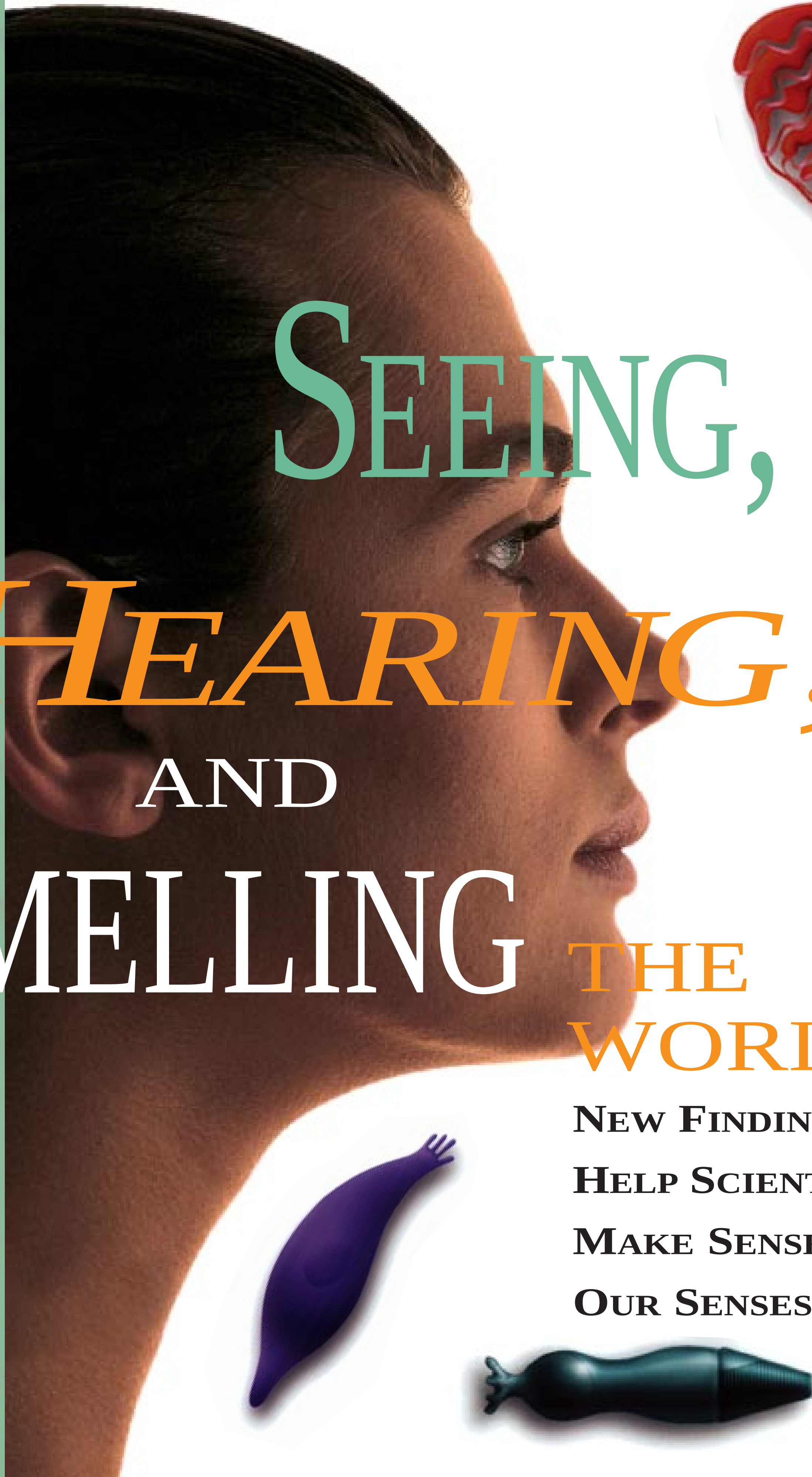
A REPORT FROM THE HOWARD HUGHES MEDICAL INSTITUTE



SEEING, HEARING, AND SMELLING THE WORLD

NEW FINDINGS HELP SCIENTISTS MAKE SENSE OF OUR SENSES

The Howard Hughes Medical Institute was founded in 1953 by the aviator-industrialist Howard R. Hughes. Its charter reads, in part:

"The primary purpose and objective of the Howard Hughes Medical Institute shall be the promotion of human knowledge within the field of the basic sciences (principally the field of medical research and medical education) and the effective application thereof for the benefit of mankind."

This is the fifth in a series of reports about biomedical science. For further information, please contact the Howard Hughes Medical Institute,
4000 Jones Bridge Road,
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FOREWORD

It is a pleasure to introduce the latest of the biomedical research reports that the Howard Hughes Medical Institute publishes for general readers. *Seeing, Hearing, and Smelling the World*, like the four previous publications in the series, takes us to the frontiers of science. It guides us on a journey into the fascinating world of the senses and the nervous system, where researchers are working to understand problems of great potential benefit.

The most routine, everyday occurrences, such as recognizing a friend on the street and exchanging greetings, demonstrate the biological complexity of the puzzles that scientists are attempting to solve. Although such encounters seem simple, they require hundreds of millions of cells to act in precise ways to receive the sights and sounds and translate them into electrical impulses. These impulses flow through the nervous system to carry the messages to the brain, where they can be understood and acted upon at astonishing speed.

Centuries of effort by thousands of scientists in laboratories throughout the world have been required to bring us to our current, deepening understanding about how we hear, see, and smell. Thanks to the new analytical tools provided by molecular biology, progress toward understanding the senses and the nervous system has been rapid during the past decade. Indeed, many neuroscientists believe that biomedical science is poised to make substantial progress toward understanding how the brain works, not only in terms of the senses, but also complex functions like learning and memory. It is an exciting prospect.

This series is published by the Institute as a public service in order to make the results of current biomedical research available to readers who are not scientists. It is clear that a basic grasp of biology is increasingly essential for citizens who have to make difficult decisions about health care, drug abuse, the environment, and other critical issues.

Teachers are particularly enthusiastic about these reports, and surveys tell us that they preserve their copies and use them year after year. Nearly 4,000 class sets have been requested by high school, college, and even medical school teachers in the United States and abroad; altogether, more than 400,000 copies of the publications have been printed.

The Institute's interest in science education continues to deepen and its commitment to education reform to grow. Its grants program, which was established in 1987, has now become the largest private science education effort in U.S. history. Through its financial support and other activities, the Institute is seeking to make science come alive for today's students, which is exactly what we hope *Seeing, Hearing, and Smelling the World* will do.

