



The background features a light beige color with a decorative illustration of a skull on the left side. The skull is rendered in a light, sketchy style, with intricate floral and scrollwork patterns overlaid on its surface. The overall aesthetic is elegant and artistic.

COMMON

FLAVORS, AROMAS, AND CHEMICAL SIGNALS RELEASED FROM ANIMALS CAN MAKE THE MOUTH WATER, EVOKE VIVID MEMORIES, AND PERHAPS EVEN SIGNAL STRESS OR FERTILITY. HOW DOES THE BRAIN SORT IT ALL OUT?

SENSES

BY RICHARD SALTUS ILLUSTRATION BY HANNAH STOUFFER

Twenty-five years ago, the physician and writer Lewis Thomas predicted that the progress of biological research would be measured by how long it took to gain a complete understanding of odor. “It may not seem a profound enough problem to dominate all the life sciences,” he observed, “but it contains, piece by piece, all the mysteries.”

How an organism recognizes a “vast universe” of odors is indeed “a fascinating problem in molecular recognition and perceptual discrimination,” agrees Richard Axel, an HHMI investigator at New York’s Columbia University.

Put simply, how do we know what we’re smelling? Scientists are exploring this question in everything from worms to fruit flies to mice to humans, bringing a variety of new molecular tools and computational methods to bear.

Only in the last decade and a half, scientists, including Axel and HHMI investigator Linda Buck at Seattle’s Fred Hutchinson Cancer Research Center, have begun breaking the code the olfactory system uses to define different incoming odor molecules—the first step in recognizing them.

They have revealed how the coded information for a smell is represented or

“mapped” in certain parts of the brain. Now the scientists are in hot pursuit of the next steps. “How does the brain transform that map into meaningful neural information so that odors will elicit appropriate cognitive responses and behaviors?” Axel says. “This is the central problem facing my laboratory.”

The nasal cavity and the tongue are laced with cells that detect chemical compounds—millions of neurons in the nose and specialized taste bud cells on the tongue. These cells are wired to relay stations and processing centers in the brain, which are thought to create sensory “images” of the perceived odors or flavors.

In parallel with the main olfactory system used for odor sensing, evolution has also spawned a separate “accessory olfactory system” in some animals for detecting “pheromones”—chemical signals used by individuals of the same species to mark territory, warn of danger, identify close relations, and induce mating.

The lack of accessory olfactory structures in humans has suggested a corresponding lack of human pheromones. But interesting new discoveries are rewriting the textbook, demonstrating that in some mammals, at

least, pheromones can be detected by the odor-sensing olfactory system as well.

**“READING”
AN ODOR**

A smell begins when volatile odor molecules (odorants) dissolve in nasal mucus and bind with receptors in the olfactory epithelium—specialized tissue located in the upper-rear nasal cavity. (The convoluted olfactory epithelium in humans, if flattened out, would be the size of a cookie, while the equivalent area in a bloodhound, for example, would be the size of a small pizza.) The odorant receptors are located on olfactory sensory neurons, which transmit signals through their axons to the olfactory bulb, a relay station in the front of the brain. Olfactory bulb neurons, in turn, transmit signals to the olfactory part of the cortex, which distributes olfactory information to yet other brain areas.

Mammals can detect at least 10,000 different odors. How the mammalian olfactory system can distinguish so many odorant chemicals was a longstanding mystery until 1991, when Axel and Buck, then his post-doctoral associate, made a discovery that opened a new chapter in olfactory research. They identified a gene family that encodes about 1,000 different types of olfactory receptors in the mouse, and a smaller number, about 350, in humans, and then independently went on to explore how olfactory information is organized and encoded in the nervous system. In 2004, Axel and Buck were awarded the Nobel Prize in Physiology or Medicine for “their discoveries of odorant receptors and the organization of the olfactory system.”

They discovered that each neuron in the nose expresses only one receptor gene. Thousands of neurons with the same receptor are scattered in the nose, but their axons all converge in a few specific glomeruli (spheroid structures) at two spots in the olfactory bulb.

