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The flair of research

It was probably with good reason that the most feared of all dinosaurs was the giant Tyrannosaur. The inordinate size of its olfactory system gave it an unfair advantage over other animals. Humans – a species that evolved millions of years later – are neither hunters nor prey to be tracked down in a radius of several kilometers and, as a result, a keen sense of smell certainly does not appear to be essential to us. Despite this apparent deficiency and lack of importance of the sense of smell in humans, Linda Buck and Richard Axel, two American researchers, set out to study the human olfactory system on the molecular level and discovered – thanks to revolutionary biotechnology techniques – the largest family of genes known to this day! They were awarded the 2004 Nobel Prize of Physiology and Medicine.

A whiff of the unknown

Our sense of smell, like our sense of taste, is the oldest in the history of evolution, and yet it was disregarded for years. Could this have been due to the fact that it was considered a marginal faculty in the process of hominization? In the evolution of Primates, sight took precedence over the sense of smell which humans now use very little, except perhaps in the event of 'bad' smells such as decaying food, for instance, which could cause poisoning. Has the human nose become a luxury? This would be a hasty conclusion, which in any case is countered by the fact that people who suffer from hay fever or some kind of trauma, either suddenly lose their sense of smell (anosmia) or worse are inadvertently incommoded by nauseating smells (cacosmia).

Humans are capable of distinguishing 400'000 odors – which seems phenomenal yet it is, in effect, insignificant with regard to other animals. Dogs, for instance, have 40 times more olfactory cells – the cells that allow the recognition of a smell – than humans do. As a result, a dog has 200 million olfactory cells against 5 million in humans…

What exactly is a smell? A smell is the sum of several molecules that we shall call scent molecules. Take the fragrance of a flower. To put it simply, it is both sweet and perfumed. So its fragrance is composed of both a sweet scent molecule and a perfumed scent molecule.

How does the olfactory system – in humans as in dogs – distinguish between scents that are so

abundant, so varied and sometimes so similar? The olfactory system is a particularly dense network of millions of olfactory neurons, all located in a specific tissue – the olfactory epithelium – which lines the nasal cavity.

Research come to a standstill

Towards the end of the 1980s, research on the mechanisms of odor perception had come to a standstill. The techniques then in use were inadequate. These techniques, the so-called electrophysiological methods, consisted in inserting electrodes into the nostrils so as to measure the olfactory system's response to odors. The only inconvenience was that such methods gave no insight into what was happening on the molecular level.

Then it was suggested that the detection of a smell could be the result of a link between a scent molecule and a specific protein – called an olfactory receptor – on the surface of a neuron. Since an odor is the sum of scent molecules, it would stimulate as many olfactory receptors which would then transmit the message to the brain where it would be deciphered. In other words, the smell would be…smelled.

Two mechanisms were put forward. First, it was assumed that there were only a few olfactory receptors that interacted with a large number of different scent molecules. In the second mechanism, it was assumed that there were as many different receptors as there were scent molecules, or that one specific receptor recognized only very few scent molecules.

This was when Linda Buck and Richard Axel struck upon a brilliant idea. Instead of seeking to identify the receptors in the epithelium of the nasal cavities, why not seek out their genes in the genome? Indeed, every protein is the product of a gene found in our genome; our genome being the sum of all our genes. A gene could be likened to a recipe which our body cells refer to for producing proteins, our system's workers as it were.

But why did Buck and Axel seek out the gene, when they might have dealt directly with its product: the protein? Especially in the knowing that the human genome contains 20 to 25'000 genes… Might as well search for a needle in a haystack. It is in fact no easy task to isolate a specific protein in an organism and even then in sufficient quantities to study it. In the past few years, a revolutionary technique in the field of molecular biology has been developed: the polymerase chain reaction (PCR). A technique which enables a specific gene not only to be located in a genome but also to be 'photocopied' millions of times over thus providing genes and especially their product, in large enough quantities for researchers to be able to proceed in their work.

Fig.1 *Linda Buck et Richard Axel, the 2004 Nobel Prize in Physiology or Medicine*

The biomolecular genie

Buck and Axel were to take great advantage of this biomolecular revolution. But, first, how were they to find the gene? How do you find an object when its shape is unknown? Perhaps indirectly, by associating it with a known partner? Much in the way a criminal can be found out by way of a footprint… It is precisely in this manner that Buck and Axel proceeded. And eventually succeeded. Results had already been published on the olfactory neurons that synthesize a protein known as the G protein. There is a well-known family of receptors that are intimately linked to the G proteins: the family of G protein coupled receptors (GPCR).