Purnell W. Choppin

Purnell W. Choppin, M.D.
President
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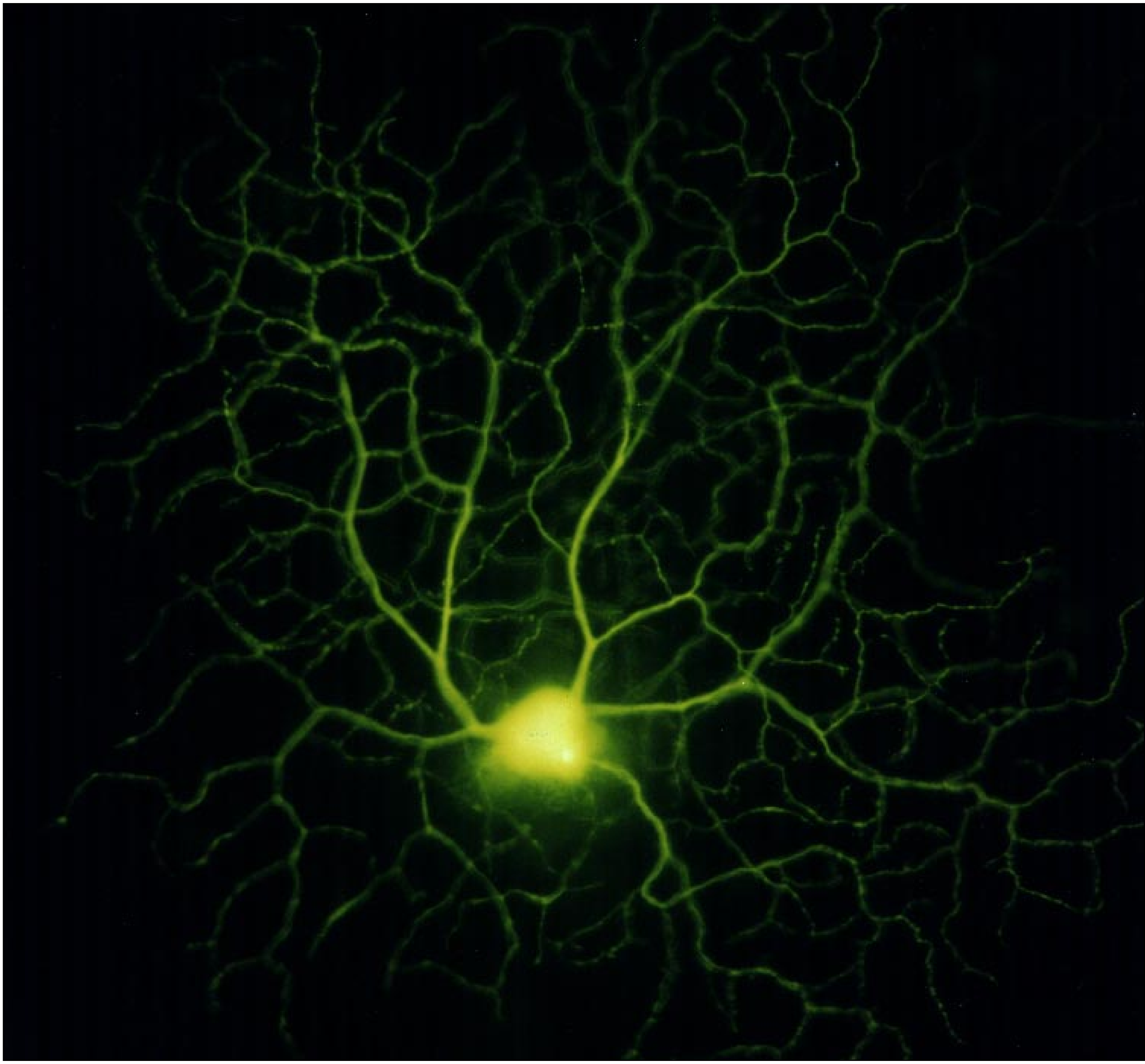
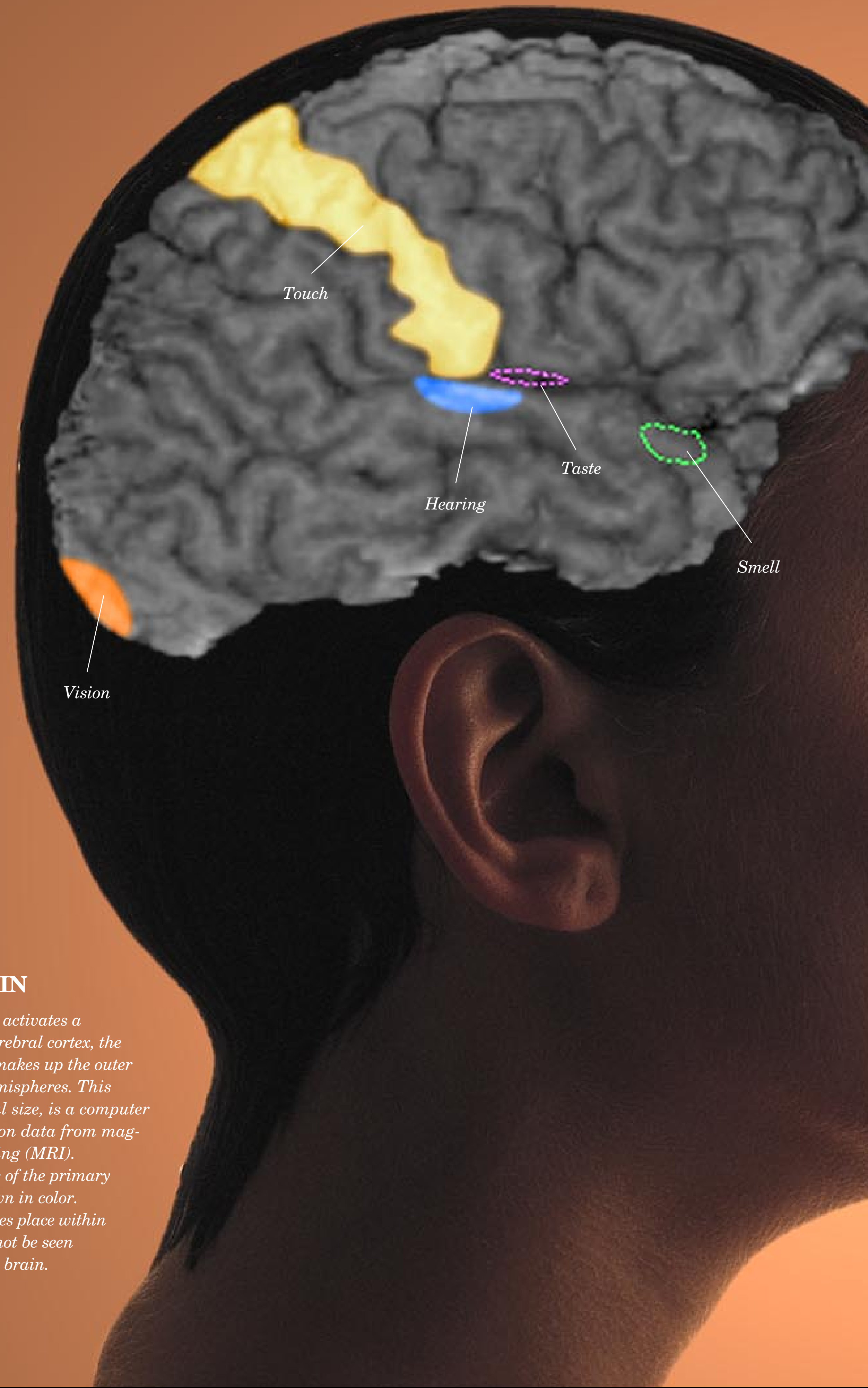
Laboratory of Richard Masland, Massachusetts General Hospital



A nerve cell that can detect in what direction an object is moving branches out to make contact with many other cells in a rabbit's visual system. The cell glows yellow because it was injected with fluorescent dye.

SEEING, HEARING, AND SMELLING THE WORLD

New Findings Help Scientists Make Sense of Our Senses



A Life-Size Human Brain

Each of the five senses activates a separate area of the cerebral cortex, the sheet of neurons that makes up the outer layer of the brain's hemispheres. This brain, shown in actual size, is a computer reconstruction based on data from magnetic resonance imaging (MRI). Approximate locations of the primary sensory areas are shown in color. Most of the activity takes place within convolutions that cannot be seen from the surface of the brain.

Our Common Senses

We can recognize a friend instantly—full-face, in profile, or even by the back of his head. We can distinguish hundreds of colors and possibly as many as 10,000 smells. We can feel a feather as it brushes our skin, hear the faint rustle of a leaf. It all seems so effortless: we open our eyes or ears and let the world stream in.

