observation that humans can detect, and assign an odour percept to, molecules never previously encountered. This could be a consequence of all molecules having vibrational spectra. However, once we probe the consequences of assuming the inelastic tunneling mechanism of Turin, questions arise.

We specified the requirements for the mechanism to work early in this review. Do they in fact hold? Let us start with the most fundamental: do the electron donor and acceptor sites exist? They have never been observed, and without a very reliable structure for an olfactory receptor, it is hard to see how strong evidence can be acquired. However, there are clues. As explored earlier, examining the statistics of residues for sequenced receptors, we can see what is highly conserved, and what is not. According to Fuchs et al. (Figure 4(b)) [64], there is a highly conserved tryptophan in the middle of the fourth helix; this might act as a donor. Turin [21] identified a strongly conserved CGSHL sequence that might bind a Zn ion; according to Fuchs it appears in helix 6 in Figure 4(b). This might be the acceptor.

Computer calculations of the electronic structure should be a useful way to see if the proposed sites can take their suggested roles. However, they require a known structure to work with. In the absence of measured olfactory receptor structures, we are using bovine rhodopsin [119] as it exhibits a number of the characteristics we are looking for, as described above, namely:

(1) it is a GPCR;
(2) there is a small hydrophobic ligand bound to it (retinal);
(3) there are tryptophan and histidine residues close by the retinal;
(4) there is a Zn ion bound to the histidine.

We need a source of electrons to drive the receptor. While there is no definitive account of where they originate, we note that electron transfer occurs at multiple points within a cell. Thus, if electrons are needed, it is plausible that a way to deliver them could be determined. Related to this is the question of time scales. It has been estimated that the electron transfer takes of order 1 ns [53] which is far too short a time for the neurons to resolve. It has been shown [120], however, that by including at least part of the journey of the electron to the

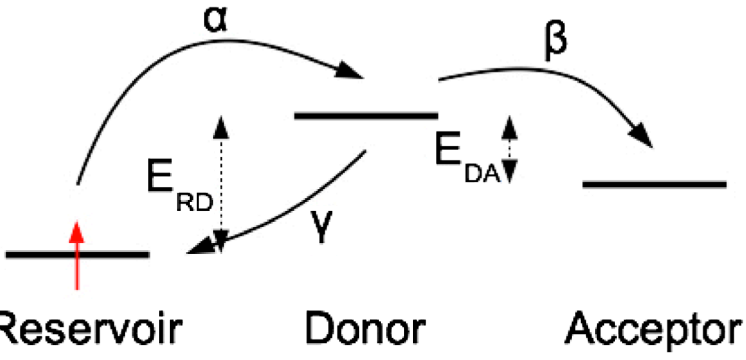


Figure 8. A cartoon of the model used to estimate the effect of the source of electrons (reservoir) on the rate at which an electron reaches the acceptor.

donor site, the same ability to distinguish between the presence and absence of the required vibrational frequency remains, but the overall time scales can be stretched significantly. We now present the mathematical argument more fully.

The model for the Turin theory of olfaction we have considered so far only treats the donor and acceptor explicitly. Here we consider, by means of a very simple model, the possible effect of including the source of electrons (reservoir) explicitly. The model system now has three parts (see Figure 8): a reservoir where an electron starts, an intermediate donor state, and a final acceptor state. We are interested in the rate at which an electron is transferred from the reservoir to the acceptor. The rate at which the electron moves from the reservoir to the donor is α, the rate at which it moves from the donor back to the reservoir is γ, and the rate at which it moves from the donor to the acceptor is β. There is no transfer of the electron from the acceptor back to the donor as we assume that there is some fast irreversible process that takes place once the electron reaches the acceptor (such as the release of the G-protein). If both α and γ are given by the standard Marcus rate equations we can easily show that they satisfy $\frac{\alpha}{\gamma} = \exp\left(-\frac{E_{RD}}{k_B T}\right)$, where E_{RD} is the energy difference between the reservoir and donor states (see Figure 8). The important point is that it is probable that $\alpha \ll \gamma$.

At some given time t the probability of the electron being in the reservoir is $R(t)$, on the donor is $D(t)$ and on the acceptor is $A(t)$. Let us define the probability vector $\vec{P}(t) = \big(R(t), D(t), A(t)\big)$. The master equation for the system is then $\frac{\partial \vec{P}}{\partial t} = M\vec{P}$ where

$$M = \begin{pmatrix} -\alpha & \gamma & 0 \\ \alpha & -\gamma - \beta & 0 \\ 0 & \beta & 0 \end{pmatrix} \tag{2}$$

The master equation can be solved exactly to find the occupation of the acceptor population as a function of time assuming the electron began in the reservoir

$$A(t) = \frac{\lambda_+}{\lambda_+ - \lambda_-}\left(1 - e^{\lambda_- t}\right) - \frac{\lambda_-}{\lambda_+ - \lambda_-}\left(1 - e^{\lambda_+ t}\right) \tag{3}$$