When this type of receptor is stimulated by a signal, the G protein recognizes it and transmits it to the cell. Then by a chain reaction, the information reaches our brain. Knowing this, Buck and Axel proposed that olfactory receptors might also belong to the GPCR family; such an assumption considerably restricted their field of action.

It is a fact that technical progress often paves the way to great scientific progress. And Linda Buck and Richard Axel seized the opportunity. The PCR technique was crucial in their quest. In assuming that olfactory receptors belonged to the GPCR family, they provided themselves with a valuable means to proceed. They made use of the known GPCR genes, 'attached' them to a hook and went fishing. Literally. This fishing game resulted in their being able to isolate the GPCR genes in the genome as well – as luck would have it – as the olfactory receptors associated with them.

Their flair rewarded

Success followed. In 1991, Buck and Axel identified a new family of genes: the olfactory receptors. This newly discovered family comprises about 200 members! But it was just the tip of the iceberg: in some mammals, like rats, the number of genes encoding olfactory receptors reaches the thousand mark.

And so it is that almost 1% of our genome is dedicated to the genetic information of our olfactory receptors making it the largest family of proteins in the human body known to this day…

The hunting of the smell!

Let us recapitulate. A smell is perceived in our nasal cavities where several scent molecules – which constitute the smell *senso stricto* – bind to specific receptors. Each one of these receptors will act as a relay and transmit the olfactory message further down the line. So breathing in a smell stimulates the neurons to send a specific signal. This signal is then 'processed' by our brain ultimately bringing about its perception and the creation of specific sensations.

So there are two stages in the perception of odors. Our nose transcribes the olfactory message brought by a smell. And then our brain deciphers it thus enabling us to identify it, with all its associated emotions and memories. So in order to respond to tens of thousands of scents, our olfactory system must trump up something both in the structure of its olfactory receptors and in the transmission of their signals.

Twists and turns

Buck and Axel confirmed that the olfactory receptors belonged to the large GPCR family. As a consequence, olfactory receptors present a particular structure, characteristic of GPCR receptors, which resembles the twists and turns of a snake.

Indeed, each receptor crosses the cell membrane seven times, weaving in and out like sewing thread through a piece of cloth. Each segment of the receptor which spans the membrane is called a 'transmembrane domain'. As a result, every olfactory receptor has seven transmembrane domains.

The three middle transmembrane domains vary from one receptor to another whereas the other four flanking them (two either side) are conserved in all GPCR families. The explanation for the diversity of olfactory receptors is latent in the three variable transmembrane domains. Indeed, research over the years has suggested that together they form a cavity in which a scent molecule can lodge. Consequently, every alteration that occurs in the transmembrane domains forms a specific cavity adapted to a specific scent molecule. This variability of the cavity's shape provides an initial explanation for the great diversity of scent molecules that can be detected and selected.

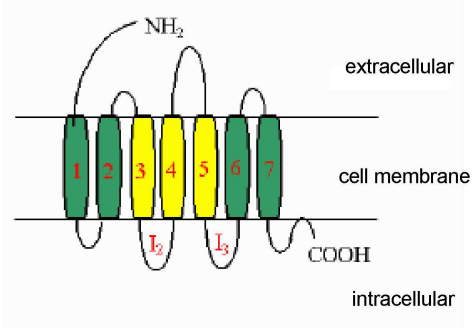

Fig.2 *Characteristic structure of the GPCR family with the transmembrane domains numbered 1 to 7. The three middle domains (3, 4 and 5) are variable.*

How is such variability expressed on the molecular level? The answer to this is found in the sequence of amino acids. Every protein is a chain of molecules called amino acids of which there are about 20 in the animate world. Each specific olfactory receptor has a sequence of amino acids that is not quite the same in each of the three central domains.

Take a mouse and a rat for example. Mice have a receptor – named receptor 17 – which has a strong affinity for a molecule whose smell evokes that of fat. Rats have the same receptor but recognize the smell as rancid fat. The difference between these two receptors lies in one amino acid located in one of the central domains, and such a slight variation is enough to turn a delicious scent into a nauseating whiff.

This tiny difference between the mouse and the rat receptors is enough to alter the cavity's structure, consequently altering the receptor's affinity for scent molecules. When – with the help of genetic engineering – the two amino acids are interchanged, the mouse develops the rat's tastes and vice-versa. This example illustrates how Nature has created such a marvelous diversity in olfactory sensitivity with a minimum of effort!

