OLFACTION

RECEPTORS, LIGANDS, INTERACTION, PROCESSING

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Neli Prota – 20/03/08

INTRODUCTION

The Nobel Prize in Physiology or Medicine 2004

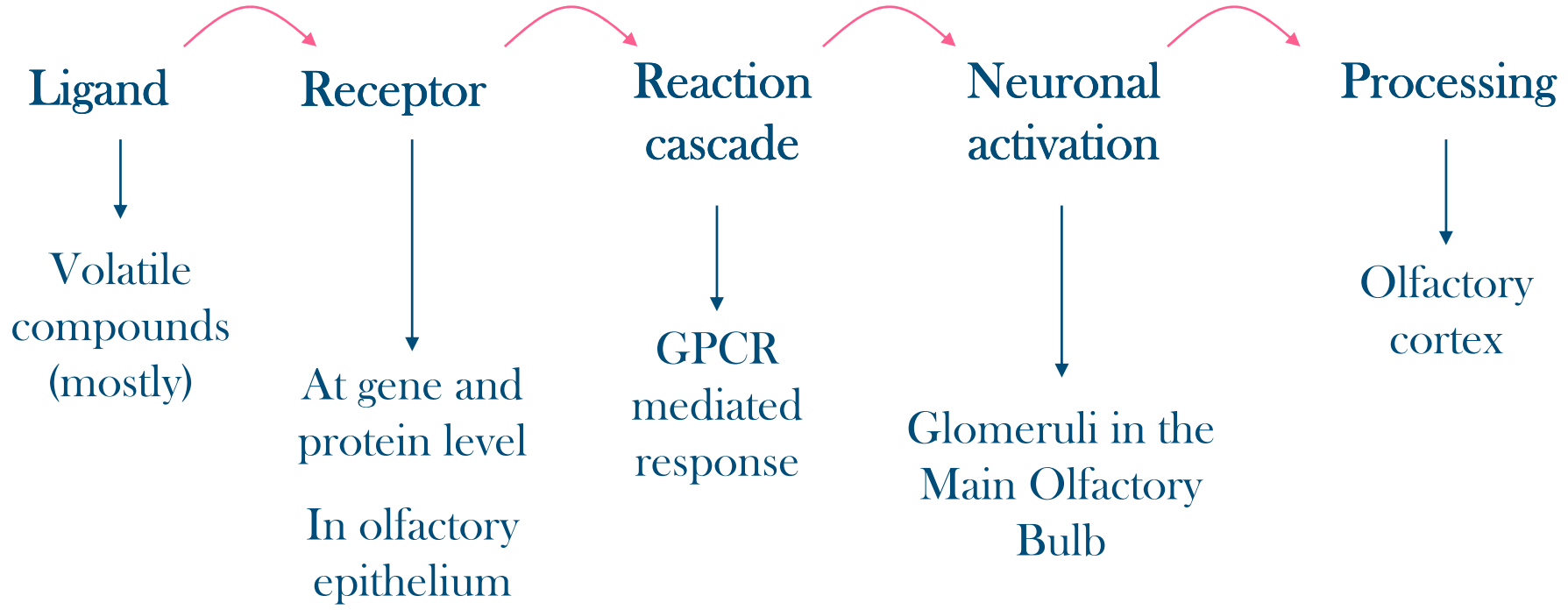
Richard Axel and Linda B. Buck

"for their discoveries of odorant receptors and the organization of the olfactory system"

Buck, L. and Axel, R. (1991) Cell, vol. 65, 175-187.

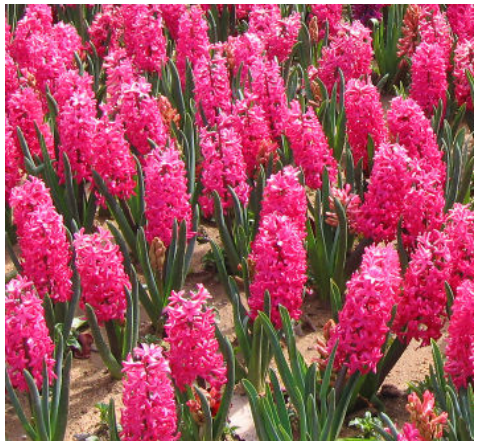
INTRODUCTION

ORIGIN OF ODOUR PERCEPTION: the chemical interaction of odorant receptors with volatile molecules is transformed into electrical signals that will carry information about the external world to the brain