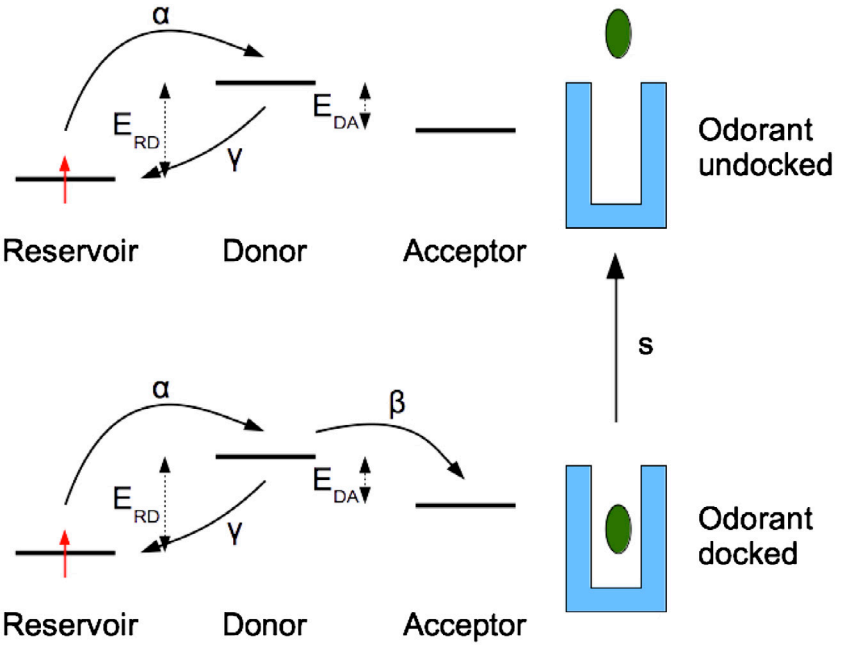


Figure 9. A cartoon of the model used to estimate the combined effect of the source of electrons and the lifetime of the odourant in the receptor on the rate at which an electron reaches the acceptor.

where λ_+ and λ_- are given by

$$\lambda_{\pm} = -\frac{1}{2}\left(\alpha+\beta+\gamma\right)\left[1 \pm \sqrt{1-\frac{4\alpha\beta}{\left(\alpha+\beta+\gamma\right)^2}}\right] \tag{4}$$

If we make the reasonable assumption that $\alpha \ll \beta, \gamma$ then we can simplify Equation (3) to

$$A(t) \approx 1 - \mathrm{e}^{-\alpha^* t} \tag{5}$$

where $\alpha^* = \alpha/(1+\frac{\gamma}{\beta})$. Thus we see that the rate of transfer of the electron from the reservoir to the acceptor is of the same order of magnitude as that for the escape of the electron from the reservoir, but can be substantially modulated by the other two rates even if the processes are very much faster. The rate that is responsible for selection in an olfactory receptor is β. If this varies by a factor of 2 or 3 between molecules that have a correct frequency and those that do not, this could lead to very substantial changes in the overall transfer rate, with the change in times possibly being of the order of ms, if that is the timescale for electron escape from the reservoir. Thus by considering more of the system we find that the timescales of the receptor can be brought into line with the rest of the signalling.

We have stated that the odourant must remain within the receptor long enough for detection to take place. We now try to quantify this by extending our

simple model. For the purpose of our calculation we assume that the removal of the odourant from the receptor makes it impossible for the electron to move from the donor to the acceptor (see Figure 9). If we set the escape rate for the odourant to be s, require that at $t = 0$ the odourant is docked and the electron is in the reservoir, assume α is small, and follow the mathematical steps given above we get

$$A(t) \approx \frac{\alpha^*}{\alpha^* + s}\left(1 - \mathrm{e}^{-(\alpha^*+s)t}\right) \tag{6}$$

If $s \ll \alpha^*$ we simply get our earlier result, corresponding to the lifetime of the odourant in the receptor being long compared to the electron transfer time. As s becomes comparable with α^* we see that the maximum probability of occupation of the acceptor drops below 1: once the odourant leaves the receptor the electron is no longer able to travel from the donor to the acceptor. Finally, we see that the rate at which the acceptor reaches its maximum population increases as a result of the odourant leaving the receptor as the time available for the transfer is reduced. Without a detailed atomistic model of a receptor, it is hard to give firm values to the relevant parameters. But it is very probable that the odourant is able reside in a receptor long enough for an electron transfer to take place (the shortest time the transfer could take place over being about 1 ns).

