Small but mighty: In mice, around ten million olfactory sensory neurons analyze incoming air for promising odors. The corresponding figure for humans has not been identified, but it is assumed to be significantly lower.

The Genetic Story **behind a Good Nose**

Peter Mombaerts is as familiar with the world of molecules, genes and cellular signals as he is with the world of odors. The Belgian, who is now Director at the **Max Planck Institute of Biophysics** in Frankfurt/Main, is one of the researchers who have clarified what pathway odors take through the nose and brain – or at least the basic principles of it.

TEXT **KLAUS WILHELM**

The importance of the world of olfaction for humans, and even more so for other mammals like dogs, cats and mice, is immediately obvious to anyone who thinks about it. But the genetics and biochemistry that underlie the sense of smell are beyond most people's imaginations. Depending on the mammalian species in question, between 300 and 1,200 genes form the basis of the ability to discriminate between the unquantifiable abundance of existing odors. These olfactory receptor genes contain the instructions for proteins that recognize the various structures of odorant molecules. Moreover – and in Mombaerts's view this is the most significant of his many findings – they also control the navigation of axons from the olfactory sensory neurons to the brain. The entire procedure of olfactory processing takes a good 200 milliseconds. Researchers like Andreas Schäfer, who heads an Independent Junior Research Group at the Max Planck Institute for Medical Research in Heidelberg, take a stopwatch in their hands when they put mice on the trail of a smell.

But many of the questions in olfactory research remain "baffling," Mombaerts notes. There are several reasons for this. For one thing, the secrets of olfactory processing in mammals can be decoded only by carrying out complex and time-consuming animal experiments in which the olfactory receptor genes are genetically modified in a specific way using increasingly finely-tuned methods. Faster experiments using cell cultures do not work. For another, only a small, select group of researchers have taken up this difficult scientific challenge. Peter Mombaerts estimates that there are only "around a thousand people" working on olfaction throughout the world.

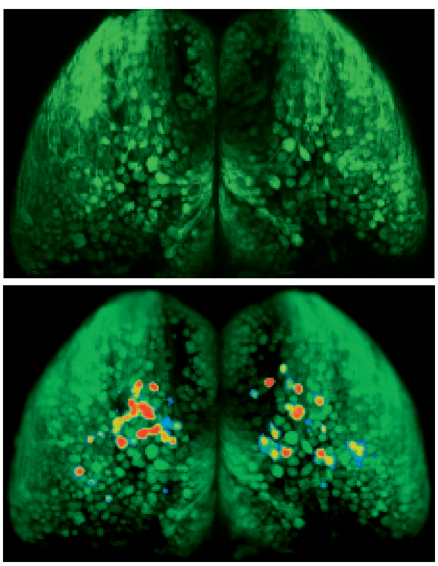
A SMELL SAYS MORE THAN A THOUSAND WORDS

When we consider that Germans alone spend billions of euros every year on the pleasure of surrounding themselves with scents like vanilla, rose and musk, it may come as a surprise to learn that so few scientists are involved in researching the pathway odors take through the nose and the brain. It is also surprising in view of the fact that women, in particular, strongly affirm that their partners must "smell good" to be attractive. There is hardly anything more repellent to us than a bad smell. Biologists, physicians and psychologists are well aware of just how loaded natural smells are with information – for humans and, to an even greater extent, for other mammals. When a dog sniffs the urine of another member of its species, it can, without seeing the other animal, immediately identify its gender. When a mouse breaks out in a cold sweat, it sends out a warning to the other animals in the group. The teat secretion of a mouse guides the sucking behavior of her pups. As Peter Mombaerts stresses, there is no doubt that "olfaction is the most elementary and fleeting of all senses." An ideal detector of body and environmental chemistry, the sense of smell is almost impossible to mislead.

Mombaerts became enamored with the science of olfaction back in 1991. At that time, a new era in olfactory research had just begun. Prior to this

Photo: fotolia

» Since the breakthrough of 1991, it has gradually become clear how an odor literally rushes to the brain and is assessed, decoded, and stored there.