“OUR SENSORY EXPERIENCES REPRESENT A BEAUTIFULLY ORCHESTRATED RESPONSE TO A WIDE RANGE OF STIMULI.” — Charles Zuker



Because of research by HHMI investigators **CHARLES ZUKER**, University of California, San Diego, **LINDA BUCK**, Fred Hutchinson Cancer Center, and colleagues, we know a lot more about taste-sensing cells than we did a decade ago.

How does the olfactory system distinguish among thousands of odorants, some with nearly identical structures? Buck's group discovered that odorant receptors are used combinatorially to encode odor identities. "Just as letters of the alphabet can be used in different combinations to form a multitude of different words, odorant receptors are used in different combinations to create a vast array of different odor perceptions," says Buck. In the olfactory bulb, each odorant is thus represented by a unique combination, or "map," of glomeruli at differing positions, with a similar activation profile in every individual.

This map must be conveyed in some form on to the next, more complex, levels of processing in the brain. Tracing these pathways and events is, for Axel, something most easily done in the simple nervous system of the fruit fly. With powerful imaging techniques, he can visualize individual neurons connecting the fly's antennal lobe (analogous to the olfactory bulb in mammals) to higher brain structures. In collaboration with HHMI investigator David J. Anderson and Seymour J. Benzer at the California Institute of Technology, Axel has traced the path of nerve signals that are activated when the fly detects carbon dioxide gas, a component of a "stress odorant" that traumatized flies emit to warn other flies away. The researchers found that a single type of

olfactory neuron detects the CO₂ and that those neurons connect to a single glomerulus in the antennal lobe. By tracing this simple "dedicated circuit" at successively higher levels, Axel hopes ultimately to close the entire loop from input to output.

In the mouse, Buck and her colleagues have traced the pathway from single types of odorant receptors in the nose through the olfactory bulb to the olfactory cortex. This work revealed that the cortex also has a stereotyped map of odorant receptor inputs. However, while inputs from different receptors are segregated in the olfactory bulb, they are mapped onto the cortex in a partially overlapping fashion. Moreover, single cortical neurons appear to receive signals from combinations of odorant receptors, suggesting that they might integrate signals from different receptors that recognize the same odorant.

Buck's group also reported that some cortical neurons respond to a mix of two odorants, but not to either one alone. "We think that what we are seeing in the cortex may be an initial step in the reconstruction of an odor image from its deconstructed features, which are encoded by combinations of odorant receptor inputs," Buck says.

THE TASTE IS IN THE RECEPTOR

Flavors are made up of inputs from the taste buds of the tongue as well as from olfactory information stimulated by the aromas of food wafting up into the rear of the nasal cavity. Taste-bud neuronal receptors are sensitive to five basic qualities—bitter, sweet, sour, salty, and umami (glutamate or "savory"). These ancient senses likely evolved for seeking nutrients and avoiding toxins or spoiled food.

The receptors were unknown until 1999, when research groups headed by Charles S. Zuker, an HHMI investigator at the University of California, San Diego, and Nicholas Ryba of the National Institutes of Health isolated the first candidate taste receptors, now known as T1R1 and T1R2. Then in 2000, Zuker, Ryba, and Buck reported the isolation of the receptors for bitter taste, collectively known as the T2Rs. More than 30 different T2Rs exist, reflecting the importance of avoiding a wide range of bitter substances, many of which are poisons. Notably, experiments by Zuker, Ryba, and colleagues demonstrated that the bitter taste receptors are a population of bitter-sensing cells in the tongue that trigger hardwired aversion signals.

If so, what explains the popularity of coffee, with its bitter caffeine taste, as well as beer and certain other bitter foods? For one thing, says Zuker, who likes darkly



HHMI investigators **RICHARD AXEL**, Columbia University, and **CATHERINE DULAC**, Harvard University, are revealing the wiring of the systems involved in smell and pheromones, respectively.

roasted coffee and takes it black, with sugar, “There is a reward associated with coffee and beer.” For another, “We like to live on the edge and have new sensory experiences.” Of course, individuals vary in their taste for bitter substances, causing some people to steer away from strong ales and to dose their coffee with cream and sugar.