Once the electron reaches the acceptor, it must be able to generate a signal. Given we have rather limited knowledge about the structure of a receptor, once again we have to fall back on speculation. Turin [21] suggests that a disulphide bond between the receptor and the G protein is reduced, and then breaks, initiating the next stage of the signalling. A suitable cysteine residue exists in the Zn binding motif noted earlier.

The greatest difficulty for the Turin mechanism, and hence the source of the most pointed and careful criticism [58], has always been the need to keep the interaction of the itinerant electron with the environment sufficiently small [53]. The problem is simple enough to identify: we require a receptor that is a protein embedded in a lipid, surrounded above and below by aqueous media, to respond to the vibrations of a small odourant while ignoring the vibrations from the extended surrounding molecules. The contribution from the environment can be characterised by the reorganisation energy, which typically has a value of 1 eV. For good odourant recognition it needs to be below 0.1 eV [53,57,58]. Without a proper model of the olfactory receptor it is not really possible to compute a reliable value. However, there is a small amount of evidence from simulations by Reese et al. [63] that such low values might be achievable.

6. Conclusions

The jury is still out on what the precise mechanism is by which the chemical identity of odourant molecules is established by olfactory receptors. As discussed in this review, evidence is mounting both in support of some form of vibrational theory, and against it.

Experimental tests of vibrational theories have focused on regularities either of the functional group region or the fingerprint region. The most striking example of the former is the similarity in odour character between boron hydrides (boranes) containing the -BH group and thiols containing the -SH group. This was noted by Stock [121] in the early days of borane chemistry and rediscovered by one of us (LT) [21]. The fact that boranes and thiols alone smell 'sulfuraceous' and that they alone share a vibration around 2600 cm^{-1} seems beyond coincidence. Modification of the fingerprint region, by contrast, is most easily achieved by deuteration of part or all the hydrogens contained in the odourant. As we have seen, it has been shown that flies [87,122], honeybees [88,90], and humans [75] can tell hydrogen from deuterium odourants. Remarkably, a connection between fingerprint and functional group regions was also found when flies who had been trained to avoid deuterium odourants also avoided nitriles and vice versa, suggesting that the -CD stretch and -CN stretch, both around 2150 cm^{-1} were being confused by the flies [87].

However, the information from receptor experiments is far less supportive. In particular the study by Block et al. [58] suggests that receptors are unable to distinguish between hydrogenated and deuterated versions of a molecule, even when they have different odours. The difficulty though is that the absence of any positive signal carries ambiguity with it, as it could be an artefact of the experimental technique. So more experiments are needed to bring clarity.

For physicists, maybe the single most important development that can be envisaged is the production of a high quality X-ray structure of a one or more olfactory receptors. This would open the door to large scale computer simulations that would help shed light on both the mechanical and electrical responses of olfactory receptors.

Notes

1. The system would not be in equilibrium if any empty orbital were lower in energy than a filled orbital. This configuration thus has to be seen as a state arrived at kinetically: the lower energy state is able to pass its electron onto another site more quickly than it can be refilled.

Acknowledgements

Useful conversations with Seogjoo Jang during the writing of this article are gratefully acknowledged.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

APH gratefully acknowledges funding from the Leverhulme Trust [grant number RPG-2014-125] and support from the Thomas Young Centre [grant number TYC-10]. AH gratefully

acknowledges funding from the Province of Trento (Research unit IBRAIM) and the Province of Bolzano (B26J16000310003). LT gratefully acknowledges funding from the Stavros Niarchos Foundation.

ORCID

A. P. Horsfield http://orcid.org/0000-0003-4533-666X
A. Haase http://orcid.org/0000-0002-8324-0047
L. Turin http://orcid.org/0000-0003-0878-6696