The images show the two olfactory bulbs of a mouse: The olfactory sensory neurons in the olfactory epithelium are stimulated here by butenal. The degree of neuronal activity is shown using different colors (bottom).

date, the nose was viewed as the most mysterious of the sensory organs. This was clearly due to the nature of the beast: hearing, for example, is based on a linear system of sound waves that can be detected by a biological structure with relative ease and transformed into sensory impressions. Similarly, sight is also based on the detection of wavelengths that can be understood in numerical ranges – for red, green and blue. But how can a mammal, with its limited genetic resources, discriminate between the hundreds of thousands of odorant molecules with different chemical structures? "From today's perspective, there were some bizarre ideas floating around the scientific world back then," remembers the Max Planck researcher, such as the theory relating to the existence of seven "primary" odors, similar to the primary colors in the visual system.

A HUGE FAMILY OF OLFACTORY RECEPTOR GENES

An article published in 1991 revolutionized the field of olfactory research. American biologists Richard Axel and Linda Buck, who have since been awarded a Nobel Prize, reported to their stunned colleagues that there are more than 1,000 genes for the detection of odors in the genome of rats. "The largest family of genes in mammals overall," says Mombaerts. Of the approximately 30,000 genes in mice and rats, around 1,000 are involved in olfaction. This figure is lower in humans, but at 350, significant nonetheless. This genetic abundance corroborates the immense significance of olfaction. Without it, the sense of taste would be helpless – not only in mice, but also in humans. The final say on all culinary creations – be it tuna steak, hamburger, pasta, or wine – goes to the olfactory epithelium.

The family of olfactory receptor genes is remarkable in a number of respects. Unlike almost all other genes, these genes are not interrupted by so-called introns – DNA segments that do not encode information about the structure of a protein. This intronless gene structure probably made it easier for the olfactory receptor genes to proliferate with new variants in the genome over the course of evolution and to become such a huge family of genes. Moreover, in all of the mammals studied so far, the genes appear to be located seemingly haphazardly on all chromosomes. "There's no identifiable logic here," says Mombaerts. The team working with the scientist succeeded in demonstrating that the control areas for gene expression are "inconceivably small," even in terms of genetic dimensions. Such control areas determine when and where a gene is activated and how its information is ultimately implemented in a protein.

Since the breakthrough in 1991, it has gradually become clear how an odor literally rushes to the brain and is assessed, decoded, and stored there. All smelly living creatures or things release volatile molecules that are almost always complex mixtures. Smells consist of hundreds of chemical components