Not long after the bitter-receptor discovery, Zuker and Ryba functionally characterized the receptors and cells for sweet and umami tastes; in the fall of 2006, the scientists reported that sour taste is detected by a completely separate population of taste cells expressing an ion channel protein called PKD2L1. The receptor for salt has still not been found.

Zuker concludes that taste coding in the tongue and mouth is configured with “elegant simplicity.” He writes: “It is now clear that distinct cell types, expressing unique receptors, are tuned to detect each of the five basic tastes. And while certain areas of the tongue are more sensitive to some flavors than to others, every area can respond to every flavor.”

A corollary that emerged from these studies is that taste is a property of the cells that are activated, not of the food molecules—or even the receptors. Zuker and colleagues inserted receptors for a tasteless opioid compound into the sweet-responsive cells of mice, and the animals reacted as if the compound tasted sweet. Zuker and Ryba also generated mice that taste bitter

compounds as sweet. “There’s nothing bitter about bitter tastants, and there’s nothing sweet about sucralose [a sugar substitute],” observes Ryba. “It tastes sweet because when we put it on our tongue we get a particular pattern of neural firing.”

Ultimately, Ryba and Zuker hope to trace taste signals from the tongue up into the brain, where they are mingled with olfactory and other sensory information, leading to cognitive and behavioral responses.

FASCINATION WITH PHEROMONES Communication via pheromones occurs in insects, fish, reptiles, and mammals—though whether they’re significant in the lives of human beings is a fascinating and controversial question. “Pheromones have to do with making shortcuts in the brain to certain behaviors,” explains HHMI investigator Catherine Dulac at Harvard University. “In a sense, these pheromones are a by-product of the animal’s internal state,” she says. “For example, if an animal has a high level of testosterone, the metabolites in its urine will be high and they act as a pheromonal signal of dominance” that goes out to both males and females in close proximity.

It has long been thought that pheromones may be sensed exclusively by the accessory olfactory system. In this system, chemicals are detected in the vomeronasal

organ (VNO) in the nasal septum and signals are then transmitted through special pathways separate from those that carry odor signals. Most mammals and reptiles have a VNO, whereas if a VNO exists in humans at all, it is nonfunctioning.

While working in Axel’s lab, Dulac in 1995 was the first to identify a family of receptors in the VNO. Two years later, the Buck and Ryba groups as well as Dulac at Harvard discovered a second family of VNO receptors. In further studies, Dulac identified additional components of the VNO signaling machinery.

Given that the human nose lacks a functional VNO, is there any reason to think that people can communicate through pheromones? Many scientists have been skeptical, yet in the popular imagination a belief in some sort of sexual “chemistry” between human beings continues to thrive. Whether this is true remains unknown, but several recent experiments have revealed a previously unsuspected capacity of the main olfactory system to detect pheromone signals—at least in some mammals—possibly representing an evolutionary backup to the VNO for receiving these crucial communications.

In 2002, Dulac reported that mice whose VNO function had been knocked out were nevertheless able to respond to pheromonal mating signals. Even without a working VNO, they sensed fertility pheromones in the environment and went into mating mode. But, to the researchers’ surprise, males could no longer distinguish between

a male and female mouse—nor did they show stereotypical aggression against other males. The experiment “told us the VNO function is not required for mating behavior,” says Dulac. “What people were saying was the detector of the ‘love potion’—the VNO—was not true. So there is something else, in the olfactory system, that is detecting cues and getting animals to mate.”

In another intriguing finding, Buck and postdoctoral fellow Stephen Liberles reported in the August 10, 2006, issue of *Nature* that they had identified a second family of chemosensory receptors in the olfactory epithelium of the mouse that is unrelated to the odorant receptor family and that may detect pheromones. These receptors, called “trace amine-associated receptors,” or TAARs, were previously proposed to function in the brain as receptors for chemical messengers called trace amines. However, the researchers could not detect any of the 15 mouse TAARs in the brain, whereas they found that 14 of the 15 are expressed by olfactory neurons in the nose. Each TAAR gene is expressed by a unique set of neurons, just like each odorant receptor gene.