References

[1] L. Buck and R. Axel, Cell 65 (1991) p.175.
[2] K. Palczewski, T. Kumasaka, T. Hori, C. Behnke, H. Motoshima, B. Fox, I.L. Tong, D. Teller, T. Okada, R. Stenkamp, M. Yamamoto and M. Miyano, Science 289(5480) (2000) p.739.
[3] O. Man, Y. Gilad and D. Lancet, Protein Sci. 13 (2004) p.140.
[4] C.D. Hanlon and D.J. Andrew, J. Cell Sci. 128 (2015) p.3533.
[5] R. Strotmann, K. Schröck, I. Böselt, C. Stäubert, A. Russ and T. Schöneberg, Mol. Cell. Endocrinol. 331 (2011) p.170.
[6] S. Jang and C. Hyeon, J. Phys. Chem. B 121 (2017) p.1304. doi:10.1021/acs.jpcb.7b00486.
[7] K. Kristiansen, Pharmacol. Ther. 103 (2004) p.21.
[8] C.A. de March, S.K. Kim, S. Antonczak, W.A. Goddard and J. Golebiowski, Protein Sci. 24 (2015) p.1543.
[9] R. Sounier, C. Mas, J. Steyaert, T. Laeremans, A. Manglik, W. Huang, B. Kobilka, H. Dmn and S. Granier, Nature 524 (2015) p.375.
[10] A. Manglik and B. Kobilka, Curr. Opin. Cell Biol. 27 (2014) p.136.
[11] B. Kobilka, Biochim. Biophys. Acta 1768 (2007) p.794.
[12] A.J. Venkatakrishnan, X. Deupi, G. Lebon, C.G. Tate, G.F. Schertler and M.M. Babu, Nature 494 (2013) p.185.
[13] A. Couto, M. Alenius and B.J. Dickson, Curr. Biol. 15 (2005) p.1535.
[14] H. Kazama, Neuroscience 296 (2015) p.3.
[15] M.E. Denno, E. Privman, B.J. Venton and A.C.S. Chem, Neurosci. 6 (2015) p.117. doi:10.1021/cn500261e.
[16] M. Stengl and N.W. Funk, J. Comp. Physiol. A 199 (2013) p.897. doi:10.1007/s00359-013-0837-3.
[17] L.B. Vosshall, Proc. Nat. Acad. Sci. USA 112 (2015) p.6525.
[18] O. Susumu, *Evolution by Gene Duplication*, Springer, Berlin, 1970.
[19] G.C. Conant and K.H. Wolfe, Nat. Rev. Genet. 9 (2008) p.938.
[20] L. Turin, Inference 2 (2016).
[21] L. Turin, Chem. Senses 21 (1996) p.773.
[22] E. Block, V.S. Batista, H. Matsunami, H. Zhuang and L. Ahmed, Nat. Prod. Rep 34 (2017) p.529.
[23] R. Moncrieff, *The Chemical Senses*, John Wiley and Sons, New York, NY, 1944.
[24] L. Pauling, Chem. Eng. News. Arch. 24 (1946) p.1064.
[25] J. Amoore, Nature 199 (1963) p.912.
[26] B. Malnic, J. Hirono, T. Sato and L. Buck, Cell 96 (1999) p.713.
[27] E.A. Hallem and J.R. Carlson, Cell 125 (2006) p.143.
[28] D. Münch and C.G. Galizia, Sci. Rep. 6 (2016) p.21841.

[29] Y. Miao and J. McCammon, Curr. Opin. Struct. Biol. 41 (2016) p.83.
[30] K. Stierand, P. Maaß and M. Rarey, Bioinformatics 22 (2006) p.1710.
[31] M.S. Kim, A. Repp and D.P. Smith, Genetics 150 (1998) p.711.
[32] R. Bentley, Chem. Rev. 106 (2006) p.4099.
[33] J.C. Leffingwell. Available at: http://www.leffingwell.com/chirality/chirality.htm.
[34] W. Li, J.D. Howard, T.B. Parrish and J.A. Gottfried, Science 319 (2008) p.1842.
[35] J. McConathy and M.J. Owens, Prim. Care Companion J. Clin. Psychiatry 5 (2003) p.70.
[36] C. Linster, B.A. Johnson, E. Yue, A. Morse, Z. Xu, E.E. Hingco, Y. Choi, M. Choi, A. Messiha and M. Leon, J. Neurosci. 21 (2001) p.9837.
[37] C. Sell, *Chemistry and the Sense of Smell*, John Wiley and sons, Hoboken, NJ, 2014.
[38] A. Arzi and N. Sobel, Trend. Cognit. Sci. 15 (2011) p.537.
[39] A. Manglik, T. Kim, M. Masureel, C. Altenbach, Z. Yang, D. Hilger, M. Lerch, T. Kobilka, F. Thian, W. Hubbell, R. Prosser and B. Kobilka1, Cell. 161 (2015), p.1101.
[40] C.S. Sell, Angew. Chem. Int. Ed. 45 (2006) p.6254.
[41] L. Turin and F. Yoshii, *Handbook of Olfaction and Gustation*, 2nd ed., CRC Press Boca, Boca Raton, FL, 2003, p.275.
[42] W. Ogle, Med. Chir. Trans. 53 (1870) p.263.
[43] G.M. Dyson, Perfumery essent Oil Record 28 (1937) p.13.
[44] G. Malcolm Dyson, J. Soc. Chem. Industry. 57 (1938), p.647.
[45] R.H. Wright, Nature 209 (1966) p.571.
[46] R.H. Wright, Experientia 31 (1975) p.530.
[47] B.R. Havens and C.E. Meloan, *Developments in Food Science*, Vol. 37, Elsevier, 1995, p.497.
[48] C.E. Meloan, V.S. Wang, R. Scriven and C.K. Kuo, *Developments in Food Science*, Elsevier, 1988.
[49] R.H. Wright, Ann. N.Y. Acad. Sci. 116 (1964) p.552.
[50] T.J. Leitereg, D.G. Guadagni, J. Harris, T.R. Mon and R. Teranishi, J. Agric. Food Chem. 19 (1971) p.785.
[51] I. Kufareva, M. Rueda, V. Katritch, G. Dock, R.C. Stevens and R. Abagyan, Structure 19 (2011) p.1108.
[52] H.L. Klopping, J. Agric. Food Chem. 19 (1971) p.999.
[53] J.C. Brookes, F. Hartoutsiou, A.P. Horsfield and A.M. Stoneham, Phys. Rev. Lett. 98 (2007) p.038101.
[54] J. Lambe and R.C. Jaklevic, Phys. Rev. 165 (1968) p.821.
[55] A. Checinska, F.A. Pollock, L. Heaney and A. Nazir, J. Chem. Phys. 142 (2015) p.025102.
[56] R.A. Marcus, J. Chem. Phys. 24 (1956) p.966.
[57] I.A. Solov'yov, P.Y. Chang and K. Schulten, Phys. Chem. Chem. Phys. 14 (2012) p.13861.
[58] E. Block, S. Jang, H. Matsunami, S. Sekharan, B. Dethier, M.Z. Ertem, S. Gundala, Y. Pan, S. Li, Z. Li, S.N. Lodge, M. Ozbil, H. Jiang, S.F. Penalba, V.S. Batista and H. Zhuang, Proc. Nat. Acad. Sci. 112 (2015) p.E2766.
[59] E.R. Bittner, A. Madalan, A. Czader and G. Roman, J. Chem. Phys. 137 (2012) p.22A551.
[60] C.G. Don and S. Riniker, J. Comput. Chem. 35 (2014) p.2279. doi:10.1002/jcc.23757.
[61] S. Sekharan, M. Ertem, H. Zhuang, E. Block, H. Matsunami, R. Zhang, J. Wei, Y. Pan and V. Batista, Biophys. J. 107 (2014) p.L5.
[62] L. Gelis, S. Wolf, H. Hatt, E.M. Neuhaus and K. Gerwert, Ange. Chem. Int. Ed. Engl. 51 (2012) p.1274.
[63] A. Reese, N. List, J. Kongsted and I. Solov'yov, PLoS ONE 11 (2016) p.e0152345.