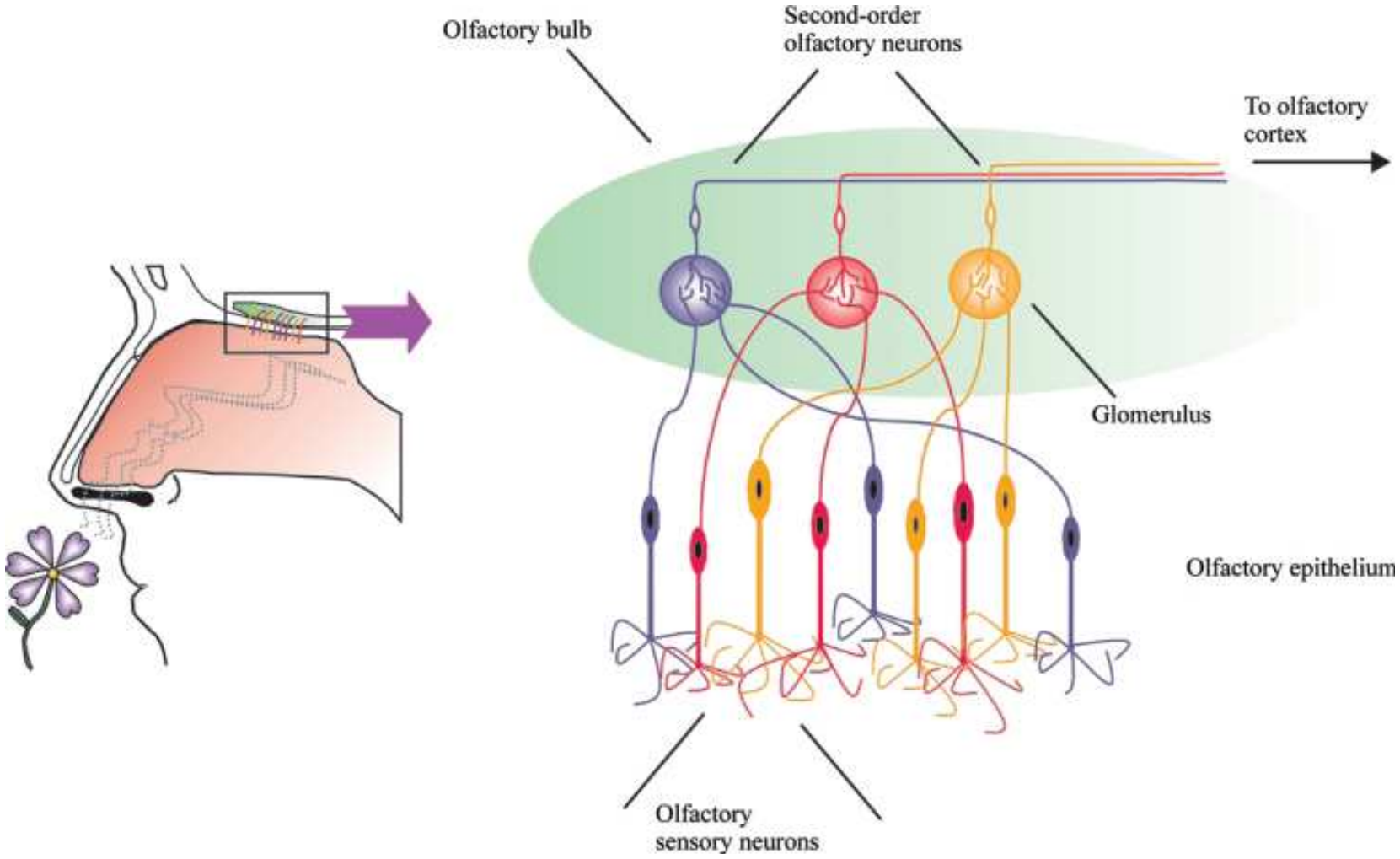


INTRODUCTION

*In humans, smell is rather considered to be an esthetic sense in contrast to most other species, which rely on olfaction to detect **food**, **predators** and **mates**; further more it helps avoiding eating toxic substances. Terrestrial animals, including humans, smell air-borne molecules, whereas aquatic animals smell water-soluble molecules with low volatility, such as amino acids.*



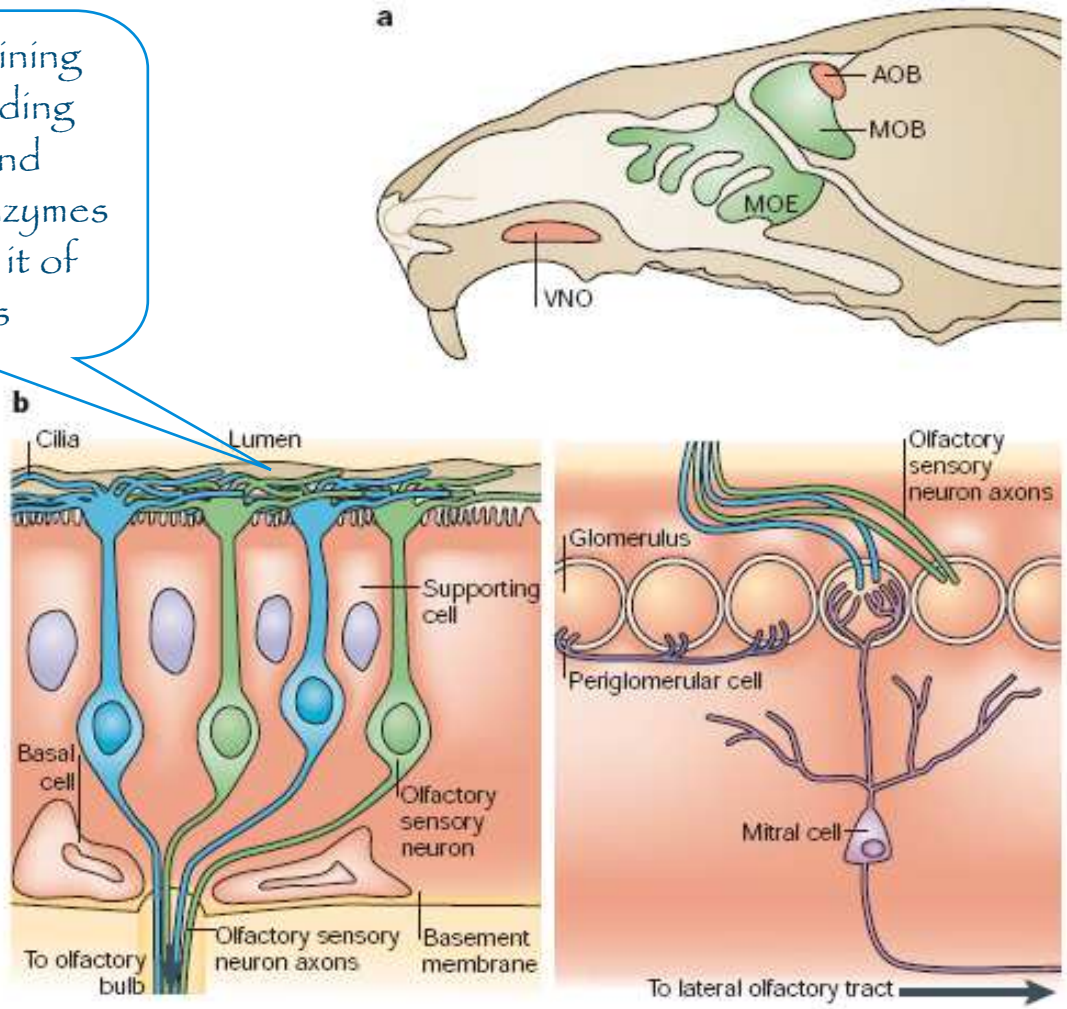
Anatomy of human olfactory system...