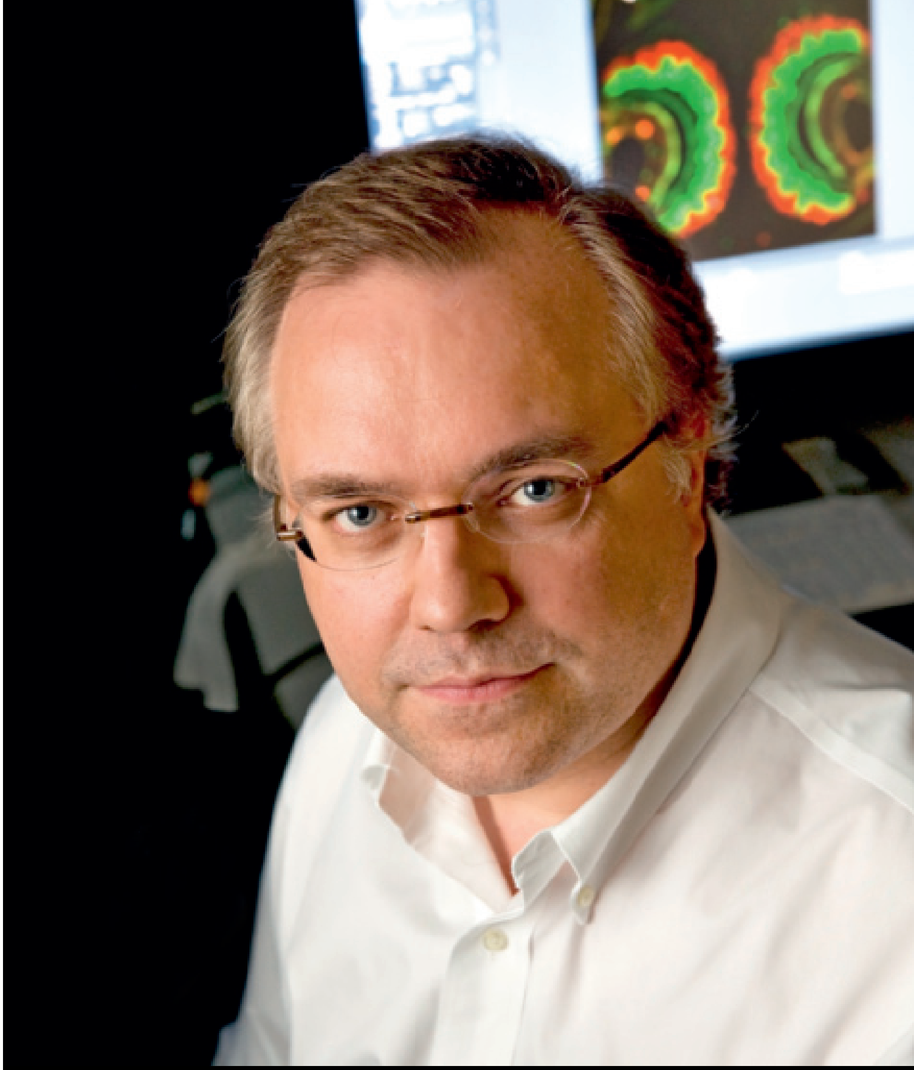
Photos: Peter Mombaerts (2)

that waft into the nose and collide there with the olfactory epithelium. This patch of tissue is located within the olfactory mucosa and is more or less the size of a postage stamp in humans, but much larger in dogs and mice relative to their body size. The olfactory epithelium consists of three cell types: the supporting cells, which provide important assistance in olfaction, the olfactory sensory neurons, and the basal cells – adult stem cells that replace the olfactory sensory neurons and thus ensure "the strongest neurogenesis of all in the adult body," says Mombaerts.

RECEPTORS FISH FOR ODORANT MOLECULES

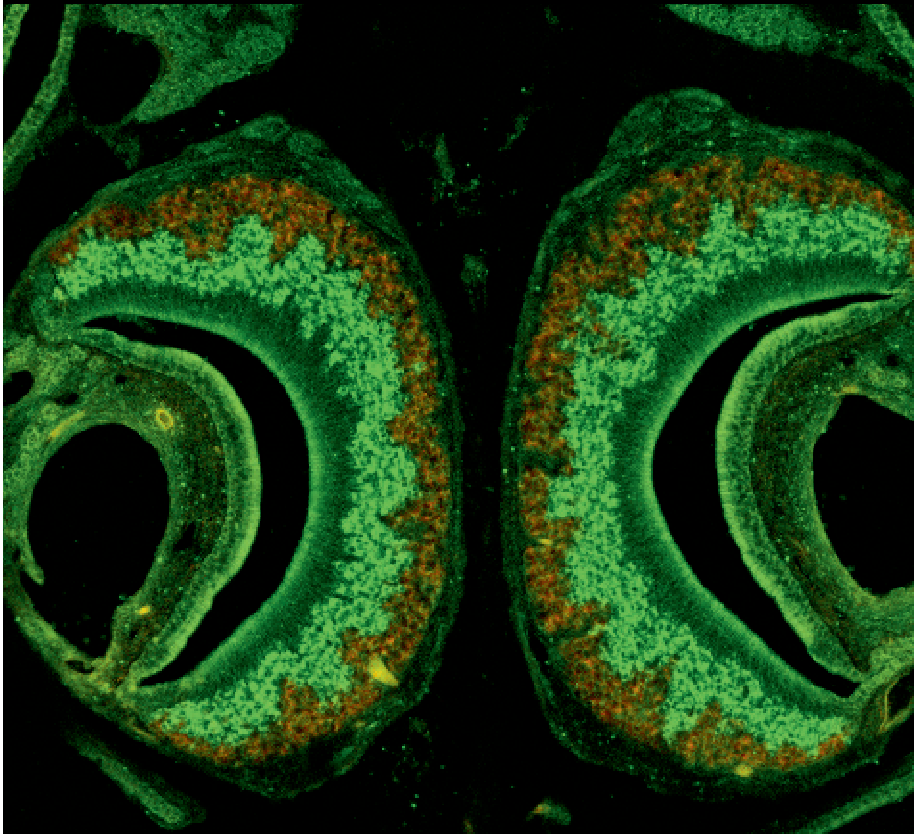
Approximately ten million olfactory sensory neurons in the mouse "appraise" the incoming air; the number of neurons in humans is unknown. Around 20 fine sensory hairs, known as the cilia, protrude into the nasal mucosa. Their cell membrane houses all of the molecular components that ensure that humans can perceive several million smells, even in low concentrations, and can discriminate between thousands of them – despite having only 350 types of molecular receptors that are encoded by the olfactory receptor genes. In the olfactory epithelium of mice and dogs, around 1,200 different types of receptor proteins scan the incoming odorant molecules.

