Making Sense of Scents

Scientists begin to decipher the alphabet of odors

By JOHN TRAVIS

Linda Buck can't smell musk. This comes as a great disappointment to her because Buck loves perfumes. She recalls dabbing them—especially Chanel No. 5, Marilyn Monroe's favorite—under her nose for fun when she was a little girl. Of course, remarks Buck, placing perfume that close to the nose can overwhelm the olfactory system to the point where the aroma is no longer sensed.

She should know. A Howard Hughes Medical Institute (HHMI) investigator at Harvard Medical School in Boston, Buck is a leader in the effort to tease out how the nose works with the brain to make sense of scents.

In 1991, she and HHMI researcher Richard Axel of Columbia University thrilled the olfaction field with the long-awaited discovery of cell-surface proteins in the mammalian nose that detect odor molecules, or odorants. Mammals appear to have around 1,000 genes for these odorant receptors, the largest gene family ever found.

In two recent papers, Buck's group and one led by HHMI investigator Randall Reed of the John Hopkins Medical Institutions in Baltimore have started to tally the odorants recognized by various receptors. This endeavor is expected to eventually reveal how one molecule can carry a pleasant scent of flowers while an almost identically shaped molecule has the stench of rotting garbage.

The preliminary answer: Distinct odorants bind to different arrays of receptors, a strategy that allows people to discriminate more than 10,000 odors even though there are only 1,000 or so odorant receptors. Buck compares this to employing the alphabet's 26 letters to form an entire dictionary of words.

"By using the letters in different combinations, you can describe an almost unlimited number of things with words. That's what the olfactory system is doing," she says

The basics of how the nose detects smells have been known for some time. Odorants waft up through the two nasal cavities until they strike a region that contains approximately 50 million olfactory neurons, the cells that bear odorant receptors. These sensory cells extend long fibers, known as axons, from the nose to the olfactory bulb, the brain region that first processes olfactory information and then sends signals to other areas of the brain (SN: 8/15/98, p. 106).

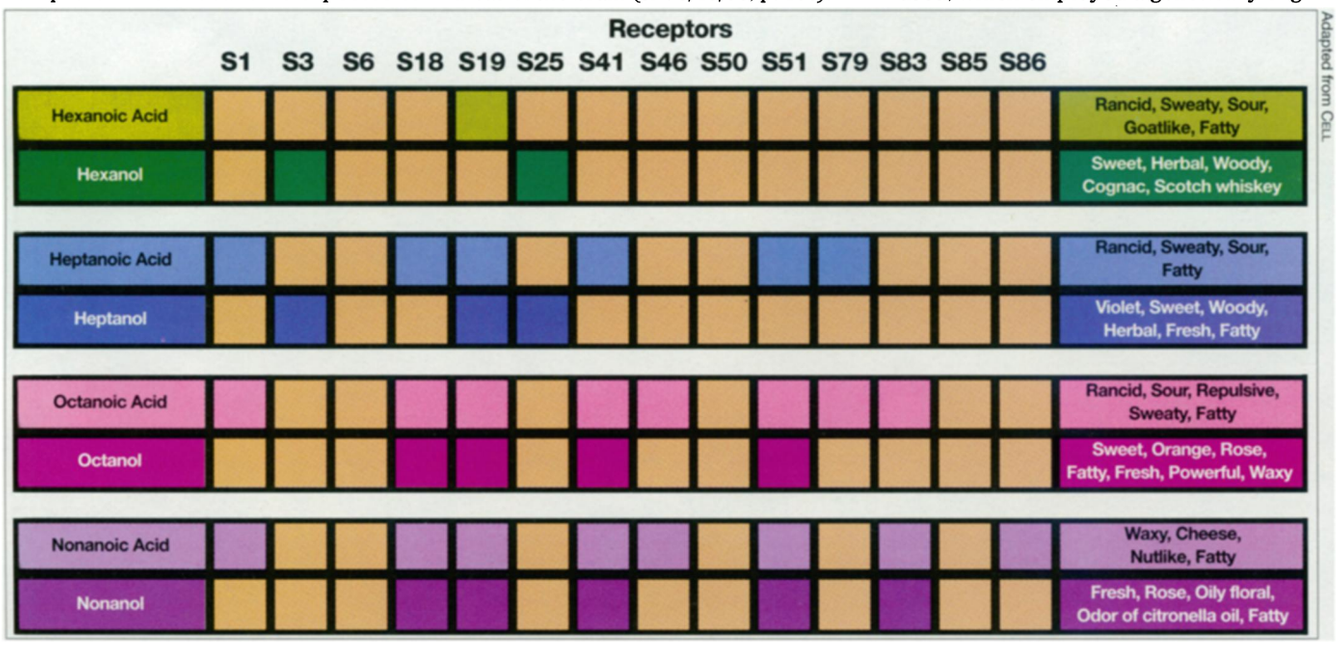
The 1991 discovery of odorant receptors spurred many new investigations into the mammalian sense of smell. In the past few years, for example, Buck and her colleagues have shown that each nasal cavity has four so-called expression zones. Any receptor type appears in only one of them.