News Physiol Sci 19: 101104, 2004

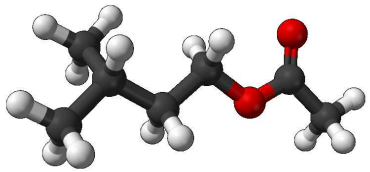
...and that of other mammals

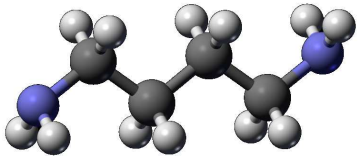
mucus containing odorant-binding proteins and detoxifying enzymes that cleanse it of odorants

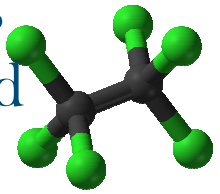


Odorants – an immense chemical variety

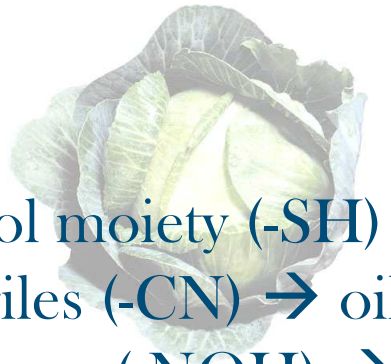
- Typically small organic molecules < 400 Da
- Can vary in shape, size, functional group and charge
- Examples:
  - Alchohols
  - Aliphatic acids
  - Aldehydes and ketones
  - Esthers
  - Amines
  - Chemicals with aromatic, alicyclic, polycyclic or heterocyclic ring structures
  - Substituted chemicals of these types and their combinations
- Subtle differences (even 2 enantiomers) can lead to pronounced differences in odour quality

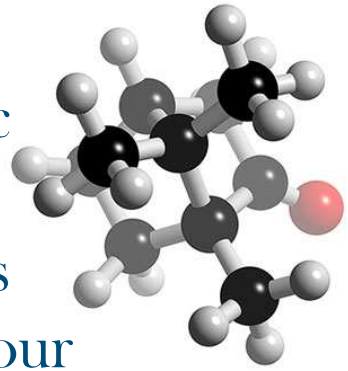






Odorants – Some examples



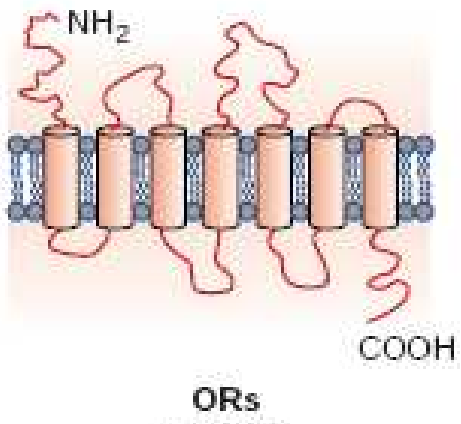


- Thiol moiety (-SH) → rotten eggs or garlic
- Nitriles (-CN) → oily-metallic character
- Oximes (-NOH) → green-camphoraceous
- Nitro groups ($-NO_2$) → sweet-ethereal odour
- Isothiocyanate (-NS) → mustardy smell
- Arsine groups (AsH_2) → cabbage
- Esters [-(C=O)-O-] → fruity
- Amines ($-NH_2$) → fishy-urinous odour

Olfactory Receptors (ORs)

- 1991 – discovery of large multigene family of ORs
- ~1000 genes encoding different types of ORs in mouse and human
- OR genes constitute the largest gene family in the vertebrate genome (2-7% of all genes)
- They belong to the G protein coupled receptors (GPCR) superfamily
- Located in the cilia of the olfactory sensory neurons (OSNs)
- Only one type expressed in each Olfactory Sensory Neuron (OSN)
- Monoallelic expression

Functional ORs initiate a feedback signal to ensure that no other OR gene will be expressed in each OSN

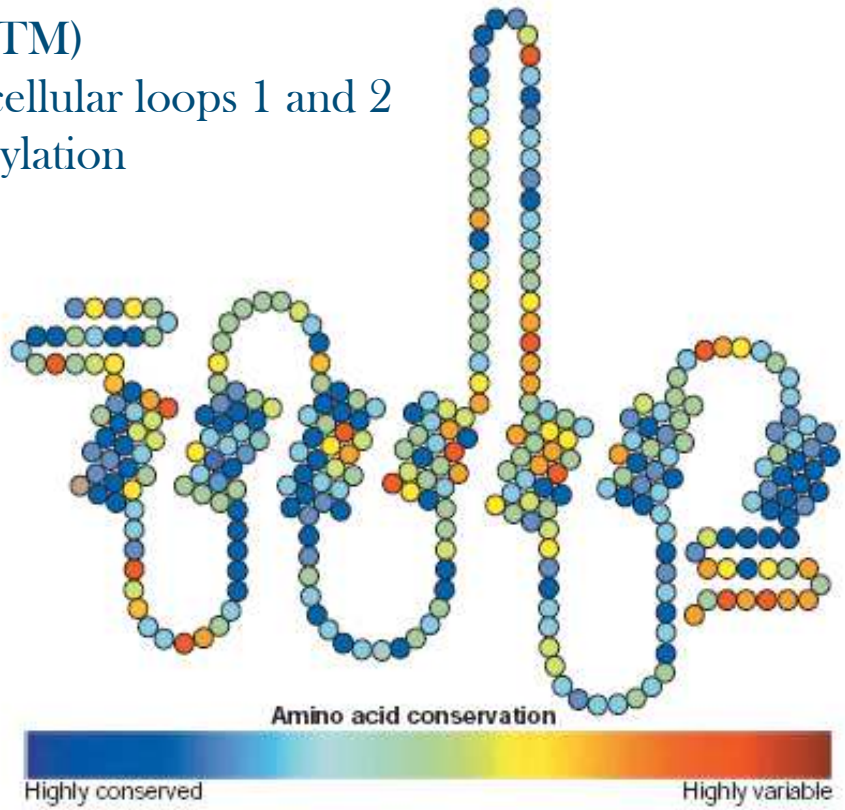


OR genes

- First detected in rat and then identified in other species by PCR with degenerate primers derived from conserved motifs
- The coding sequences of the OR are intronless
- Characterized in mammals, birds, amphibians, fish and invertebrates
- Tandemly organized into clusters in the genome
- Present on virtually every chromosome (not on 20 and Y in humans)
- Chromosome 11 >40% of all human OR genes
- In mammals 900-1500 members, in ancient vertebrates and invertebrates from 100 to few hundreds members
- In humans 70% pseudogenes → 300-400 functional genes
- In mouse only 20% pseudogenes → 1200 functional genes (3x more)
- Two main classes: I OR (*activated by water-soluble compounds*) and II OR (*activated by volatile odorants*)