Yet anything we see, hear, feel, smell, or taste requires billions of nerve cells to flash urgent messages along linked pathways and feedback loops in our brains, performing intricate calculations that scientists have only begun to decipher.

"You can think of sensory systems as little scientists that generate hypotheses about the world," says Anthony Movshon, an HHMI investigator at New York University. Where did that sound come from? What color is this, really? The brain makes an educated guess, based on the information at hand and on some simple assumptions.

When you look at the illustration below, for instance, you see an X made of spheres surrounded by cavities. But if you turn the page upside down, all the cavities become spheres, and vice versa. In each case, the shapes seem real because "your brain assumes there is a single light source—and that this light comes from above," says Vilayanur Ramachandran, a professor of neuroscience at the University of California, San Diego. As he points out, this is a good rule of thumb in our sunlit world.

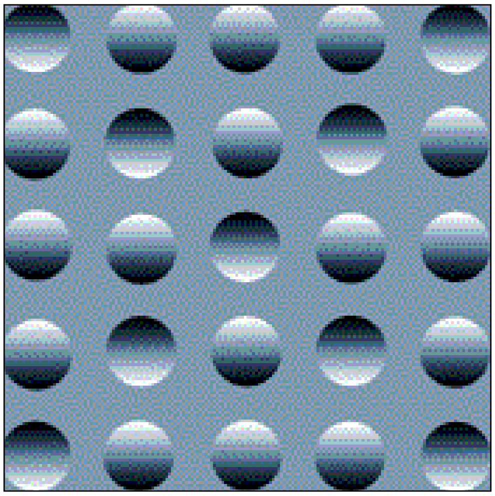
To resolve ambiguities and make sense of the world, the brain also creates shapes from incomplete data, Ramachandran says. He likes to show an apparent triangle that was developed by the Italian psychologist Gaetano Kanizsa. If you hide part of this picture, depriving the brain of certain clues it uses to form conclusions, the large white triangle disappears.

We construct such images unconsciously and very rapidly. Our brains are just as fertile when we use our other senses. In moments of anxiety, for instance, we sometimes "hear things" that are not really there. But suppose a leopard approached, half-hidden in the jungle—then our ability to make patterns out of incomplete sights, sounds, or smells could save our lives.

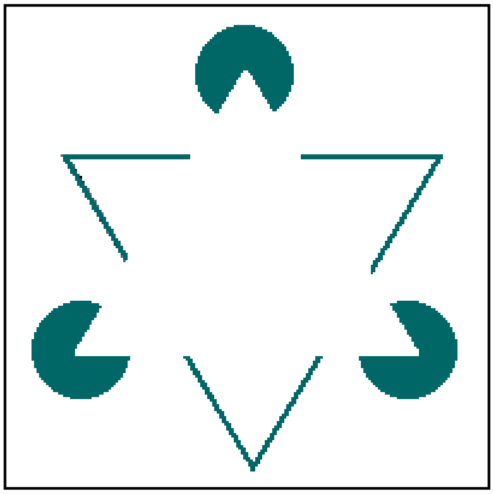
Everything we know about the world comes to us through our senses. Traditionally, we were thought to have just five of them—vision, hearing, touch, smell, and taste. Scientists now recognize that we have several additional kinds of sensations, such as pain, pressure, temperature, joint position, muscle sense, and movement, but these are generally included under "touch." (The brain areas involved are called the "somatosensory" areas.)

Although we pay little attention to them, each of these senses is precious and almost irreplaceable—as we discover, to our sorrow, if we lose one. People usually fear blindness above all other disabilities. Yet deafness can be an even more severe handicap, especially in early life, when children learn language. This is why Helen Keller's achievements were so extraordinary. As a

ILLUSIONS REVEAL SOME OF THE BRAIN'S ASSUMPTIONS

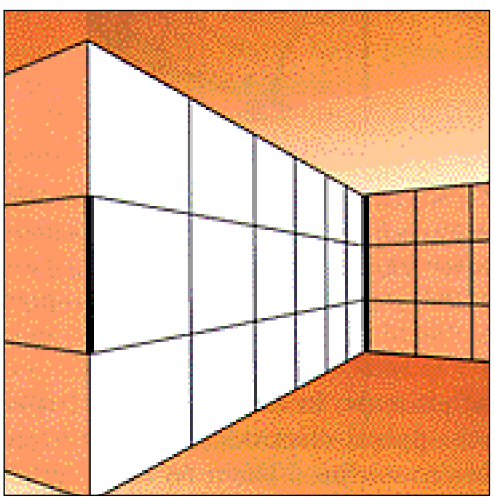


The shaded circles seem to form an X made of spheres. But if you turn the page upside down, the same circles form an X made of cavities, since the brain assumes that light comes from above.



Are these triangles real? They appear to be, because the brain automatically fills in lines that are missing. But if you block out parts of the picture, the triangles vanish.

result of an acute illness at the age of 19 months, she lost both vision and hearing and sank into a totally dark, silent universe. She was rescued from this terrible isolation by her teacher, Anne Sullivan, who managed to explain, by tapping signs into the little girl's palm, that things have names, that letters make up words, and that these can be used to express wants or ideas. Helen Keller later grew into a writer (her autobiography, *The Story of My Life*, was published while she was still an undergraduate at Radcliffe College) and a well-known advocate for the handicapped. Her remarkable development owed a great deal to her determination, her teacher, and her family. But it also showed that when a sense (or two, in Helen Keller's case) is missing, another sense (in her case, touch) may be trained to make up for the loss, at least in part.



The black line in the back seems much longer than the one in the front because your brain assumes it is seeing the effects of perspective. Take a ruler to find out for yourself.

What we perceive through our senses is quite different from the physical characteristics of the stimuli around us. We cannot see light in the ultraviolet range, though bees can, and we cannot detect light in the infrared range, though rattlesnakes can. Our nervous system reacts only to a selected range of wavelengths, vibrations, or other properties. It is limited by our genes, as well as our previous experience and our current state of attention.