[64] T. Fuchs, G. Glusman, S. Horn-Saban, D. Lancet and Y. Pilpel, Human Genet. 108 (2001) p.1.
[65] H. Jia, O.M. Pustovyy, Y. Wang, P. Waggoner, R.J. Beyers, J. Schumacher, C. Wildey, E. Morrison, N. Salibi, T.S. Denney, V.J. Vodyanoy, G. Deshpande, Chem. Senses 41 (2015) p.53.
[66] T.N.C. Wells, M. Stedman and R.J. Leatherbarrow, Ultramicroscopy 42 (1992) p.1200.
[67] M.Y. El-Naggar, G. Wangerb, K.M. Leung, T.D. Yuzvinskya, G. Southame, J. Yang, W.M. Laud, K.H. Nealson and Y.A. Gorby, Proc. Nat. Acad. Sci. 107 (2010) p.18127.
[68] R. Pethig and A. Szent-Györgyi, Proc. Nat. Acad. Sci. 74 (1977) p.226.
[69] M. Takeuchi, Z. Makita, R. Bucala, T. Suzuki, T. Koike and Y. Kameda, Mol. Medicines 6 (2000) p.114.
[70] R. Cecil and P.D.J. Weitzman, Biochem. J. 93 (1964) p.1.
[71] Z.H. Pan, X. Zhang and S.A. Lipton, Neuroscience 98 (2000) p.333.
[72] S.G.F. Rasmussen, B.T. DeVree, Y. Zou, A.C. Kruse, K.Y. Chung, T.S. Kobilka, F.S. Thian, P.S. Pil, Seok Chae, E. Pardon, D. Calinski, J.M. Mathiesen, S.T.A. Shah, J.A. Lyons, M. Caffrey, S.H. Gellman, J. Steyaert, G. Skiniotis, W. Weis, R.K. Sunahara and B.K. Kobilka, Nature 477 (2011) p.549.
[73] G.A. Jeffrey, J.R. Ruble, R.K. McMullan and J.A. Pople, *The crystal structure of deuterated benzene*, In *Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, Vol. 414, The Royal Society, London, 1987, p.47.
[74] D.J. Lacks, J. Chem. Phys. 103 (1995) p.5085.
[75] S. Gane, D. Georganakis, K. Maniati, M. Vamvakias, N. Ragoussis, E.M.C. Skoulakis and L. Turin, PLoS One 8 (2013) p.e55780.
[76] A.B. Foster, Trend. Pharmacol. Sci. 5 (1984) p.524.
[77] E. Drimyli, A. Gaitanidis, K. Maniati, L. Turin, and E.M.C. Skoulakis, eNeuro. 3 (2016), p.ENEURO-0152.
[78] G. Nemethy and H.A. Scheraga, J. Chem. Phys. 41 (1964) p.680.
[79] U.B. Kaupp, Nat. Rev. Neurosci. 11 (2010) p.188.
[80] K. Sato, M. Pellegrino, T. Nakagawa, T. Nakagawa, L.B. Vosshall and K. Touhara, Nature 452 (2008) p.1002.
[81] R. Vassar, S.K. Chao, R. Sitcheran, J.M. Nunez, L.B. Vosshall and R. Axel, Cell 79 (1994) p.981.
[82] J. Joerges, A. Küttner, C.G. Galizia and R. Menzel, Nature 387 (1997) p.285.
[83] G. Laurent and H. Davidowitz, Science 265 (1994) p.1872.
[84] M. Paoli, N. Weisz, R. Antolini and A. Haase, Eur. J. Neurosci. 44 (2016) p.2387.
[85] C.G. Galizia, Eur. J. Neurosci. 39 (2014) p.1784.
[86] A. Haase, E. Rigosi, E. Frasnelli, F. Trona, F. Tessarolo, C. Vinegoni, G. Anfora, G. Vallortigara and R. Antolini, Eur. Biophys. J. 40 (2011) p.1247.
[87] M.I. Franco, L. Turin, A. Mershin and E.M.C. Skoulakis, Proc. Nat. Acad. Sci. USA 108 (2011) p.3797.
[88] W. Gronenberg, A. Raikhelkar, E. Abshire, J. Stevens, E. Epstein, K. Loyola, M. Rauscher, and S. Buchmann, Proc. R. Soc. London B: Biol. Sci.. 281 (2014), p.20133089.
[89] M.E. Bitterman, R. Menzel, A. Fietz and S. Schäfer, J. Comp. Psychol. 97 (1983) p.107.
[90] M. Paoli, A. Anesi, R. Antolini, G. Guella, G. Vallortigara and A. Haase, Sci. Rep. 6 (2016) p.21893.
[91] A. Haase, E. Rigosi, F. Trona, G. Anfora, G. Vallortigara, R. Antolini and C. Vinegoni, Biomed. Opt. Express 2 (2010) p.131.
[92] M. Paoli, M. Andrione and A. Haase, *Imaging techniques in insects*, In *Lateralized Brain Functions: Methods in Human and Non-Human Species*, 1st ed., chapter 16, L.J. Rogers and G. Vallortigara, eds. Springer, New York, NY, 2017, p.471.