"The olfactory receptors are the basis of olfactory perception in mammals," stresses Mombaerts. The receptor proteins, which consist of around



above | A pioneer in the realm of the senses: Flemish scientist Peter Mombaerts is one of the researchers who have clarified what pathway odors take through the nose and brain.

below | Cross-section of the vomeronasal organ of a mouse. The different colors show that the epithelium consists of two types of neurons.



Photos: Christophe VanderEecken (top)/Tomohiro Ishii

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320 amino acids, are similar in overall structure; they traverse the cell membrane of the olfactory sensory neurons seven times. Certain parts of the receptors display the greatest diversity: the binding pocket, the area where the interaction between the odorant molecule and receptor takes place, is probably located there. Every olfactory sensory neuron in the mouse has all 1,200 receptor genes in its genome, but produces only a single receptor type. "Even though, for technical reasons, it's not really possible to provide definitive proof, and the evidence that exists is somewhat weak, this hypothesis has now become dogma," says Mombaerts. Thus, for better or for worse, he works on the assumption of the "one neuron-one receptor hypothesis."

There is, however, some evidence that olfactory sensory neurons can actually produce several receptor types during their maturation, but ultimately opt for a single receptor type. Such details are important for Mombaerts, as he wants to understand how and why a cell chooses only one olfactory receptor gene for expression and – while "in biology nothing is really perfect" – why the mechanism appears to be so good. Mombaerts's team discovered in experiments with genetically modified mice that this selection process does not involve so-called DNA rearrangements. Experts had favored this mechanism for a long time because the immune system uses it to ensure that it can recognize countless pathogens based on just a few gene segments. The Frankfurt-based researcher believes that the unusually short control elements in front of the olfactory receptor genes determine the likelihood with which a neuron chooses a particular receptor for expression.