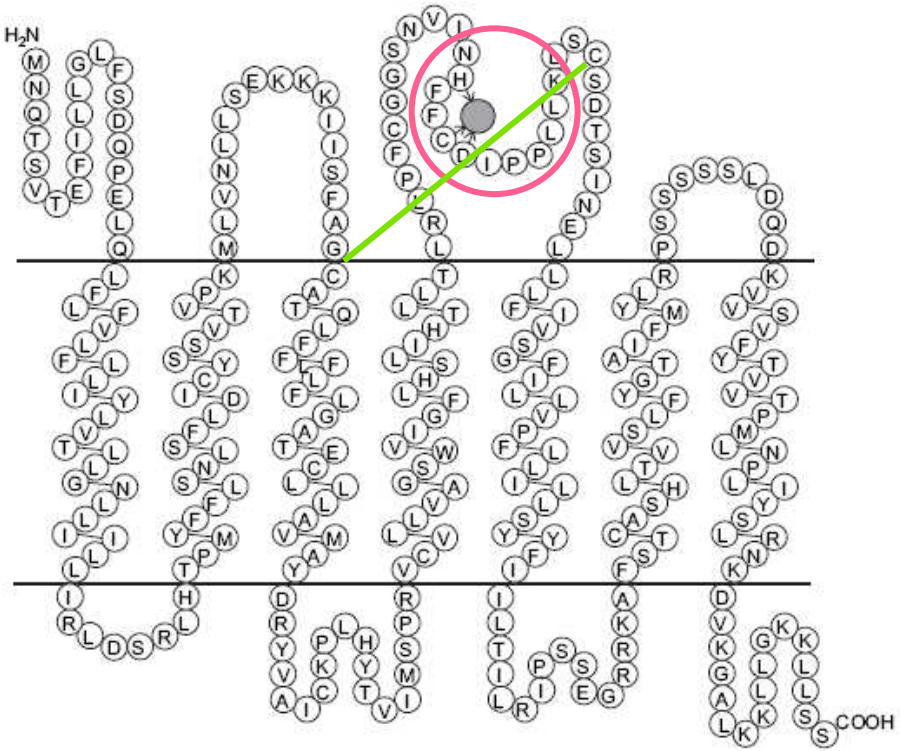
OR protein structure

- Circa 300-350 amino acids long
- Features common to all GPCRs:
  - 7 hydrophobic transmembrane domains (7TM)
  - Potential S-S bond between 2 Cys in extracellular loops 1 and 2
  - Conserved consensus for N-terminal glycosylation
- Many specific conserved motifs in TM domains 1, 2, 6 and 7
- Hypervariable region in TMD 3, 4 and 5 (odorant binding pocket) involved in the diversity of ligand recognition

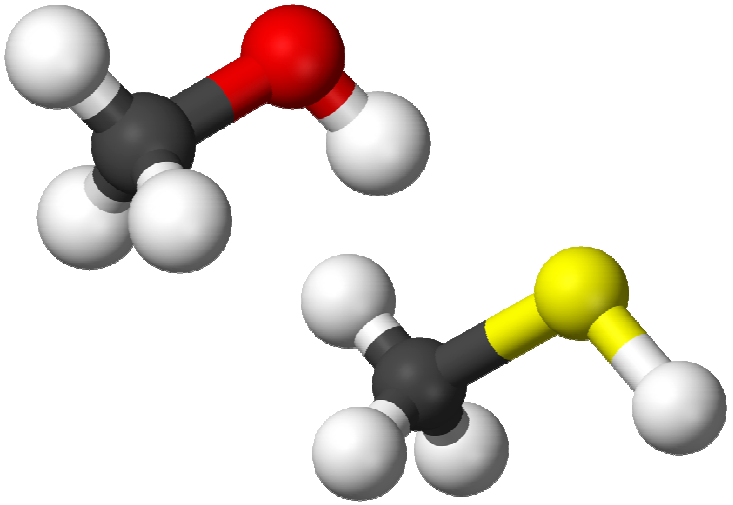


OR as metalloproteins

- According to some authors the extracellular loop could have a binding site for a metal ion (like Zn(II) for instance), which would interact with some odorants to mediate the response



Example:
difference in smell perception between -OH and -SH



Biol. Rev. (2007), 82, pp. 455–479

Matching odorants to cloned ORs

- It took 7 yrs from the discovery of ORs for the first unambiguous OR-ligand pair to be reported
- Co-expression of rat I7 receptor with GFP in rat olfactory mucosa (*in vivo*) trough adenoviral infection
- Electro-olfactograms used to record response
- 74 odorants tested
- Octanal was the main odorant eliciting response
- Later on
  - developed heterologous expression systems (Human Embrionic Kidney cells – HEK293)
  - Developed different assays such as *whole-cell patch clamp* and *Fura-2 Ca^{+} imaging*