[93] Q. Wang, G. Hasan and C.W. Pikielny, J. Biol. Chem. 274 (1999) p.10309.
[94] F. Younus, T. Chertemps, S.L. Pearce, G. Pandey, F. Bozzolan, C.W. Coppin, R.J. Russell, M. Maïbèche-Coisne and J.G. Oakeshott, Insect. Biochem. Mol. Biol. 53 (2014) p.30.
[95] S.P. Wang, X.X. Hu, Q.W. Meng, S.A. Muhammad, R.R. Chen, F. Li and G.Q. Li, Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol. 166 (2013) p.7.
[96] M. Paoli, D. Münch, A. Haase, E. Skoulakis, L. Turin and C.G. Galizia, eNeuro 4 (3) (2017), ENEURO.0070-17.2017. "Significantly to Olfactory Receptor Ligand Studies: Tales from Testing the Vibration Theory".
[97] H. Kakidani and M. Ptashne, Cell 52 (1988) p.161.
[98] N. Webster, J.R. Jin, S. Green, M. Hollis and P. Chambon, Cell 52 (1988) p.169.
[99] A. Keller and L.B. Vosshall, Nat. Neurosci. 7 (2004) p.337.
[100] H.J. Sanders, H.F. Keag and H.S. McCullough, Ind. Eng. Chem. 45 (1953) p.2.
[101] T. Maegawa, Y. Fujiwara, Y. Inagaki, H. Esaki, Y. Monguchi and H. Sajiki, Angew. Chem. Int. Ed. 47 (2008) p.5394.
[102] A.S. Williams, Synthesis 1999 (1999) p.1707.
[103] A.N. Gilbert and S.E. Kemp, Chem. Senses 21 (1996) p.411.
[104] K. Nara, L.R. Saraiva, X. Ye and L.B. Buck, J. Neurosci. 31 (2011) p.9179.
[105] H. Saito, Q. Chi, H. Zhuang, H. Matsunami and J.D. Mainland, Sci. Signal. 2 (2009), p.ra9.
[106] J.D. Mainland, A. Keller, Y.R. Li, T. Zhou, C. Trimmer, L.L. Snyder, A.H. Moberly, K.A. Adipietro, W.L.L. Liu, H. Zhuang, S. Zhan, S.S. Lee, A. Lin and H. Matsunami, Nat Neurosci 17 (2014) p.114. doi:10.1038/nn.3598.
[107] D. Colquhoun, bioRxiv (2017). Available at: http://www.biorxiv.org/content/early/2017/08/07/144337.
[108] L. Turin, S. Gane, D. Georganakis, K. Maniati and E.M. Skoulakis, Proc. Nat. Acad. Sci. 112 (2015) p.E3154.
[109] E. Block, S. Jang, H. Matsunami, V.S. Batista and H. Zhuang, Proc. Nat. Acad. Sci. 112 (2015) p.E3155.
[110] D.E. Koshland Jr, Proc. Natl. Acad. Sci 44 (1958) p.98.
[111] M. Zarzo, Biol. Rev. 82 (2007) p.455.
[112] K. Mori and G. Shepard, Semin. Cell. Biol. 5 (1994) p.65.
[113] L. Melander and W.H.S. Jr, *Reaction Rates of Isotopic Molecules*, Wiley, New York, NY, 1980.
[114] K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds, in Handbook of Vibrational Spectroscopy*, John Wiley & Sons, New York, 2006.
[115] G. Dyson, Chem. Ind. 57 (1938) p.647.
[116] R.H. Wright, *The Science of Smell*, George Allen and Unwin, London, 1964.
[117] S.Y. Takane and J.B.O. Mitchell, Org. Biomol. Chem. 2 (2004), p.3250.
[118] J.C. Brookes, A. Horsfield, A. Stoneham and J.R. Soc, Interface 6 (2009) p.75.
[119] T. Okada, M. Sugihara, A. Bondar, M. Elstner, P. Entel and V. Buss, J. Mol. Biol. 342 (2004) p.571.
[120] J.C. Brookes, A.P. Horsfield and A.M. Stoneham, Sensors 12 (2012) p.15709.
[121] A. Stock and C. Massenez, Ber. Dtsch. Chem. Ges. 45 (1912) p.3539.
[122] E. Drimyli, A. Gaitanidis, K. Maniati, L. Turin and E.M. Skoulakis, Eneuro. (2016), p.ENEURO-0152.
[123] A. Horsfield, D. Bowler, H. Ness, C. Sanchez, T. Todorov and A. Fisher, Rep. Prog. Phys. 69 (2006) p.1195.