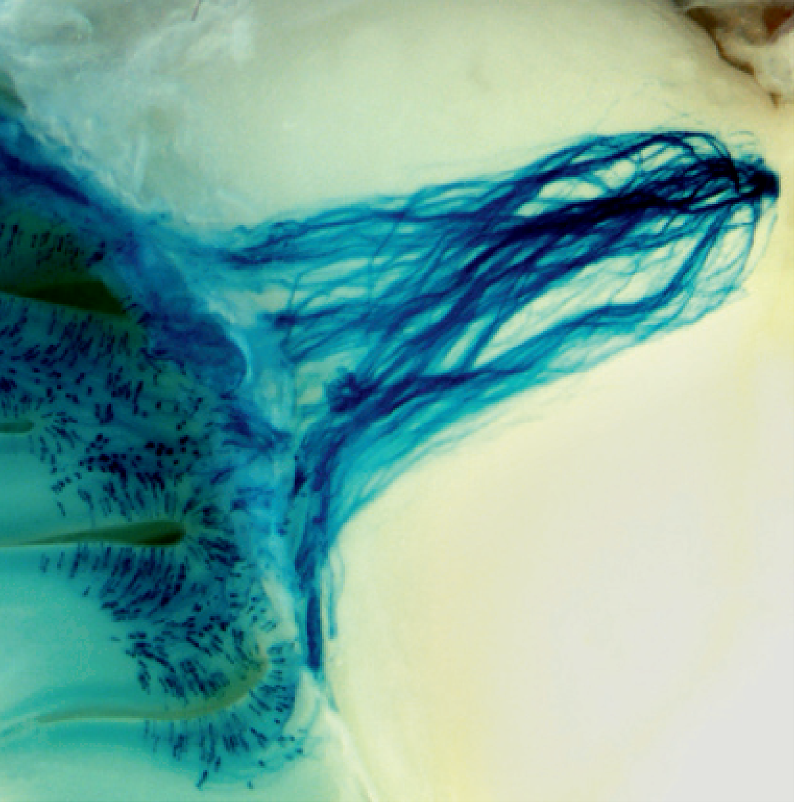
What is clear is that, in humans, tens of thousands of neurons of each olfactory receptor type are distributed throughout the olfactory epithelium. Equipped in this way, humans can differentiate between the smell of lemons and oranges, for example. When a hu-

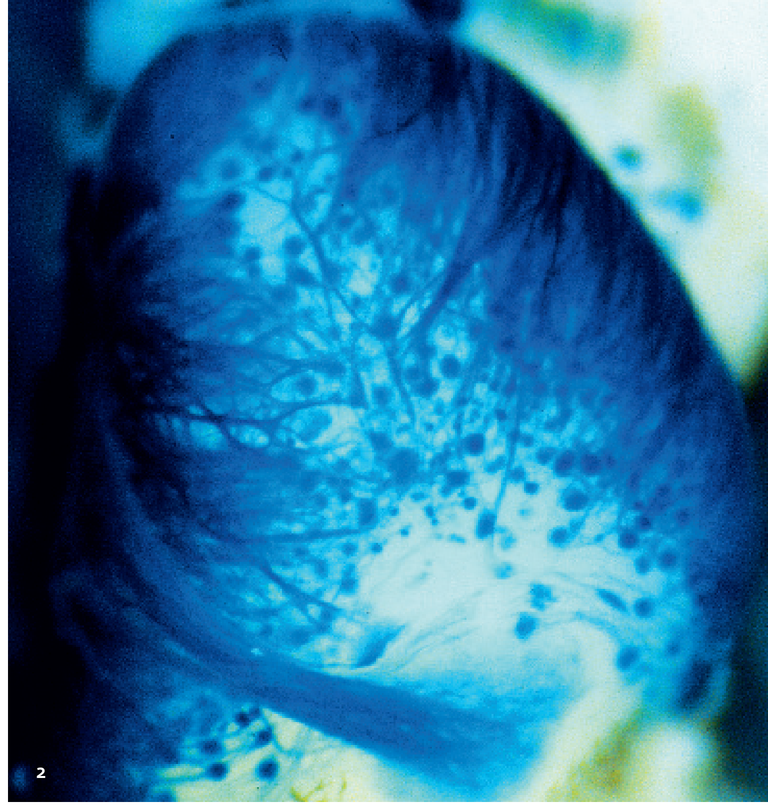
WHERE SMELLS TRIGGER EMOTIONS

The vomeronasal organ (VNO) looks like a tiny thread and huddles against the base of the nasal mucosa. "We demonstrated for the first time in mice that the cells of the VNO detect pheromones with their special receptors," says Peter Mombaerts of the Max Planck Institute for Biophysics in Frankfurt. Furthermore, the VNO regulates sex and aggression in a very direct way through the perception of the pheromones of conspecifics.