ORs and odorants: structure

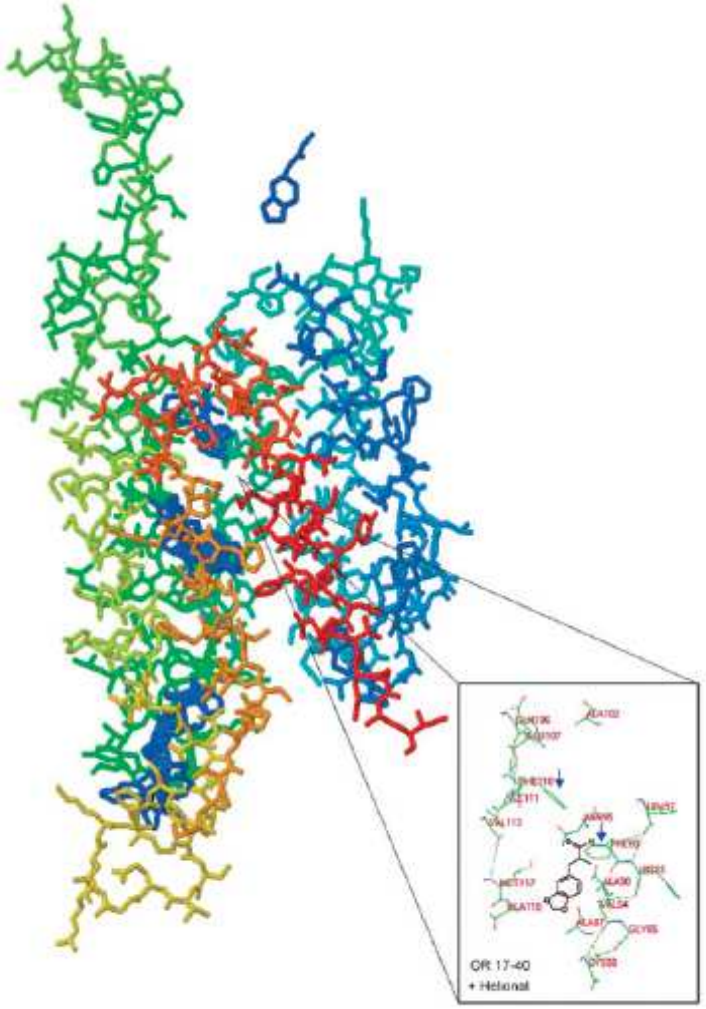
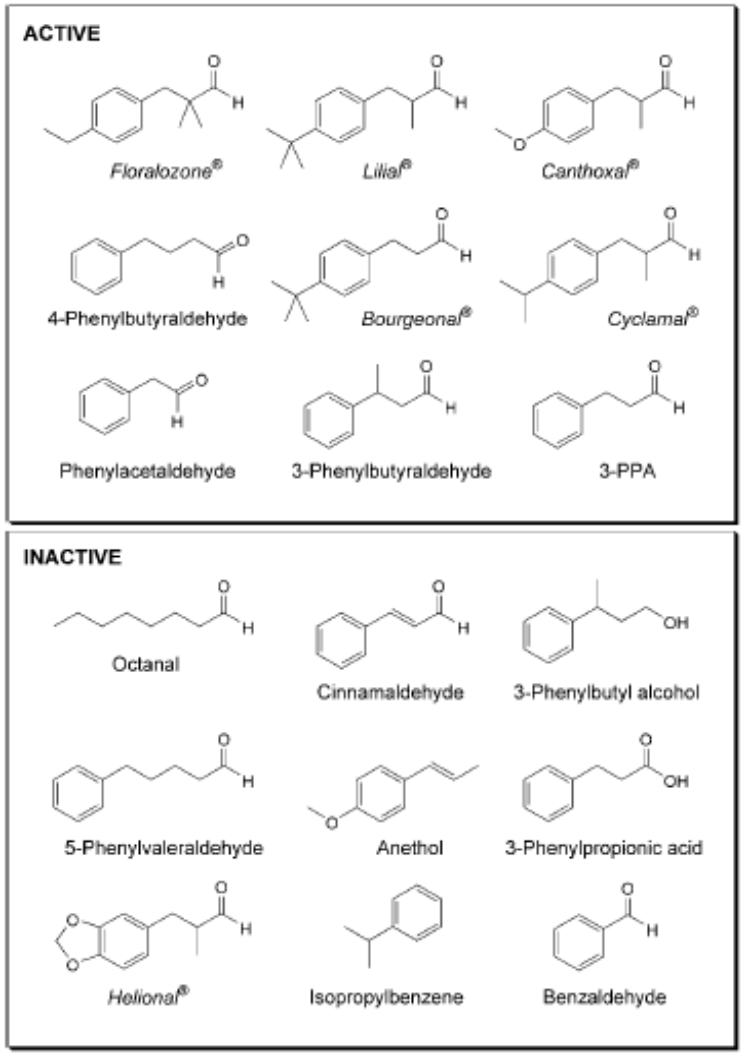


Fig. 7. *Molecular modeling of the human olfactory-receptor hOR17-40. A possible olfactory binding pocket for Helional® is shown in the box.*



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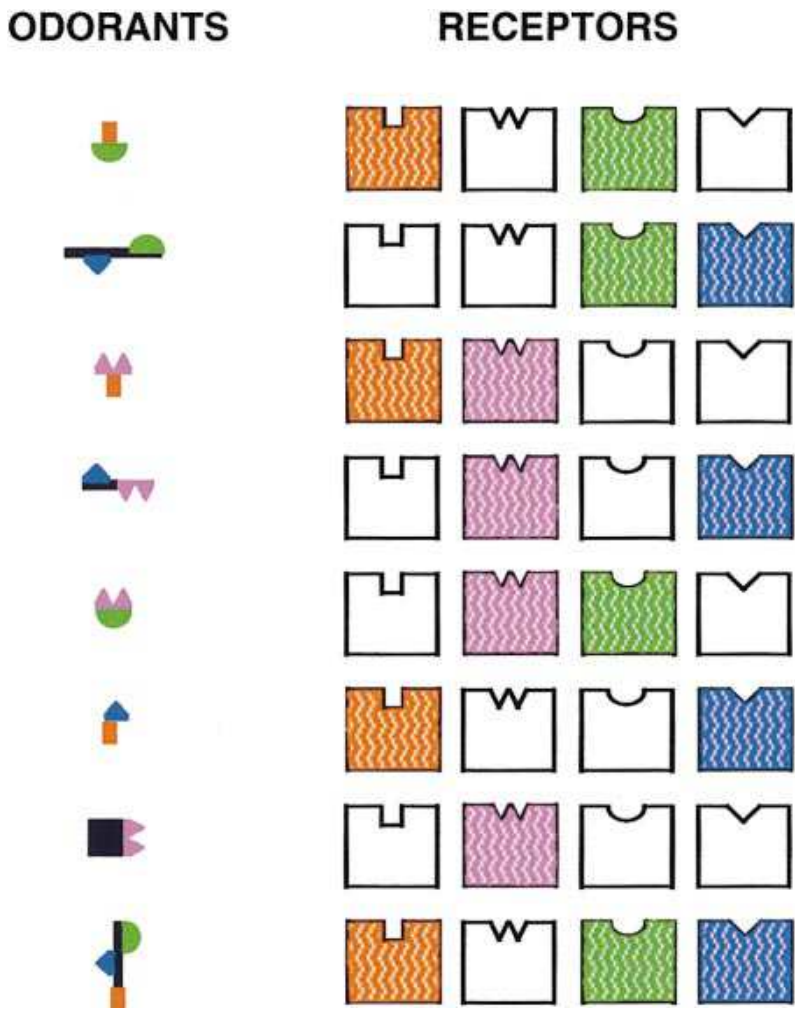
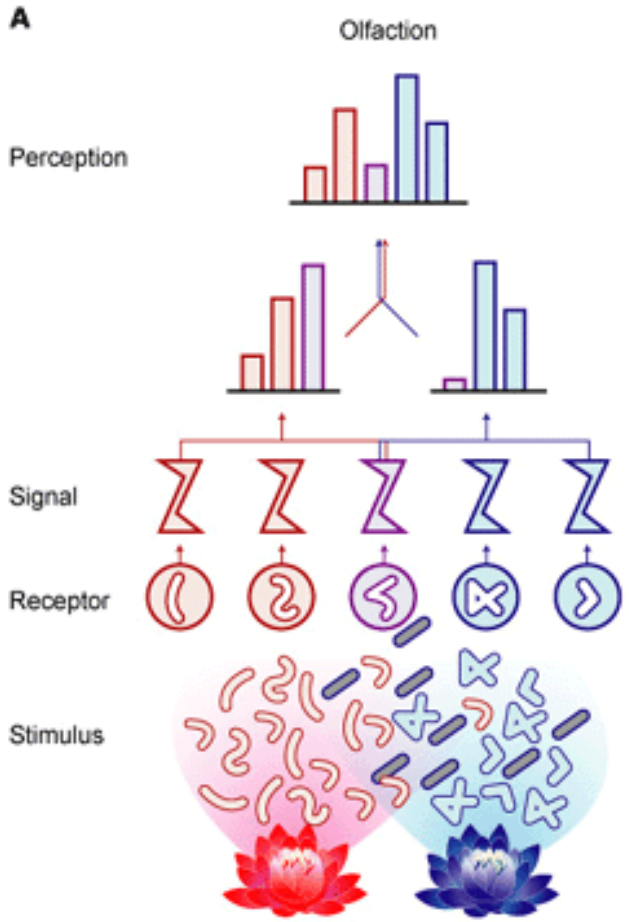
ORs and odorants: combinatorial receptor code