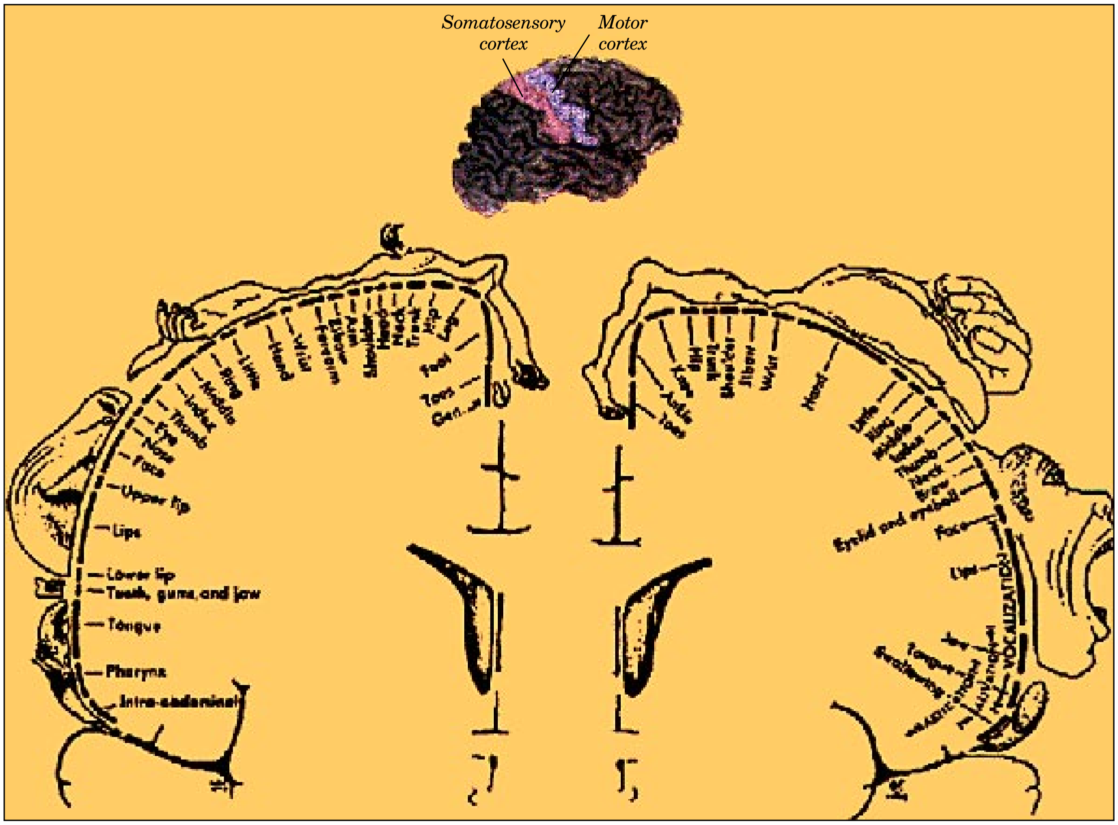
What draws our attention, in many cases, is change. Our senses are finely attuned to change. Stationary or unchanging objects become part of the scenery and are mostly unseen. Customary sounds become background noise, mostly unheard. The feel of a sweater against our skin is soon ignored. Our touch receptors, "so alert at first, so hungry for novelty, after a while say the electrical equivalent of 'Oh, that again,' and begin to doze, so we can get on with life," writes Diane Ackerman in *A Natural History of the Senses*.

...the patients were awake, since the brain does not feel what is happening to it.

If something in the environment changes, we need to take notice because it might mean danger—or opportunity. Suppose an insect lands on your leg. Instantly the touch receptors on the affected leg fire a message that travels through your spinal column and up to your brain. There it crosses into the opposite hemisphere (the right hemisphere of the brain receives signals from the left side of the body, and vice versa) to alert brain cells at a particular spot on a sensory map of the body.

This map extends vertically along a strip of cerebral cortex near the center of the skull. The cortex—a deeply wrinkled sheet of neurons, or nerve cells, that covers the two hemispheres of the brain—governs all our sensations, movements, and thoughts.

The sensory map in humans was originally charted by the Canadian neurosurgeon Wilder Penfield in the 1930s. Before operating on patients who suffered from epilepsy, Penfield stimulated different parts of their brains with electrodes to locate the cells that set off their attacks. He could do this while the patients were awake, since the brain does not feel what is happening to it. In this way, Penfield soon learned exactly where each part of the body that was touched or moved was represented in the brain, as he showed in his famous "homunculus" cartoons of the somatosensory



These famous maps by Wilder Penfield show that each part of the body is represented on two strips of the brain's cerebral cortex, the somatosensory cortex (left), which receives sensations of touch, and the motor cortex (right), which controls movements. Fingers, mouth, and other sensitive areas take up most space on both maps. Penfield called these cross sections the "sensory homunculus" and the "motor homunculus."

and motor areas.

Surprisingly, these maps do not accurately reflect the size of body parts but rather, their sensitivity. Arms and legs take up very little space, despite their length. The face and hands, which have greater sensitivity, are given more space—especially the tips of the fingers. Nevertheless, the signal that a mosquito has landed on the back of your left leg comes through loud and clear. In a fraction of a second, through a decision process that is not yet understood, this signal leads you to swat the insect at just the right place.

Ever since humans have wondered about where their thoughts came from, they have tried to understand the senses. Much was learned from observing the results of head injuries and tumors, as well as by dissecting postmortem human brains and the brains of animals. In the 1930s and 1940s, scientists applied electrodes to the surface of the brain or placed them on the skull of humans to study "evoked responses," the changing rhythms of electrical signals in the brain in response to specific stimuli such as light or sound. Unfortunately, these signals from billions of brain cells proved almost impossible to unscramble.

When extremely thin microelectrodes became available in the late 1950s, researchers implanted them into the brains of living animals to spy on the activity of individual cells. Sharp popping sounds could be heard as specific neurons fired, and the scientists tried to find out what provoked these electrical discharges.

This is how David Hubel and Torsten Wiesel, who were then at Johns Hopkins University, began the groundbreaking experiments on the visual cortex of cats and monkeys, for which they later won a Nobel prize. They discovered that one neuron in the primary visual cortex at the back of a cat's brain might fire only when the animal's eye was exposed to a line at a particular location and angle, while another next to it would fire only in response to a line at a slightly different angle. No one had suspected that these neurons would dissect a scene—and respond to particular elements of it—with such amazing specificity. Hubel and Wiesel's success led to a general focus on the abilities of single neurons, especially in the visual system.

The past decade has seen an explosion of research on all the senses, partly because of the new tools supplied by molecular biology. Scientists can now analyze sensory neurons far more precisely, down to the level of specific genes and proteins within these neurons. This publication will describe some recent research on three of our senses—vision, hearing, and smell—in which there have been particularly interesting developments.

The visual system, which involves roughly a quarter of the human cerebral cortex, has attracted more research than all the other sensory systems combined. It is also the most accessible of our senses. The retina, a sheet of neurons at the back of the eye that any physician can see through an ophthalmoscope, is the only part of the brain that is visible from outside the skull. Research on the visual system has taught scientists much of what they know about the brain, and it remains at the forefront of progress in the neurosciences.

Research on hearing is also gathering momentum. One group of scientists recently discovered how receptor neurons in the ear—the so-called "hair cells"—respond to sounds. Another group explored how animals use sounds to compute an object's location in space. This may be a model of similar operations in the auditory system of humans.

The olfactory system, which was almost a total mystery until a few years ago, has become the source of much excitement. The receptor proteins that make the first contact with odorant molecules appear to have been identified with the help of molecular genetics, and researchers are beginning to examine how information about smells is coded in the brain.

The use of molecular biology has enabled scientists to discover just how receptor neurons respond to light, to vibrations in the air, to odorant molecules, or to other stimuli. The receptor neurons in each sensory system deal with different kinds of energy—electromagnetic, mechanical, or chemical. The receptor cells look different from one another, and they exhibit different receptor proteins. But they all do the same job: converting a stimulus from the environment into an electrochemical nerve impulse, which is the common language of the brain (see p. 11). Recently, researchers have uncovered many of the genes and proteins involved in this process of sensory transduction.

From their understanding of this first step on the sensory pathway, researchers have edged up to analyzing how messages about a sensory stimulus travel through the brain to the cerebral cortex and how these messages are coded.

They know that nearly all sensory signals go first to a relay station in the thalamus, a central structure in the brain. The messages then travel to primary sensory areas in the cortex (a different area for each sense), where they are modified and sent on to "higher" regions of the brain. Somewhere along the way, the brain figures out what the messages mean.

Many factors enter into this interpretation, including what signals are coming in from other parts of the brain, prior learning, overall goals, and general state of arousal. Going in the opposite direction, signals from a sensory area may help other parts of the brain maintain arousal, form an image of where the body is in space, or regulate movement.

These interactions are so complex that focusing on the activity of single neurons—or even single pathways—is clearly not enough. Researchers are now asking what the central nervous system does with all the information it gets from its various pathways.