A chart of odors: complex stuff

What is known about the spatial organization – in the brain – of neurons dedicated to smell? One

extremity of the neurons are grouped together in a tissue known as the 'olfactory bulb', from where neuronal projections – or axons – bathe in the nasal mucous membrane. Each olfactory neuron bears only one type of receptor. The other extremity of the neurons are gathered in structures called glomeruli. Axons which carry the same receptor – and hence are receptive to the same scent molecule – converge towards specific glomeruli in an ordered manner.

This convergence groups the olfactory information before reaching the cerebral stage. As a result, it also constitutes a chart of odors at the level of the olfactory bulb: scent molecules that differ by their structure (size or chemical properties) stimulate specific parts of the olfactory bulb. Would this partitioning explain why we never mistake the fragrance of lilac for the smell of a car exhaust?

The sequence seems simple enough: scent molecule -> specific receptor -> message relayed to the brain -> deciphering -> perception of a smell. But it is not so straightforward. We now know that the recognition of such a vast range of fragrances is far more complex. The equation 'one scent molecule = one olfactory receptor' cannot be generalized. While it is clear that every scent molecule binds to a specific receptor, a little like a molecular key in a keyhole, it does in truth have more possibilities than one. In the mechanism described above, several keys can in fact turn in one same keyhole, and one same key can turn in a number of keyholes…

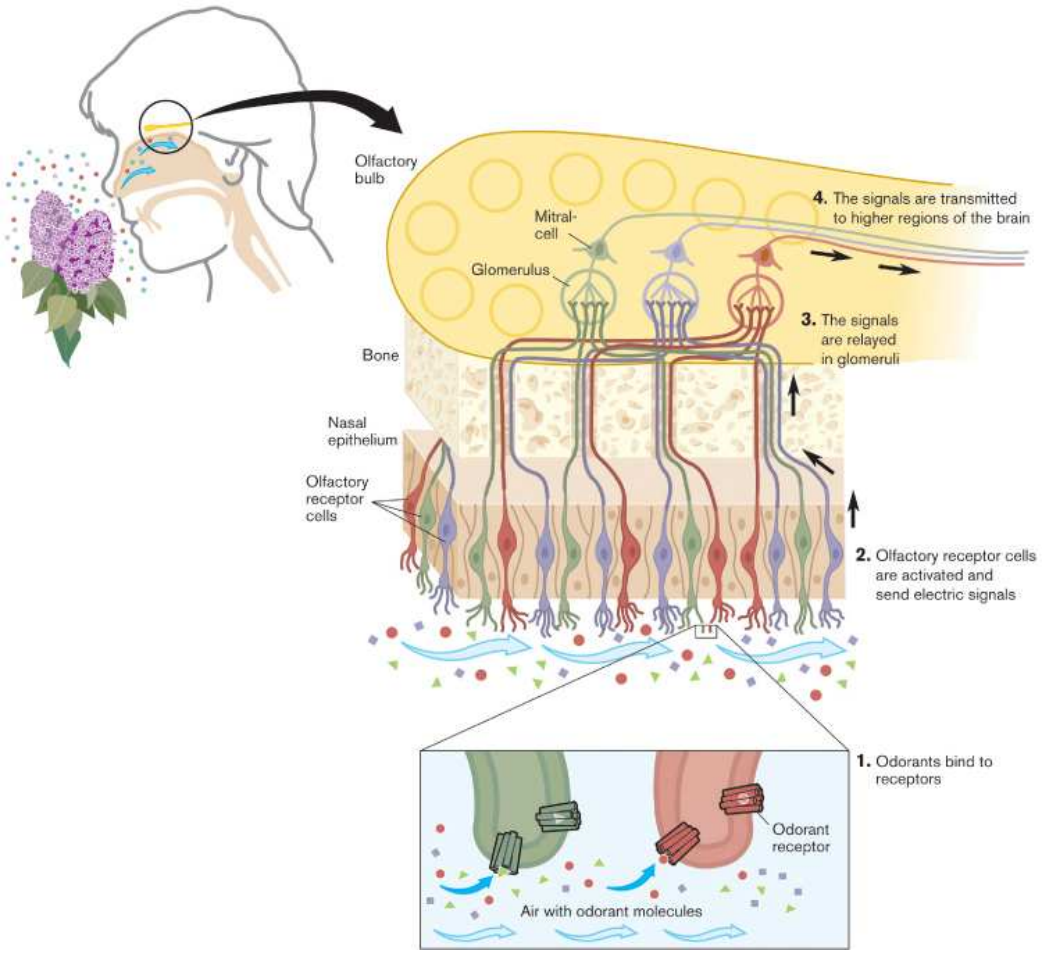

Fig.3 *From the nose to the brain: the perception of odors.*

Other researchers have investigated further into the connection between the spatial organization of the olfactory information in the brain and the perception of smell. Their results suggest that patterns of information are not the same in every case. Elsewhere in the brain, instead of converging, messages received from the same type of olfactory receptor may also be dispersed…

What are we to make of this? It is yet early days to grasp fully the meaning of this chart of odors. There are many technical limitations and progress is slow. In spite of the unremitting effort put into its understanding by various research teams, the precise mechanisms of our sense of smell remain a mystery.