- An OR recognizes multiple odorants (that must share common molecular determinants)
- Receptors present a tolerance for slightly different molecular features to which they bind with different affinity
- An odorant is recognized by multiple receptors
- Different odorants are recognized by distinct combinations of receptors
- The receptor code (the activated ORs) for an odorant may change with odorant concentration → perception of a different odor!
- We can perceive ~10000 odours, with only 350 ORs

- *The conscious perception of an odour is an image of the combinatory code of activated ORs*

ORs and odorants: combinatorial receptor code





Convergence on a single glomerulus

- Each OSN expresses only one type of OR
- Each different type of neuron converges on only one glomerulus in the Main Olfactory Bulb
- The OR guides the axon during development to the correct glomerulus

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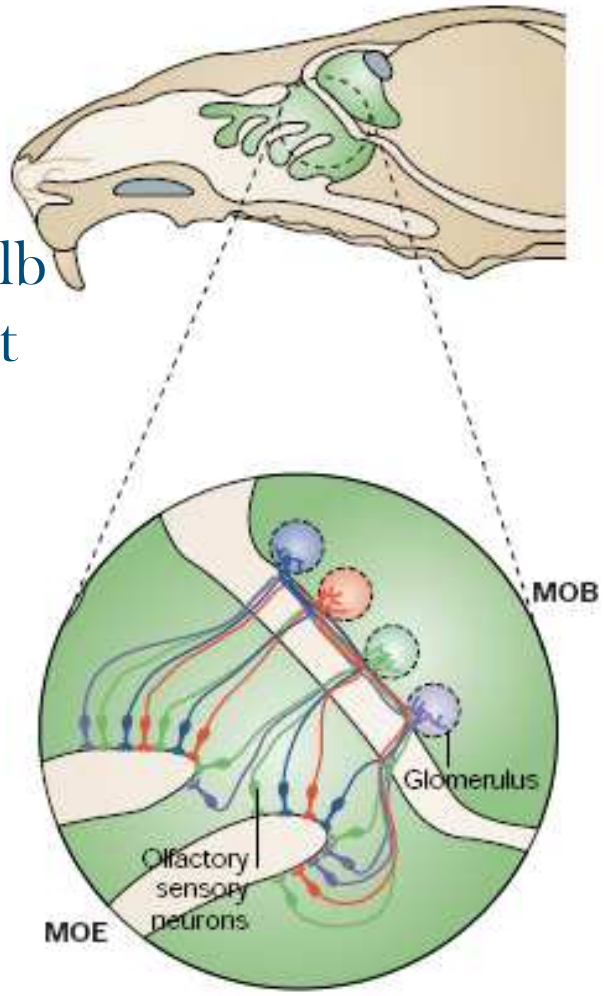
- Improved signal-to-noise ratio!

- Different odorants may activate overlapping but non-identical patterns of glomeruli



Signal transduction

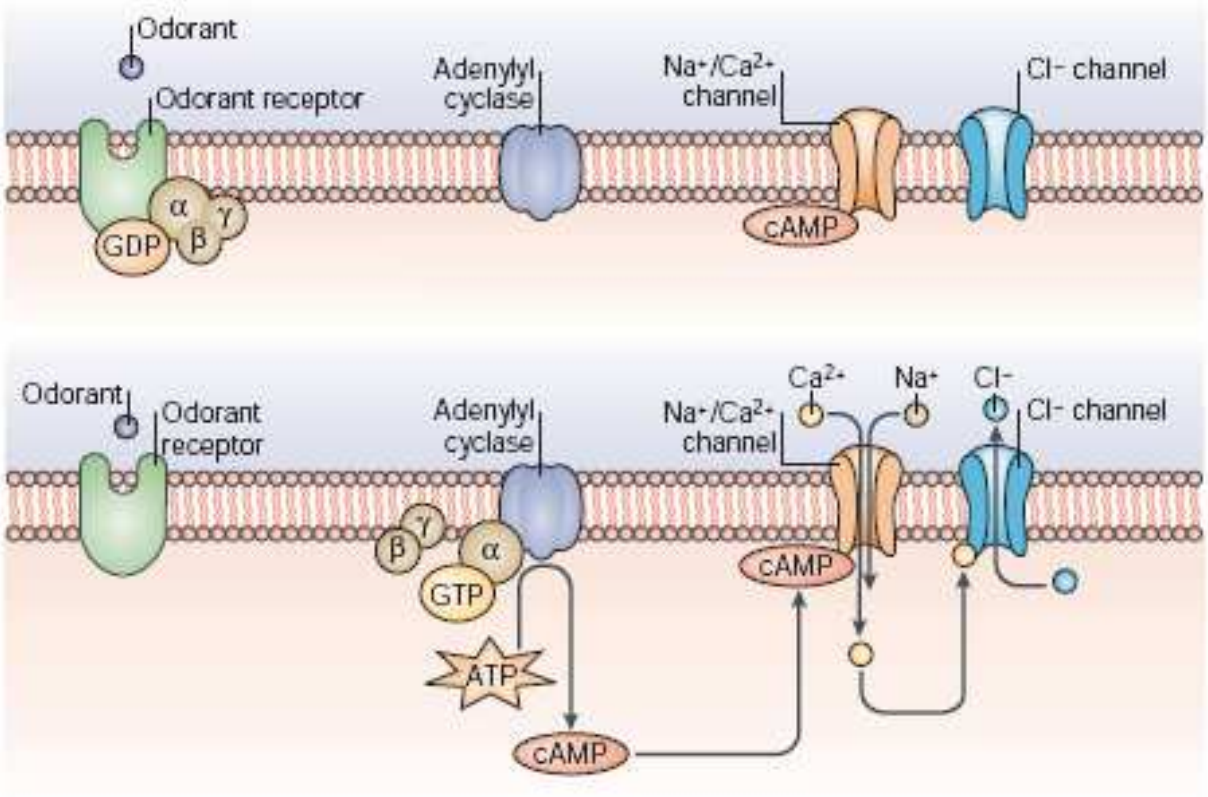


Figure 6 | **Canonical pathway of signal transduction in olfactory sensory neurons.** The odorant receptor defines odorant responsiveness. The heterotrimeric G-protein, the adenylyl cyclase, the cyclic-nucleotide gated Na^+/Ca^+ channel and the Cl^- channel are thought to be common among olfactory sensory neurons.