Within each zone, however, the sensory cells bearing the same receptor are strewn about randomly, perhaps to prevent a loss of smell if a small area becomes damaged. Axons from sensory cells bearing the same receptor all converge on identical targets in the olfactory bulb.

Despite such findings, this emerging picture of the sense of smell had a major hole. Scientists hadn't been able to match odorants with their receptors.

"We knew a lot about how the wiring was set up from the primary olfactory neurons to the olfactory bulb," says Reed. "But you'll never understand how we perceive, or code, for an odor, unless you know something about the selectivity of [odorant] receptors on cells."

Last year, Stuart Firestein of Columbia University and his colleagues finally linked a rat receptor to a specific odorant, octanal, which has a meaty smell (SN: 1/10/98, p. 23). Yet the technique used, which employed a genetically engi-



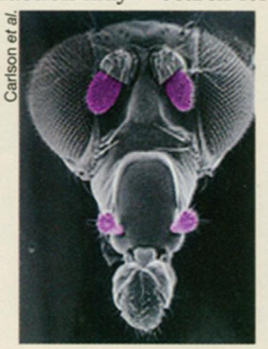
Although structurally similar, the molecules in each pairing have very different odors. Each molecule activates a unique combination of odorant receptors.

Insect odor receptors fly into view

Whether they are malaria-carrying mosquitoes homing in on human flesh, bees searching for flowers, or crop-devouring pests seeking their next meal, insects depend heavily on their sense of smell. Thus, insect olfaction is a topic of intense scientific, medical, and economic interest.

"There is a lot of motivation to learn how insect olfaction works—in the hope of finding ways to intrude into the control system and to get harmful insects to stop doing their bad behaviors," says John G. Hildebrand of the University of Arizona in Tucson. He studies the olfactory system of moths, which are major agricultural pests, for example.

Understanding insect olfaction may also provide insight into the sense of smell in higher animals. "What we learn about olfaction in flies and moths will guide us in our efforts to understand olfaction in our own kind," says Hildebrand.



Carlson et al.

Fruit fly head: In this electron microscope image, the olfactory appendages are tinted pink.

Yet insect olfactory investigators have been at a major disadvantage when compared with their vertebrate-studying counterparts. Despite years of effort by more than a dozen research groups, no one had identified any insects' odor-receptor molecules. Two research teams now report unearthing these elusive proteins in *Drosophila melanogaster*, the common fruit fly studied in many laboratories.

The findings are "truly landmark advances," says Hildebrand.

After mammalian odor receptors were identified in 1991, scientists studying fruit flies expected to quickly duplicate the feat. "Initially, we thought that [insect odor receptors] would look like the receptors in other animals," recalls Leslie B. Vosshall of the Howard Hughes Medical Institute at Columbia University, an author of one of the new reports in the March 5 CELL.

No such luck. Searches for fly genes whose DNA sequences resemble the genes for receptors in mammals bore no fruit. Last year, John R. Carlson of Yale University and his colleagues, who describe their work in the February NEURON, decided to broaden their search parameters. They scanned the growing database of fruit fly gene sequences created by researchers at the University of California, Berkeley. They looked for any gene encoding a protein that has two features considered necessary for an odor receptor. It must crisscross the cell membrane seven times and be able to couple to a molecule called a G protein.

While several dozen candidate genes emerged, just two were active only in the olfactory sensory organs in insects. "We got very excited about that. We found them in the antennae but not in the brain or abdomen, for example," says Carlson.

Further studies eventually identified 14 other members of this gene family. Each is active only in subsets of olfactory cells, further evidence that the genes encode odor receptors, says Carlson.

Vosshall, who is part of a research team led by Richard Axel of Columbia University, took a different tack in her search for fruit fly odor receptors. She and her colleagues compared the genes active in olfactory cells with those active in other parts of the insect's head or body. In this way, the researchers identified more than 50 genes specific to the olfactory organs. Only one of those genes encoded a cell-surface protein that seemed likely to be an odor receptor.

The researchers have now completed their own computer search of the fruit fly–genome database and discovered nearly a dozen relatives of their initial receptor candidate. Almost all of these matched the potential receptors found by Carlson's group.

Since only around 15 percent of the fly genome has been deciphered, the research teams estimate that the fruit fly might have 100 to 200 odor receptors. That's far fewer than the 1,000 or so odor receptors employed by worms and mammals, but Carlson cautions that the estimates are very rough. There could be one or more odor-receptor families still undetected, he says.

Demonstrating the power of fruit fly genetics, he and his colleagues have already begun work on the next question: How do you tell the neuron that recognizes the smell of a peach to turn on peach-receptor genes and the neuron that responds to the scent of an apple to turn on apple-receptor genes? "It's a really intriguing problem, and there's remarkably little known about it," says Carlson.

The researchers show that a protein called Acj6 helps certain sensory cells choose the odor receptor to display on their surface.

"I'm pretty optimistic we're going to learn a lot about how the brain sees odor," says Vosshall. "I think the true excitement will come in the next few years when we build a map that shows where all these [odor receptors] wire to in the brain." —*J.T.*

neered virus that forced sensory cells in rat nasal cavities to overproduce the receptor, is too labor-intensive to apply to all 1,000 or so receptors.