However, the discoveries made by Buck and Axel have revealed certain surprising characteristics of the olfactory neurons. For instance, if these neurons are severely damaged they can be regenerated. This is contrary to all beliefs held until now that brain cells (unlike liver or skin cells) cannot be replaced if they are damaged. How they regenerate, no one knows. And neuron regeneration remains yet another mystery to be solved.

Odorless odors

The discoveries made by Buck and Axel opened up another field of investigation: that of pheromones. These scentless molecules have a very subtle influence on us. In the course of their research, scientists discovered that pheromone receptors also belong to the GPCR family and that they have a great many of their properties.

Pheromones are emitted by animals and act as messengers between individuals of the same species. They play an essential role in the mating season to attract the opposite sex and for certain social insects, such as ants and bees, they are vital for group cohesion and function. Although produced in minute quantities, they are highly active and can be detected within a radius of several kilometers.

These odorless molecules seem to have a primordial role since evolution has deemed it necessary to provide them with a specific olfactory system that is independent from the principal system that we have been discussing. Pheromones worm their way through our nostrils to a particular sensory organ called the vomeronasal organ.

However, despite a difference in destination, pheromones are treated in the same manner as 'standard' scent molecules. In fact, the classic olfactory system and the vomeronasal one are perhaps not so independent as they might seem. In humans for instance, the vomeronasal system is atrophied. Yet we know that human behavior can be influenced by pheromones. It is common knowledge that the menstrual cycle of girls in boarding schools – or even sharing the same office – is synchronized. How can this be explained other than by the effect of pheromones?

Some rudimentary explanation may reside in experiments carried out on mice amongst which certain neurons of the olfactory bulb – an integral part of the classical system – responded to a pheromone found in the urine of male mice. This would prove that pheromones can actually use the classical pathway of olfactory perception as an alternative. Since the human vomeronasal organ is atrophied, could it be that human pheromones simply make use of the classical olfactory system to be perceived? This has yet to be proved…

Of fragrance and sperm

In the past decade, many research teams have pursued the work of Buck and Axel on the olfactory system receptors. There is still much to learn and a long way to go… We know today that the olfactory receptors belong to a super family of proteins that play a part not only in the perception of smell but also in our perception of taste, our vision and even in sperm mobility and socializing! A very wide spectrum indeed!

Evolution no doubt made use of the olfactory senses to guide superior organisms in the choices they had to make for their survival. Odor is one of the best messengers of good or bad. For these reasons alone we share the wonder of Linda Buck and Richard Axel when they state that the sense of smell and the olfactory system 'constitute a wondrous and limitless enigma'.

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For further information

On the internet:

- On the olfactory system (in French) : http://olfac.univ-lyon1.fr/sysolf/lso-frame.htm

A little more advanced:

- Menini A. et al., "Olfaction : From odorant molecules to the olfactory cortex", News Physiol. Sci. 19:101-104(2004) PMID: 15143202
- Buck L. and Axel R., "A novel multigene family may encode odorant receptors: a molecular basis for odor recognition", Cell 65:175-87(1991) PMID: 1840504

Illustrations:

- Heading illustration, Source: www.mayforth.com
- Fig.2, Adaptation : www.cf.ac.uk/biosi/staff/jacob
- Fig.3, Adaptation : http://nobelprize.org/medicine/laureates/2004/press.html

At UniProtKB/Swiss-Prot:

- Olfactory receptor 41, Rattus norvegicus (rat) : P23269
- Olfactory receptor-like protein F3, Rattus norvegicus (rat) : P23265
- Olfactory receptor-like protein F5, Rattus norvegicus (rat) : P23266
- Olfactory receptor-like protein F6, Rattus norvegicus (rat) : P23267
- Olfactory receptor-like protein F12, Rattus norvegicus (rat) : P23268

- Olfactory receptor 10A1, Homo sapiens (human) : O95223
- Olfactory receptor 10A3, Homo sapiens (human): P58181
- Olfactory receptor 10A4, Homo sapiens (human): Q9H209
- Olfactory receptor 10A5, Homo sapiens (human): Q9H207
- Olfactory receptor 10A6, Homo sapiens (human): Q8NH74

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