Signal transduction

Ca^{2+}-activated Cl^- channels produce an efflux of Cl^- from the cilia

↓

Depolarization of the Olfactory Sensory Neuron (OSN)

↓

The depolarization spreads passively to the dendrite and soma of the OSN

↓

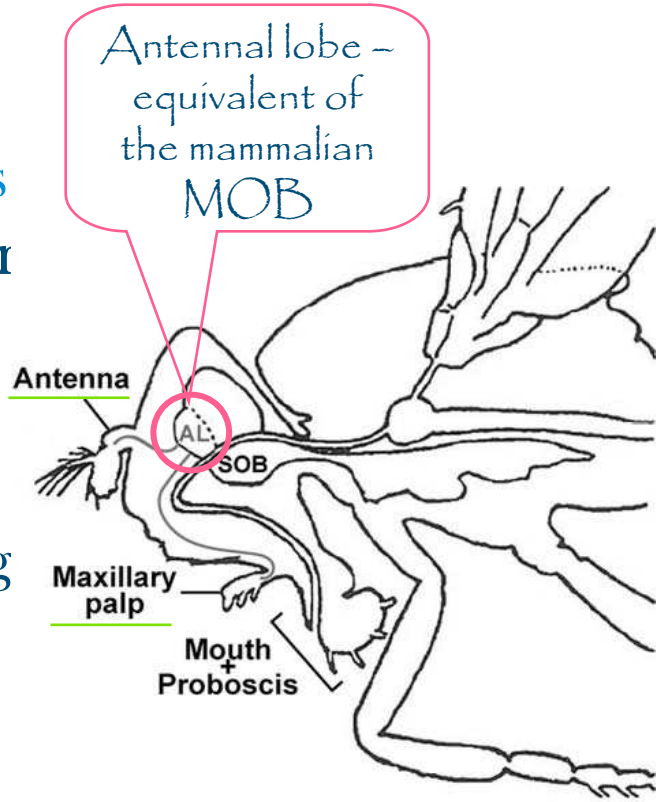
Firing of Action Potential to the Main Olfactory Bulb (MOB)

↓

Mitral cells in the MOB glomeruli are activated → signals to the cortex

In insects

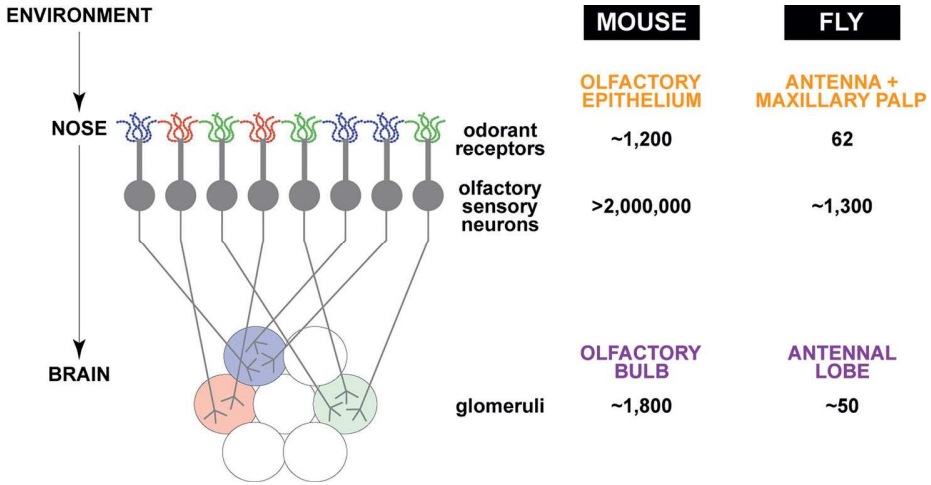
- Striking **similarity** with mammals in the olfactory physiology and neuroanatomy → odours perceived with conserved mechanisms
- Rich repertoire of olfactory-driven **behaviour** under the control of a much smaller nervous system than that of mammals
- Examples: *Anopheles gambiae* (malaria mosquito); *Manduca sexta* (hawkmoth – flying for miles in pursuit of potential mate)
- OR genes discovered in *Drosophila* only in 1999 (62 genes)
- Gene expression in the antenna (~88%) and maxillary palps ("noses" – only 7)

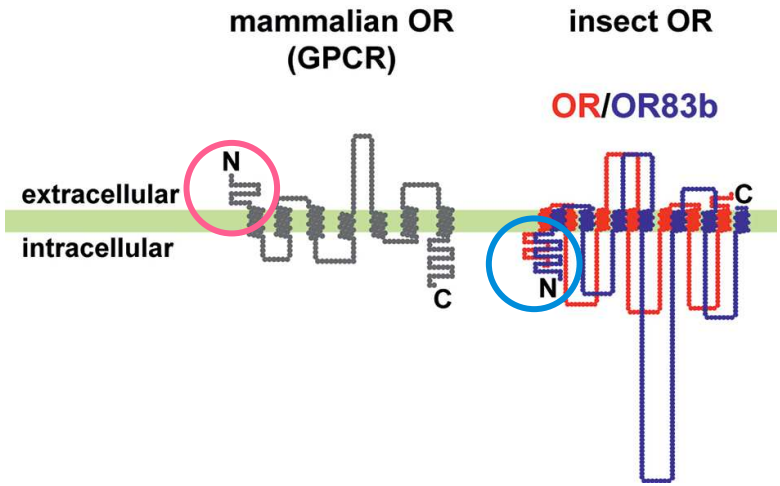


CMLS, Cell. Mol. Life Sci. 61 (2004) 456–469

In insects

- **Differences** between mouse and *Drosophila* OR proteins (functionally and structurally):
  - Insect ORs have no role in guidance of OSN axons
  - Some insect OSN express 2 OR genes (according to one review)
  - OR83b – co-expressed with other OR in almost all cells (escort)
  - Insect OR belong to a different protein family (not GPCR) (one review)