In more authoritarian times, scientists believed that the brain had a strictly hierarchical organization. Each relay station was supposed to send increasingly complex information to a higher level until it reached the very top, where everything would somehow be put together. But now "we are witnessing a

Sharp popping sounds could be heard as specific neurons fired...

paradigm shift," says Terrence Sejnowski, an HHMI investigator who directs the Computational Neurobiology Laboratory at the Salk Institute in La Jolla, California. Instead of viewing the cortex as "a rigid machine," scientists see it as "a dynamic pattern-processor and categorizer" that recognizes which categories go together with a particular stimulus, as best it can, every step of the way. "There is no 'grandmother cell' at the top that responds specifically to an image of Grandma," Sejnowski emphasizes. "We recognize a face by how its features are put together in relation to one another."

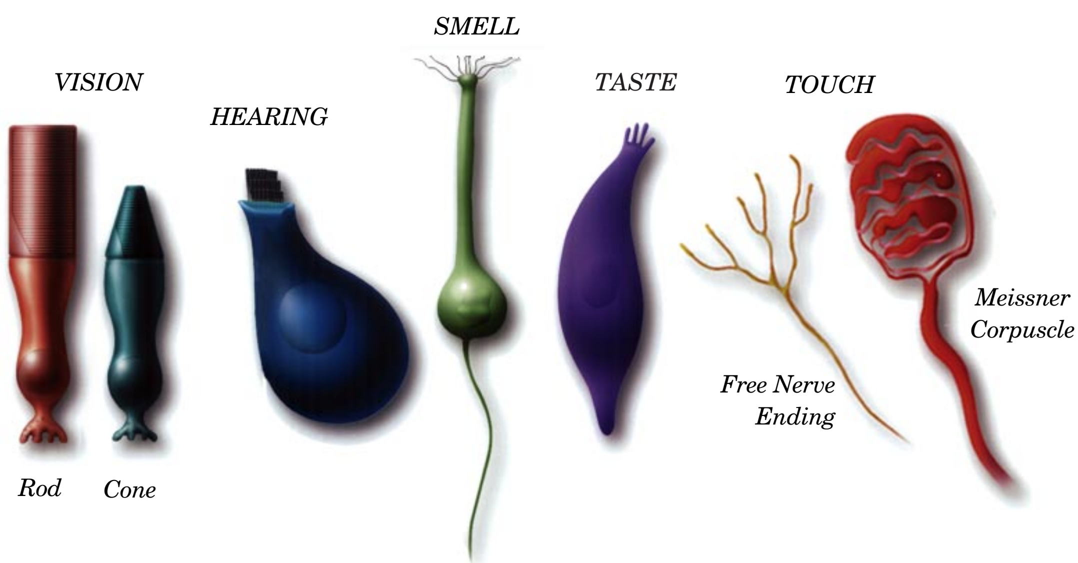
senses evolved "to help animals solve vital problems..."

Sejnowski, a leader in the new field of computational neuroscience, studies neural networks in which the interaction of many neurons produces surprisingly complex behavior. He recently designed a computer model of how such a network might learn to "see" the three-dimensional shape of objects just from their shading, without any other information about where the light came from. After being "trained" by being shown many examples of shaded shapes, the network made its own generalizations and found a way to determine the objects' curvature.

Vision and the other senses evolved "to help animals solve vital problems—for example, knowing where to flee," says Sejnowski. Large populations of sensory neurons shift and work together in the brain to make this possible. They enable us to see the world in a unified way. They link up with the motor systems that control our actions. These neurons produce an output "that is more than the sum of its parts," Sejnowski says. Just how they do it is a question for the next century.

Maya Pines, Editor

SPECIAL RECEPTOR CELLS FOR EACH OF THE SENSES



Rod and cone cells in the eye respond to electromagnetic radiation—light.

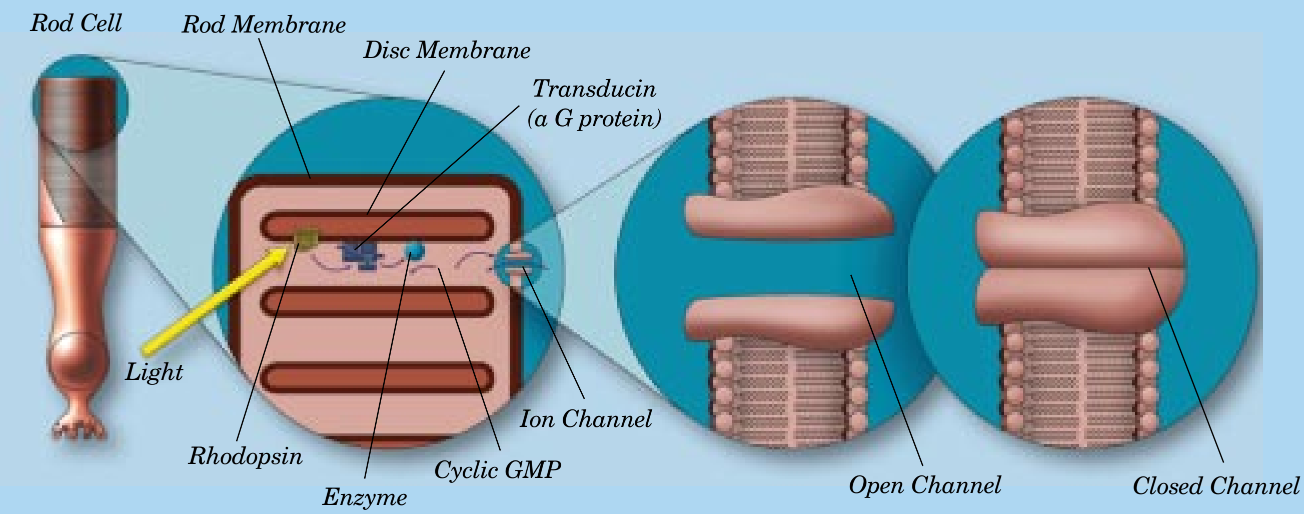
The ear's receptor neurons are topped by hair bundles that move in response to vibrations—sound.

Olfactory neurons at the back of the nose respond—and bind—to odorant chemicals .

Taste receptor cells on the tongue and back of the mouth respond—and bind—to chemical substances.

Meissner corpuscles are specialized for rapid response to touch, while free nerve endings bring sensations of pain.

A LANGUAGE THE BRAIN CAN UNDERSTAND



Almost at the very instant that light hits a cell in the retina, or a sound wave nudges the tip of a receptor cell in the ear, the receptor cell converts this stimulus into an electrical signal—the language of the brain.

This conversion, or transduction, is swift and precise. But it is also surprisingly intricate—so intricate that the process is not yet fully understood for most of the senses. In the past decade, however, it has been worked out quite thoroughly for vision.

It begins when a photon of light meets one of the photoreceptor cells of the retina (either a rod or a cone cell). A photon that strikes a rod cell is immediately absorbed by one of the 100 million molecules of a receptor protein—rhodopsin—that are embedded in the membranes of a stack of disks in the top part, or "outer segment," of each cell. These rhodopsin molecules have a snakelike shape, crisscrossing the membrane seven times, and contain retinal (a form of vitamin A), which actually absorbs the light. In the dark, the retinal fits snugly into a binding pocket in rhodopsin. But on exposure to light, it straightens out. This alters the three-dimensional structure of the entire rhodopsin molecule, activating it and triggering a biochemical cascade.

The activated rhodopsin then stimulates transducin, a protein that belongs to the large family of so-called G proteins. This in turn activates an enzyme that breaks down cyclic GMP, a "second messenger," dramatically lowering its level. Cyclic GMP carries signals from the disks, where light is absorbed, to the cell's surface membrane, which contains a large number of channels that control the flow of ions (charged atoms) into the cell. As ions move into the cell, they alter its electrical potential.