[124] M. Bixon and J. Jortner, *Electron transfer - from isolated molecules to biomolecules*, In *Electron Transfer - From Isolated Molecules to Biomolecules*, J. Jortner and M. Bixoneds. Advances in Chemical Physics. Vol. 106, John Wiley and Sons, 1999, p.35.

Appendix 1. Derivation of the vibrational rate equation

Possible mathematical models for the Turin mechanism have been discussed and criticized in some detail in the literature [53,57–59]. Here we present the core algebra again in a way that allows the various approaches to be treated in a unified way.

What we wish to compute is a rate for the transfer of an electron from D to A. To obtain this rate, we consider the following sequence of events based on the narrative given above:

(1) Initially the odourant M and the receptor are well separated from one another.
(2) M moves through the mucus and into the receptor so that it is situated between D and A.
(3) During this migration M equilibrates with its surroundings (a fast process).
(4) M enables the electron transfer from D to A by being vibrationally excited, possibly also acting as a bridge across which an electron can pass (a slow process).

The passage of the electron is slow relative to the motion of the atoms in the receptor, but fast relative to the time M remains docked between D and A. The electron transfer could be slow for up to three reasons: there is weak overlap of the electronic wavefunctions between the donor and acceptor sites; the mobile electron has to travel through the odourant, whose unoccupied states have high energy, and thus act as a tunnelling barrier. This slowness allows us to distinguish between forward and backward processes. For the forward process the odourant gains energy from the electron as it jumps from D to A. However, for the reverse process the odourant must provide the energy. Since the odourant will re-equilibrate with the receptor very rapidly after excitation [55], the reverse process is frustrated. Thus we can ignore any coherence between the electron jumping from D to A and the reverse process, and so can describe it in terms of a transition rate.

An appropriate theory for describing transitions is scattering theory, and because we want rates we use the generalized Fermi's Golden Rule [123]

$$\Gamma_{D\to A} = \frac{2\pi}{\hbar}\sum_{DA} P_D \left|\left\langle D\left|\hat{T}(E_D)\right|A\right\rangle\right|^2 \delta(E_D - E_A) \tag{A1}$$

where the states $|D\rangle$ and $|A\rangle$, and their associated energies E_D and E_A, correspond to the mobile electron being in the donor and acceptor sites respectively. They include both nuclear and electronic degrees of freedom, span the odourant M as well as the receptor and the more distant environment. There will also be a set of states $|M\rangle$ corresponding to the electron being localised on M, and they will have energies E_M. The transfer matrix $\hat{T}(E) = \hat{V} + \hat{V}\hat{G}(E)\hat{V}$ introduces the coupling $\hat{V}$ between the states $|D\rangle$, $|A\rangle$ and $|M\rangle$, and $\hat{G}(E)$ is the retarded Green's function for the whole system.