The team working with the neurobiologist – who was based at The Rockefeller University in New York at that time – deleted from the mouse genome 16 genes that are important for VNO neurons, and this genetic manipulation generated defects in the processing of pheromones. Female mice barely took care of their offspring and the sexual drive of the males declined. Male mice can usually recognize, in a matter of seconds, whether a female is available to mate via pheromone messages, or whether urine originates from a strange male – something that triggers instant aggression in rodents. The approximately 300 receptors found in the VNO neurons differ clearly from the olfactory receptors in the olfactory epithelium. And, as Mombaerts' team discovered, the axons from the VNO neurons with the same receptor do not project toward a single glomerulus in the olfactory bulb, but to 15 small glomeruli of the accessory olfactory bulb.

The scientist suspects "that the olfactory processing of pheromones takes place mainly in the accessory olfactory bulb." The accessory olfactory bulb does not send axons to the cerebral cortex, but directly to the hypothalamus and the emotional centers of the brain, which control the behavior of the mice directly through secreted hormones and messenger substances.





man breathes in olfactory compounds, only the olfactory sensory neurons that express the receptors for the cognate odorant molecules are activated. Thus, dozens of receptor types are stimulated simultaneously by each odorant, but in different combinations.

"Olfactory perception is highly combinatorial," says Peter Mombaerts. This logic is the only way humans can identify thousands of different odors with ease, while mice and dogs can potentially identify hundreds of thousands. Because small structural changes in odorant molecules alter the interaction with the receptors only gradually, the number of chemicals that can be smelled is theoretically unlimited. A receptor protein recognizes a defined part of a molecule very specifically and thus interacts only with odorants that contain this part. In higher concentrations, however, molecules with similar structures also activate the receptor.

The olfactory sensory neurons that are activated in this way transform the olfactory stimuli into electrical signals. They do this through a chain of biochemical reactions: When odorant molecules have found matching receptors on the surface of an olfactory sensory neuron and have docked there, the receptors activate, via so-called G proteins, an enzyme that produces vast quantities of the messenger substance cAMP. Positively charged ions then stream into the cell through the membrane channels that are now opened, and electrical signals, action potentials, are generated. These propagate along the nerve extension (axon) of the olfactory sensory neuron to the brain's olfactory bulb *(bulbus olfactorius)* where, in the mouse, 2,000 knots made of neural extensions known as glomeruli are found.

AN OLFACTORY BULB WITH A SYSTEM

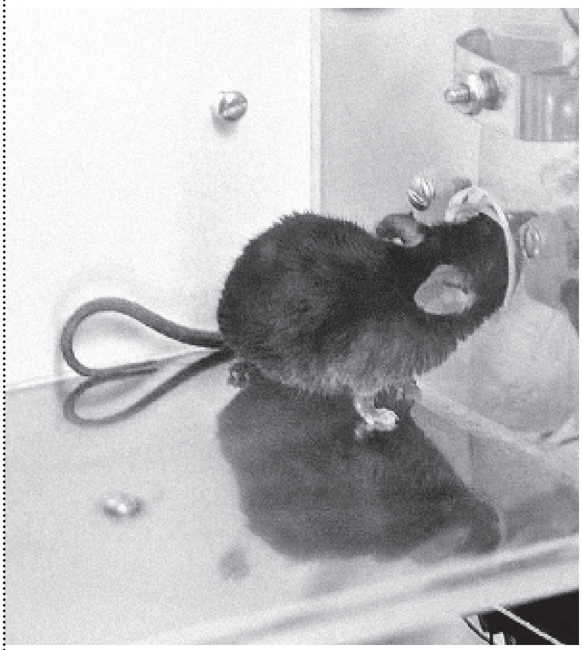
This is precisely the interface at which the initially indiscriminate receptor signals become more specific. The axons of olfactory sensory neurons do not project toward the olfactory bulb in a random fashion. Instead, axons of olfactory sensory neurons with the same receptors form bundles, which are probably held together by adhesion proteins, such that "their axons ultimately coalesce into the same glomerulus of the olfactory bulb," says Peter Mombaerts. Thus, olfactory sensory neurons with receptor A go to glomerulus A, olfactory sensory neurons with

1 Olfactory sensory neurons in the olfactory epithelium that express the same receptor gene were dyed blue. The cell bodies of the neurons are located in the epithelium (left); its axons (nerve fibers) run across the surface of the olfactory bulb (right) and culminate in a neural knot, a glomerulus.