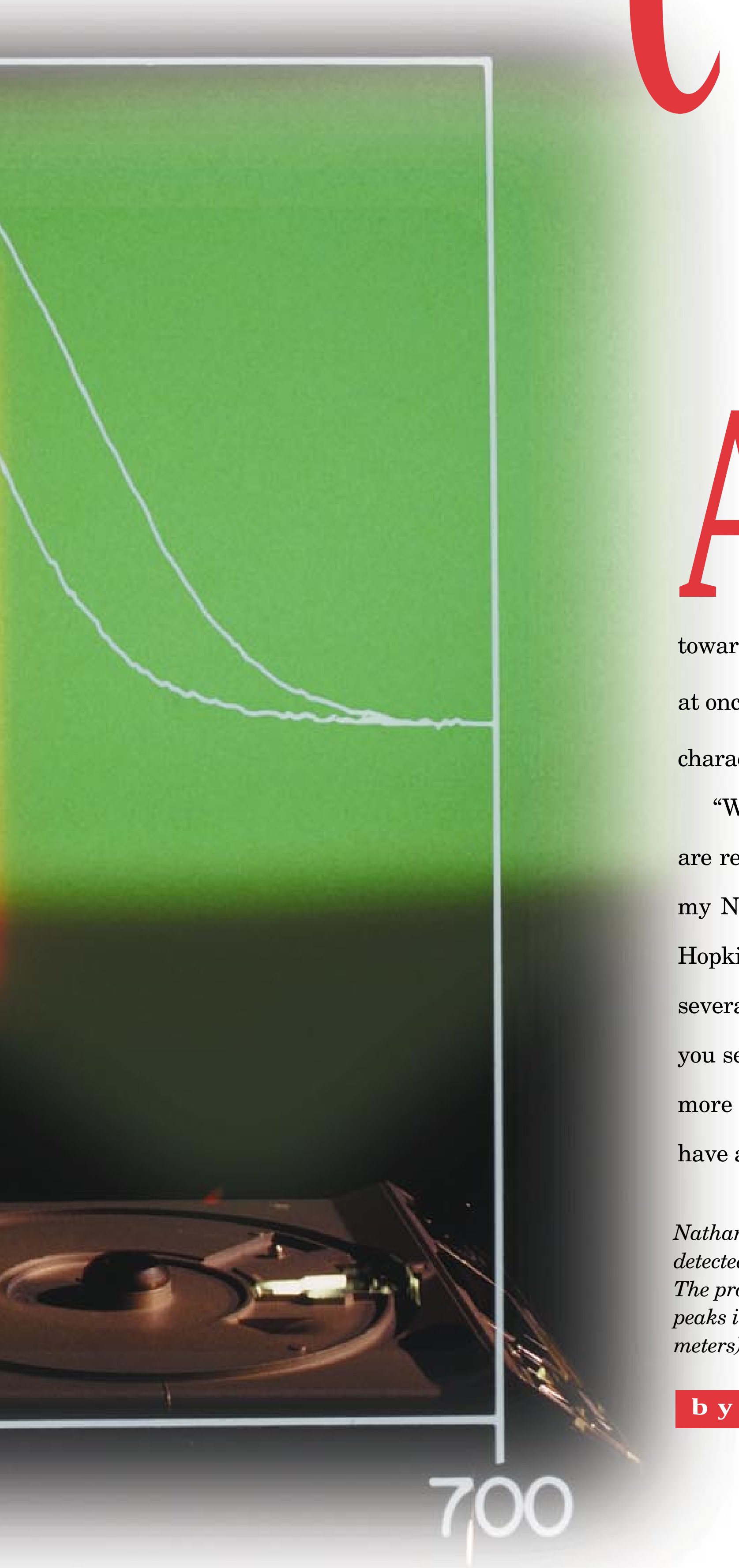
"In the dark, the channels are constantly open because of a high level of cyclic GMP. This allows sodium and calcium ions, which carry positive charges, to flow into the cell," explains King-Wai Yau, an HHMI investigator at the Johns Hopkins University School of Medicine who played an important role in deciphering the transduction process. "But in the light, the channels close. Then the electrical potential inside the cell becomes more negative. This reduces the amount of neurotransmitter that is released from the base of the cell to act on other cells"—and thus alerts neurons in the next layer of retinal cells that a photon of light has arrived.

This complex cascade of transduction events is repeated in a remarkably similar way in olfactory receptor cells, which respond to odors, says Yau. But the receptor cells that respond to sound use a very different system: their channels open and close as a direct response to a mechanical force—either tension or relaxation.

Whatever the means, the end result of transduction is the same: the cell generates an electrical signal that flashes through a dense thicket of nerve cell connections in the brain, bringing news from the outside world in a Morse-code-like language the brain can understand.

Breaking the C

ode of COLOR



A bright red beach ball comes whirling toward you. You see its color, shape, and motion all at once—but your brain deals with each of these characteristics separately.

"We need parallel processing because neurons are relatively slow computing machines," says Jeremy Nathans, an HHMI investigator at the Johns Hopkins University School of Medicine. "They take several milliseconds to go from input to output. Yet you see things in a fraction of a second—time for no more than 100 serial steps. So the system has to have a massively parallel architecture."

Nathans adjusts a slide projector to show the colors that are detected by receptor proteins in red and green cone cells. The proteins were made from human DNA in his lab. The peaks in the graph indicate the wavelengths (in nanometers) of light best absorbed by each protein.

by Geoffrey Montgomery

The First Glimmer of Color

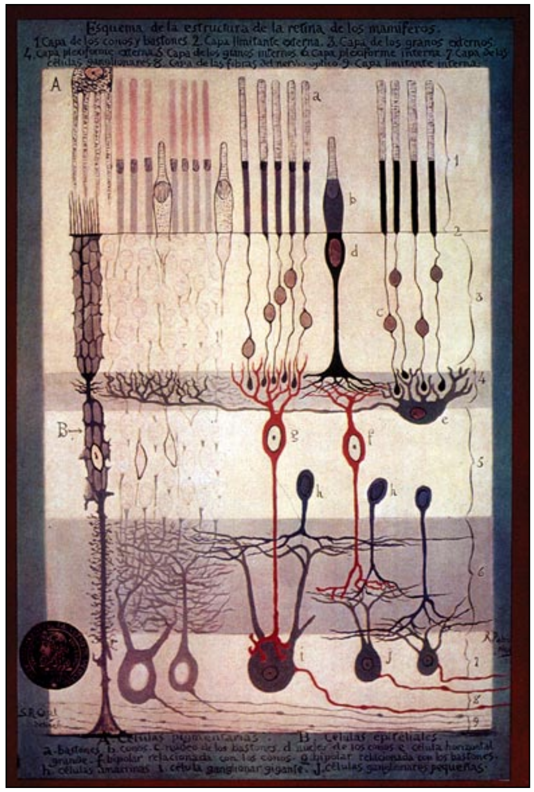
Nathans became interested in how we see in color the day he heard of new discoveries about how we see in black and white. It was 1980, and he was a student at Stanford Medical School, he recalls, when Lubert Stryer and Denis Baylor, both of Stanford, described their remarkable findings about the workings of rod cells. These cells—one of two kinds of photoreceptor cells in the retina—enable us to see in dim light, even by the muted starlight of a hazy night.

"Baylor showed that rod cells achieve the ultimate in light sensitivity—that they can respond to a single photon, or particle of light," says Nathans. "It was a beautiful experiment." (Baylor's work was done in collaboration with Trevor Lamb and King-Wai Yau.)