This Golden Rule expression in Equation (A1) contains the following contributions: a weighted sum over initial states corresponding to an incoherent average over independent initial possibilities (thereby giving us the average response of the system); the square of the matrix element of the transfer matrix originating with the rate at which the wavefunction evolves under a perturbing potential (the effective coupling between the donor and acceptor mediated by the odourant), and the energy conserving delta function combined with the sum over final states which determines the density of states into which the system can evolve. At thermal equilibrium the population of the initial states of the system is $P_D = \exp(-E_D/k_BT)/Z$ where $Z = \sum_D \exp(-E_D/k_BT)$ is a partition function.

The trickiest part of this argument is defining exactly what we mean by the states $|D\rangle$, $|A\rangle$ and $|M\rangle$. These states need to express the slowness of the mobile electrons relative to the atomic motion. This leads to all three sets of states being diabatic, with the itinerant electron being localised on D, A and M respectively. We will assume they can be well represented by a Born–Oppenheimer (BO) form $\langle rR \mid D\rangle \equiv \Psi_D(rR) = \Phi_D(rR)\chi_D(R)$ (and similarly for A and M), where r represents electronic degrees of freedom, R represents nuclear degrees of freedom, $\Phi_D(rR)$ is the diabatic electronic wavefunction, and $\chi_D(R)$ is the nuclear wavefunction.

A key quantity in Equation (A1) is the matrix element $\left\langle D \left| \hat{T}(E_D) \right| A \right\rangle$. If we introduce the full expression for the transfer matrix we get $\left\langle D \left| \hat{T}(E_D) \right| A \right\rangle = \left\langle D \left| \hat{V} \right| A \right\rangle + \left\langle D \left| \hat{V}\hat{G}(E_D)\hat{V} \right| A \right\rangle$. To keep just the lowest order terms in $\hat{V}$, we approximate $\hat{G}(E)$ by $\hat{G}_0(E)$ defined by

$$\hat{G}_0(E) = \sum_D \frac{|D\rangle\langle D|}{E - E_D + \mathrm{i}0^+} + \sum_A \frac{|A\rangle\langle A|}{E - E_A + \mathrm{i}0^+} + \sum_M \frac{|M\rangle\langle M|}{E - E_M + \mathrm{i}0^+} \tag{A2}$$

We now have two cases to consider. If the electron can travel from D to A without passing through M, we have $\left\langle D \left| \hat{T}(E_D) \right| A \right\rangle \approx \left\langle D \left| \hat{V} \right| A \right\rangle$. If, however, the electron cannot travel directly, but must spend time on M, we have

$$\left\langle D \left| \hat{T}(E_D) \right| A \right\rangle \approx \sum_M \frac{\left\langle D \left| \hat{V} \right| M \right\rangle \left\langle M \left| \hat{V} \right| A \right\rangle}{E_D - E_M + \mathrm{i}0^+} \tag{A3}$$

On going from D to A, an electron is added to the odourant. If this increases the energy by several eV, this contribution will dominate the contributions to the denominator from vibrational excitations, and we can replace the denominator by a constant for each state of the odourant. If we then make the Condon approximation, then both expressions for the transfer matrix reduce to the form

$$\left\langle D \left| \hat{T}(E_D) \right| A \right\rangle \approx W_{DA} \int \chi_D(R)\chi_A(R)\,\mathrm{d}R \tag{A4}$$

with the only difference being in the definition of W_{DA} [53]. The remaining mathematical steps leading to the Marcus–Jortner equation are now completely standard [124], and we obtain the expression given by Brookes et al. [53,57,58]:

$$\Gamma_{D\to A} = \frac{2\pi}{\hbar}|W_{DA}|^2 \frac{\sigma_n}{\sqrt{4\pi k_B T\lambda}} \exp\left(-\frac{(\epsilon_{DA} - n\hbar\omega_M - \lambda)^2}{4k_B T\lambda}\right) \tag{A5}$$

where k_B is the Boltzmann constant, T is the temperature, λ is the reorganization energy, σ_n is a measure of how strongly the itinerant electron couples to the nth vibration state of the mode in the odourant being probed, ϵ_{DA} is the difference in energy of the electron between D and A, and ω_M is the vibrational frequency for the odourant mode. We note that Solov'yov [57] generalised this to include all the vibrations in the odourant.