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Cell. Mol. Life Sci. 63 (2006) 1579–1585

In insects

- **Question:** how do insects transform odour binding into neuronal depolarisation if they don't behave like GPCRs?
- **Answer:** future studies such as...
  - Comparative sequence analysis of insect ORs to identify hypervariable residues that might be expected to contribute to ligand specificity
  - Biochemical and genetic investigation to prove the suggested hypothesis of coupling of ORs with G proteins (there is evidence of the presence of some G alpha subunits in the antennae)

In insects

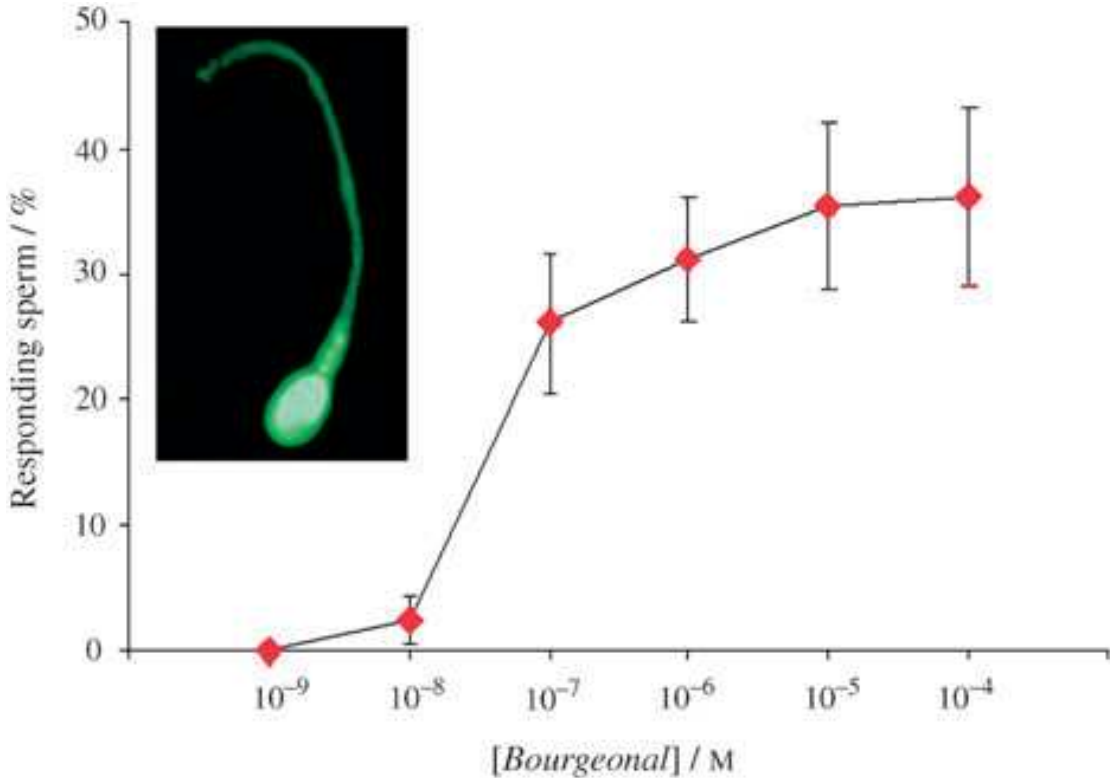
- Concluding:
  - The reliance of insect behaviours on olfactory cues makes this sensory modality an attractive target for chemical intervention
  - Opportunities for development of novel insect repellents
  - Exploiting the unique insect heteromer OR/OR83b as ideal target for specific chemical inhibitors and modulators

Or...

Use the "famous" NATURAL COMPOUNDS
to discourage insects from invading crops!

Elsewhere...

- It has been recently shown that ORs play an important functional role outside the olfactory epithelium: in human sperm cells



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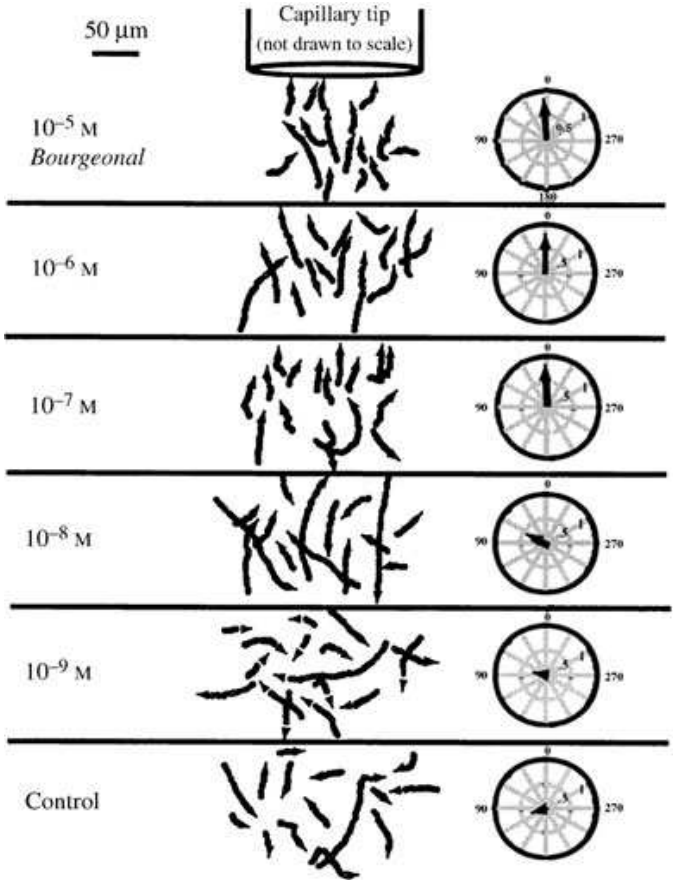
Elsewhere...

- Searching for receptors from chromosome 17 the hOR17-2 and hOR17-4 have been detected (PCR)
- Calcium imaging shown that sperm can smell Bourgeonal and Cyclamal in a concentration-dependent manner
- Sperm showed a concentration-dependent positive chemotactic behaviour and doubled their speed in the presence of the odour
- hOR17-4 potentially governs chemical **communication between sperm and egg** → the system could be used to manipulate fertilization



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Thank you for your attention