"Our real goal is to associate lots of [odorants] with lots of receptors," says Reed.

He and his colleagues therefore tried another tactic, which they described in the Dec. 23, 1998 CELL. Other scientists adding unaltered receptor genes to cells had found that the large proteins rarely make it to the cell's surface and thus seldom function. Reed and his team, however, edited the DNA sequences of various genes encoding odor receptors. The new genes resulted in surface proteins that are smaller than normal odor receptors. They retained most, but not all, of the potential odorant-binding regions.

Reed and his colleagues then joined each edited receptor gene to a gene encoding rhodopsin, a protein that they hoped would help guide the altered receptors to the cell surface. Reed's group created 80 such chimeric receptor genes, inserted them into kidney cells growing in a laboratory culture, and exposed the cells to 26 different odorants.

As indicated by changes in intracellular chemistry, each of three odorants—carvone (spearmint or carroway seeds), citronellal (citrus), and limonene (lime)—activated a different chimeric receptor. The researchers also confirmed that the rat receptor studied by Firestein's group responds to the meaty smelling octanal, but they found that the mouse version of the same receptor recognized heptanal, an herbal odor, instead. The change apparently stems from a single amino acid difference between the two receptor proteins.

This work "represents the beginning of a molecular understanding of odorant recognition," Reed and his colleagues say.

In the March 5 CELL, in a study that extends Reed's findings, Buck and her colleagues describe a more natural approach to deciphering olfactory codes. Instead of forcing nonsensory cells to make an altered odorant receptor, these researchers detect responses to odorants in olfactory sensory neurons taken from mice. Only later do they identify the receptors that the cells employ.

Buck's collaborators, Junzo Hirono and Takaaki Sato of the Life Electronics Research Center in Amagasaki, Japan, began with individual mouse olfactory neurons that they loaded with a calcium-sensitive fluorescent dye. They then exposed the cells to 24 odorants, each at several concentrations. If an odorant activated a cell, calcium ions would flow in and change the color of the light emitted by the dye.

After recording these responses, the Japanese scientists shipped the cells to Harvard Medical School, where Buck

and her colleague Bettina Mainic identified the single odorant receptor made by each cell. (In mammals, each olfactory neuron seems to use only one receptor. In contrast, a worm olfactory neuron uses many different receptors.)

By marrying their results, the two groups of scientists showed that individual odorants activate multiple receptors. They also found that individual receptors respond to multiple odorants, and different odorants trigger different combinations of receptors. The researchers conclude that the combination of receptors activated by an odorant determines the smell we perceive.

"We thought there was a combinatorial code. This is the first direct evidence for it," says Buck.

While praising her study, some olfactory researchers take issue with Buck's contention that the new evidence is the first. "The combinatorial idea has had an enormous amount of support from many people over many years," says John S. Kauer of Tufts University School of Medicine in Boston.

He points to past studies finding that odorants activate combinations of olfactory sensory neurons or of the target cells in the olfactory bulb to which they're connected. In fact, Kauer and other researchers have already incorporated the combinatorial coding strategy into artificial noses that they are constructing.

The new work by Buck and Reed provides "some of the first evidence that relates particular receptors to particular odors," Kauer says. "That's the strength of the papers."

The combinatorial idea offers fuller explanations for some fascinating features of the mammalian olfactory system. Consider that differences in concentration of an odorant can dramatically change how we perceive it. For example, a small amount of indole has a floral scent, while high concentrations have a putrid odor.

This oddity seems to stem from differences in the affinity of receptors for particular odorants. Since some receptors are activated only by large amounts of an odorant, a compound's receptor profile can differ with concentration.

"If you raise the concentration, you add new receptors to the code," explains Buck. In the case of bromooctanoic acid, varying concentrations of the odorant were recognized by between two and eight different types of odorant receptors.

The exquisite talent of the mammalian nose to discriminate different compounds is also reflected in Buck's results. "A slight change in the structure of an odor can lead to dramatic changes in smell," she notes. For instance, the rosy, orangy smell of octanol contrasts sharply with the rancid, sweaty odor of octanoic acid, although the molecules differ only in a side chain of atoms. As expected, Buck and her colleagues found that the two odorants triggered overlapping, yet distinct, arrays of receptors.

In the next year or two, predicts Reed, researchers may completely unravel the codes used to identify the world's smells. "We could scan all 1,000 or so mammalian odorant receptors to find the ones that are best at giving a response to [any given] odor," he says.

From such data, the researchers hope to discern eventually how the shapes of odorant receptors determine which odorants they recognize. Moreover, the investigators plan to examine whether the code for some odors can consist of a single receptor. Perhaps detecting rotten food has been so important that mammals evolved a specific receptor for just that odor, muses Buck.

Then, there's the musk issue, one of personal interest to her. Many people have anosmias—defects in the ability to smell certain odors. Buck's inability to sense musk is a common one; more than 10 percent of people share the problem. She hopes to test the hypothesis that such anosmias result from a lack of certain odorant receptors.

While Buck can accept not being able to enjoy the scent of some perfumes, she won't rest until she knows why. □