2 General overview of the olfactory bulb in which all mature sensory neurons were dyed blue. The small blue balloons in the center are the glomeruli toward which the nerve fibers converge. The bundle of nerves protruding from the left consists of the fibers of the vomeronasal organ and projects toward the accessory olfactory bulb.

Photos: Peter Mombaerts (2)

SPLIT-SECOND SMELLING



Mouse sniffing the apple odor in the behavioral experiment.

The brain wastes no time when it comes to processing odors – as Andreas Schäfer is only too well aware. The biophysicist heads an Independent Junior Research Group at the Max Planck Institute for Medical Research in Heidelberg, where he used a simple experiment to determine the "smelling speed" of mice: The animals first broke through a light barrier. At that moment, an odor was blown into their noses. The rodents quickly learned that, after sniffing the scent of bananas, they were allowed to lick some sugar water as a reward. After sniffing an apple scent, in contrast, they were left empty-handed – whereupon they retreated. "Only 230 milliseconds elapsed from the inhalation of the smell to the behavior ultimately triggered by the action," says the researcher. "It all takes place at unimaginable speed."

In subsequent experiments, the rodents were presented with very similar smells and, as a result, the processing time increased to 340 milliseconds – not even half a second. As part of his ongoing research, Schäfer's team is using sophisticated techniques to switch certain groups of nerve cells in the olfactory bulb on and off to observe the resulting changes in behavior. This work is being carried out in cooperation with Thomas Kuner's team at the University of Heidelberg. Initial findings indicate that the animals can better distinguish between smells when the neurons are rendered more excitable.

receptor B go to glomerulus B, and so on. The Belgian scientist gave olfactory research this fundamental insight through his pioneering experiments with genetically modified mice. When he replaced the gene for a certain receptor type with a different receptor type in the genome of the mouse, the axons of these olfactory sensory neurons did not end in their usual glomeruli. Instead, new glomeruli were formed.