Then Stryer explained how rhodopsin, the light-sensitive receptor protein in the disk membranes of rod cells, announces the arrival of this tiny pulse of light to the signaling machinery inside the cell. Stryer had found that rhodopsin could do this only with the help of an intermediary, called a G protein, which belonged to a family of proteins that was already known to biochemists from their study of how cells respond to hormones and growth factors.

Nathans immediately realized this meant that the structure of rhodopsin itself might be similar to that of receptors for hormones. His mind began racing with possibilities. "And I ran—literally ran—to the library and started reading about vision," he says.

Until then, Nathans had been studying the genetics of fruit flies. But as he read a paper by Harvard University biologist George Wald—a transcript of Wald's 1967 Nobel prize lecture on "The Molecular Basis of Visual Excitation"—Nathans set off on a different course. He determined to do what Wald himself had wished to do 40 years earlier: find the receptor proteins in the retina that respond to color.



The intricate layers and connections of nerve cells in the retina were drawn by the famed Spanish anatomist Santiago Ramón y Cajal around 1900. Rod and cone cells are at the top. Optic nerve fibers leading to the brain may be seen at bottom right.

Rod cells function only in dim light and are blind to color. "Get up on a dark moonlit night and look around," suggests David Hubel of Harvard Medical School, a winner of the Nobel prize for his research on vision. "Although you can see shapes fairly well, colors are completely absent. It is remarkable how few people realize that they do without color vision in dim light."

But the human retina also contains another kind of photoreceptor cell: the cones, which operate in bright light and are responsible for high-acuity vision, as well as color.

Rods and cones form an uneven mosaic within the retina, with rods generally outnumbering cones more than 10 to 1—except in the retina's center, or fovea. The cones are highly concentrated in the fovea, an area that Nathans calls "the most valuable square millimeter of tissue in the body."

Even though the fovea is essential for fine vision, it is less sensitive to light than the surrounding retina. Thus, if we wish to detect a faint star at night, we must gaze slightly to the side of the star in order to project its image onto the more sensitive rods, as the star casts insufficient light to trigger a cone into action.

In bright light, then, when the cones are active, how do we perceive colors? This question has attracted some of the finest minds in science. As early as 1672, by experimenting with prisms, Isaac Newton made the fundamental discovery that ordinary "white" light is really a mixture of lights of many different wavelengths, as seen in a rainbow. Objects appear to be a particular color because they reflect some wavelengths more than others. A red apple is red because it reflects rays from the red end of the visible spectrum and absorbs rays from the blue end. A blueberry, on the

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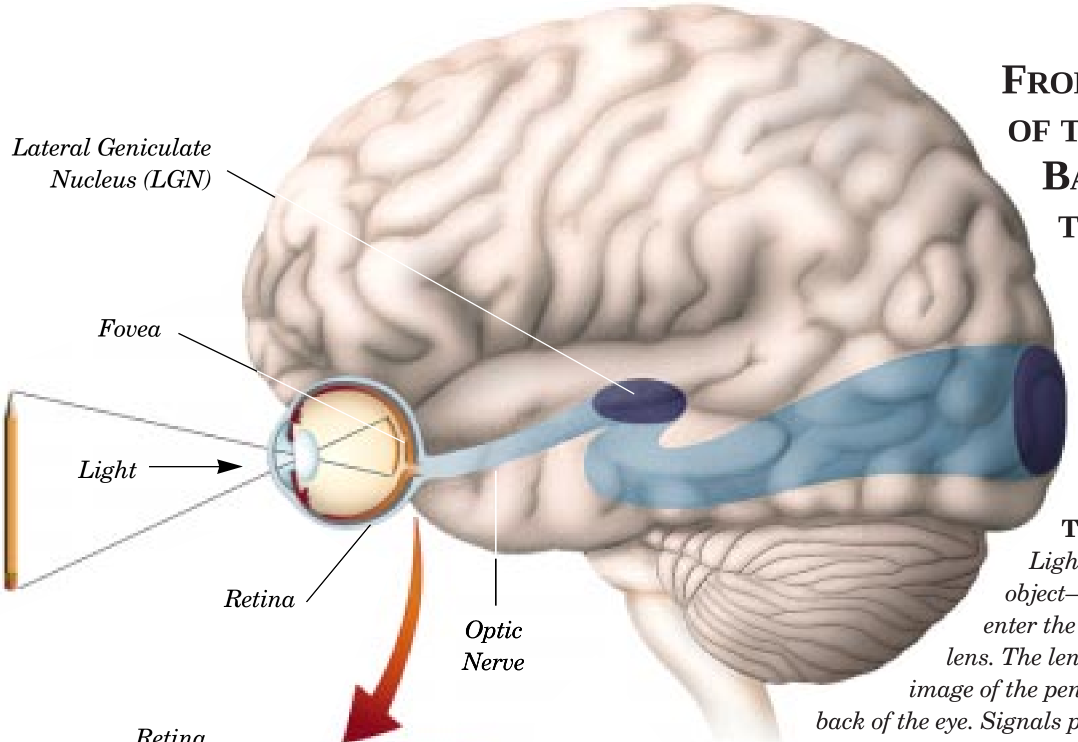
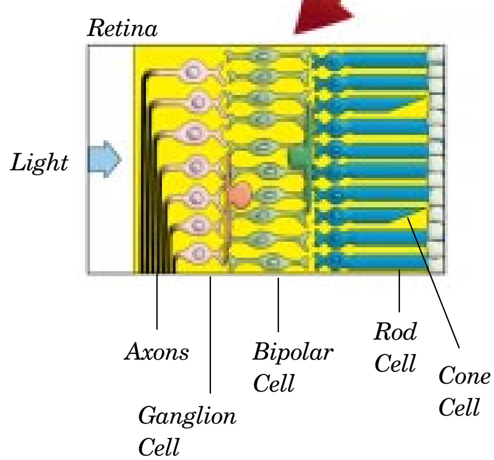
Geoffrey Montgomery, a New York-based science writer, is working on a book about vision and the brain.

FROM FRONT OF THE EYE TO BACK OF THE BRAIN





The visual pathway: *Light rays reflected by an object—for example, a pencil—enter the eye and pass through its lens. The lens projects an inverted image of the pencil onto the retina at the back of the eye. Signals produced by rod and cone cells in the retina then start on their way into the brain through the optic nerve and reach a major relay station, the LGN (lateral geniculate nucleus).*

Signals about particular elements of the pencil then travel to selected areas of the primary visual cortex, or V1, which curves around a deep fissure at the back of the brain. From there, signals fan out to "higher" areas of cortex that process more global aspects of the pencil such as its shape, color, or motion.

Surprisingly, light rays must penetrate two transparent layers of neurons in the retina before reaching the precious rods and cones at the back: a middle layer of bipolar cells, and a front layer of ganglion cells whose long axons (fibers that transmit electrical impulses to other neurons) form the optic nerve leading into the brain.

other hand, reflects the blue end of the spectrum and absorbs the red.

Thinking about Newton's discovery in 1802, the physician Thomas Young, who later helped decipher the hieroglyphics of the Rosetta Stone, concluded that the retina could not possibly have a different receptor for each of these wavelengths, which span the entire continuum of colors from violet to red. Instead, he proposed that colors were perceived by a three-color code. As artists knew well, any color of the spectrum (except white) could be matched by judicious mixing of just three colors of paint. Young suggested that this was not an intrinsic property of light, but arose from the combined activity of three different "particles" in the retina, each sensitive to different wavelengths.