Zebrafish have 57 of these receptors—many or all found in the olfactory epithelium—and humans, interestingly, have six TAARs. By testing TAARs with

more than 200 compounds, Liberles and Buck found that several mouse TAARs recognize stress or gender-linked signals in mouse urine, and one TAAR recognizes a male pheromone that stimulates puberty in female mice. “The evolutionary conservation of TAARs, their expression patterns in mouse and fish, and the TAAR ligands we have identified so far together suggest that TAARs serve a different function from that of odorant receptors, and that they may be involved in recognizing social cues such as pheromones,” Buck says. Since humans have TAARs, might they be capable of detecting pheromonal signals among people? That’s a question Buck is pursuing.

The discovery of TAARs as olfactory receptors is not the only recent finding that suggests pheromones can be detected in the nose as well as in the VNO. Dulac and Buck have also found evidence in mice of connections between the olfactory epithelium and brain neurons that control reproductive hormones and perhaps sexual behavior. “So now, if you ask the question ‘Where are the neurons that detect pheromones?’ ” Dulac says, “we can say that both the VNO and the main olfactory system are involved.” Why have animals developed two sensory systems to detect pheromones? Dulac has shown

that the brain coding of VNO information is very different from the main olfactory system and favors the processing of complex blends of pheromone compounds as contrasted with single pheromone compounds.

If these discoveries carry over to humans, they could help explain intriguing observations of what appear to be pheromone-like communications between people—even in the absence of a functioning VNO. Among them: the well-studied phenomenon that women working or living in close proximity tend to have synchronized menstrual cycles and reports that members of opposite sexes have more “chemistry” with individuals whose immune systems are genetically different. (The evolutionary explanation is that such couples would theoretically produce offspring with a broader range of disease-fighting immune cells.)

THE LURE OF THE PURSUIT

Clearly, an enormous amount of work lies ahead to reach Lewis Thomas’s goal of understanding smell—as well as taste and the mechanics of pheromone sensing. Yet the lure of the pursuit remains strong among researchers, the biotech and food industries, and curious people everywhere.

“Our sensory experiences represent a beautifully orchestrated response to a wide range of stimuli,” says Zuker. “The chemical senses capture the imagination of scientists and the public because we can so easily relate to them, and because they have the potential to lead to products that can improve diet, health, and the way we live.” ■

AT A STORE NEAR YOU?

Industry scientists are using knowledge about the codes

for taste and smell to try to trick the sensory receptors and enhance the flavors of food, create more healthful substitutes for sugar and salt, or counter the bitter taste of medicines. Most current applications involve taste receptors, even though the olfactory receptors were discovered first, says Gary Beauchamp, Ph.D., president and director of the Monell Chemical Senses Center, a nonprofit research institute in Philadelphia. >> “It was thought that once the receptors for smell were identified, they could be put into cell systems in the laboratory; then you could find out what compounds bind to the receptors,

and this might be useful in designing odors of value. But it’s been much more difficult to do than anyone would have guessed,” Beauchamp says. >> In early 2006, Monell scientists reported they had succeeded in maintaining rat taste receptor precursor cells in culture for up to two months and planned to try growing human taste cells in a similar manner. “This has the potential for practicality,” Beauchamp says.

>> **Senomyx**, a California biotechnology company of which HHMI investigator Charles Zuker is a founder and Catherine Dulac is a scientific advisory board member, is developing several products aimed at the food industry. One, a spinoff of the discovery of the umami receptor, is aimed at creating a savory flavor-enhancing substitute for MSG (monosodium glutamate) and the company plans to sell it to China, which has the world’s largest market for MSG. >> **The company is** also at work on natural and synthetic compounds as high-potency sweeteners to

reduce the need for high-calorie carbohydrate sweeteners in foods and drinks and on compounds that can block bitter receptors in taste cells. >> “**Blocking or modulating** bitter sensation and taste could make otherwise unpalatable food sources such as soy protein more desirable, as well as medicines that leave a bitter aftertaste,” says Zuker. Who knows—such a product might even lead to a cup of coffee with no bitterness, even taken black. —R.S.