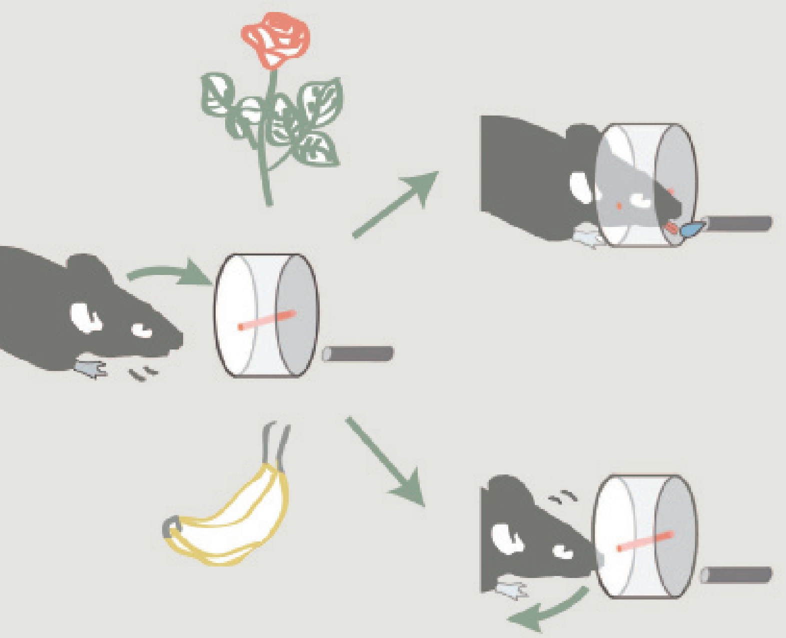
OLFACTORY PATTERNS IN THE NOSE

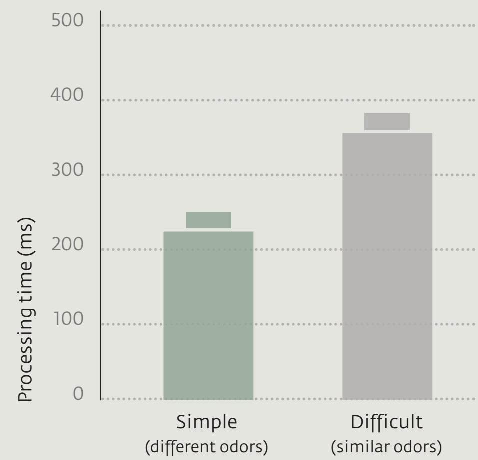
The result of this process is a characteristic and complex activation pattern – a kind of glomerular map – that, conversely, displays the mix of odorants an animal has smelled. A "camembert pattern" differs clearly from a "lemon pattern." If individual chemical components are found in both smells, the patterns of the activated glomeruli overlap. Even an individual odorant molecule can evoke a complex pattern because, in most cases, several different receptors respond. The brain constantly registers which types of olfactory receptors are activated at the same time. In the example of the typical camembert pattern, the brain then generates the matching olfactory image.

Mombaerts is fascinated by the dual function of the olfactory receptors. On the one hand, they recognize odorants. On the other hand, "the receptors also guide the axons to the right place in the olfactory bulb – a wonderfully simple solution from an evolutionary perspective," explains the olfactory researcher. At least one new glomerulus emerged in the bulb with each new gene for an olfactory receptor. Glomeruli, in turn, send their axons to the cerebral cortex where the final olfactory impression and the associated links and memories are produced.

Mombaerts and his team recently identified a second control mechanism in olfactory processing that imposes order on the cornucopia of smells at a higher level. The researchers started by showing that the olfactory mucosa can be divided into two zones with different classes of receptors. The top one, referred to as "D" for dorsal, contains olfactory sensory neurons that express class I and II receptors (class I receptors probably mainly recognize water-soluble odorant molecules). The lower zone, referred to as "V" for ventral, contains almost exclusively class II receptors. A mix of the cell bodies of the two cell classes is found in the dorsal olfactory epithelium. On the way to the brain, the axons of the class I neurons fasciculate together via a mechanism that is not dependent on the receptor that is produced. When, through genetic manipulation, the researchers forced dorsal olfactory sensory neurons that

Photo: MPI for Medical Research – Rolf Sprengel





Odor identification in mice is a fast process, but it depends on the similarity of the smells: the more similar the smells, the longer it takes.

normally produce a class I receptor to instead produce a class II receptor, the axons of these neurons still aligned themselves with axons of neurons that express a class I receptor. Therefore, one of the first phases in sorting axons on their way to the olfactory bulb appears to depend only on the class of the olfactory sensory neuron. Again, as Mombaerts notes, "such a small step to unravel the odor code."

This will have to be followed by some big steps: "We still haven't worked out the entire logic of the system," Mombaerts admits. Why do we perceive the molecule phenylethyl ethanol as the scent of a rose? "No idea," says Mombaerts. Although his team has described some of the odorant molecules that bind to the olfactory receptors in recent years, the olfactory researchers still know too little about the chemical properties of many odorant molecules. "As a result, we are a long way from being able to predict how a molecule can best be altered in the test tube to create, for example, the ultimate rose scent." Or how a scent can be produced more inexpensively and simply. The perfume industry would give almost anything for this magical code – the Holy Grail of olfactory research. ◂

GLOSSARY

Intron
A non-coding section of a gene's DNA that divides neighboring exons (the coding sections). Introns are transcribed, but then spliced out of the RNA transcript before it is exported out of the nucleus for translation into the corresponding amino acid sequence.

Olfactory epithelium
A tissue comprising three cell types; it is specialized in the detection of smells and is located in humans in the *regio olfactoria* of the nasal mucosa.

cAMP
Cyclic adenosine monophosphate, a biological molecule that is derived from adenosine triphosphate (ATP) and that acts as a messenger substance in cellular signal transmission.

Glomeruli
Spherical neural knots through which olfactory information coming from the olfactory sensory neurons is processed.

Pheromone
A messenger substance that serves biochemical communication between members of a species.

Illustration: designergold based on material provided by the MPI for Medical Research