We now know that color vision actually depends on the interaction of three types of cones—one especially sensitive to red light, another to green light, and a third to blue light. In 1964, George Wald and Paul Brown at Harvard and Edward MacNichol and William Marks at Johns Hopkins showed that each human cone cell absorbs light in only one of these three sectors of the spectrum.

Wald went on to propose that the recep-


Laboratory of Richard Masland, Massachusetts General Hospital

The many rods (small circles) and fewer cones (dark outlines) in most of the retina form a mottled pattern, as shown in this photomicrograph. The central retina, or fovea, has only cones.

tor proteins in all these cones were built on the same plan as rhodopsin. Each protein uses retinal, a derivative of vitamin A, to absorb light; and each tunes the retinal to absorb a different range of wavelengths. Wald believed that the three receptor proteins in cones probably evolved from the same primordial gene—and so did rhodopsin. They were all "variations on a central theme," Wald wrote in his Nobel lecture.

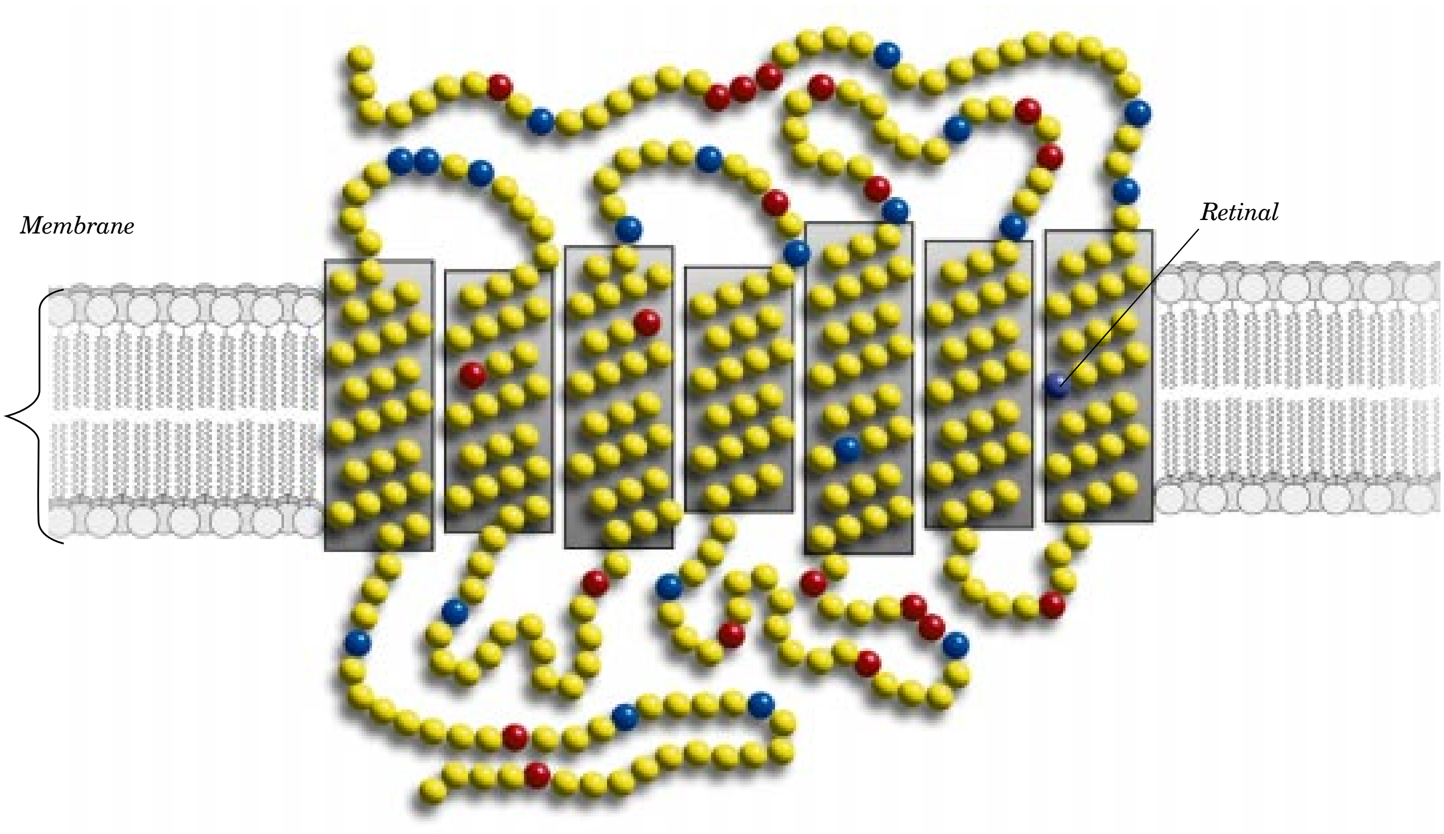
This evolutionary message was music to Nathans' ears. It meant that if the gene encoding only one receptor protein could be located, the genes encoding the other receptor proteins could be found by the similarity of the sequence of bases in their DNA.

"I realized while reading Wald's lecture," says Nathans, "that Wald had laid out the whole problem of the genetic basis of color vision, and that this problem was now solvable, completely solvable, by molecular genetic methods." Wald had taken the problem as far as he could, Nathans pointed out. "But lacking these molecular methods, he couldn't go any further." Nathans' ambitious plan to isolate the genes that coded for the three color receptor proteins depended on Wald's view that the genes all evolved from the same primordial ancestor. The only visual receptor protein that had been studied with any intensity at that time was bovine rhodopsin—from the rod cells of cows' eyes. Scientists had purified bovine rhodopsin and deduced the sequence of a fragment of the DNA that coded for it. Nathans used this information to construct a lure—a single strand of DNA—with which he fished out the complete gene for bovine rhodopsin from a sea of bovine DNA.

Next he used part of this bovine gene as a lure to catch the gene for human rhodopsin from the jumble of DNA in a human cell. This took less than a year "because the genes for human and bovine rhodopsin are virtually identical, despite an evolutionary distance of 200 million years between cattle and humans," Nathans says.

Finding the human genes for the color receptors proved more challenging, however, since these genes are less closely related to the gene for rhodopsin. Nathans began to sift through DNA from his own cells. "I figured I'd be an unlimited source of DNA as long as I kept eating," he says. Eventually he fished out some pieces of DNA that belonged to three different genes, each of them clearly related to the rhodopsin gene. "This coincidence—three genes, three types of cones—didn't escape our notice," he said. Furthermore, two of these genes were on the X chromosome—"exactly what one would expect," says Nathans, "since it has long been known that defects in red and green color vision are X-linked."

Some 10 million American men—fully 7 percent of the male population—either cannot distinguish red from green, or see red and green differently from most people. This is the commonest form of color blindness, but it affects only 0.4 percent of women. The fact that color blindness is so much more prevalent among men implies that, like hemophilia, it is carried on the X chromosome, of which men have only one copy. (As in hemophilia, women are protected because they have two X chromosomes; a



normal gene on one chromosome can often make up for a defective gene on the other.)

Wald and others had found that in color-blind men, the green or red cones worked improperly or not at all. Wald suggested that the genes for the red and green receptors were altered in these men. He also thought that these genes must lie near each other on the X chromosome. This tandem arrangement—which Nathans confirmed—probably results from the duplication of a DNA fragment in primates that occurred some 40 million years ago. The New-World monkeys of South America, which broke from the continent of Africa at about that time, possess only a single functional copy of a red or green gene, much like color-blind men. But in Old World primates—the monkeys and apes of Africa and the ancestors of humans—a primordial red-green gene must have duplicated and then diverged slightly in sequence, leading to separate receptors of the red and green type. In keeping with this picture,

Rhodopsin, the receptor protein in rod cells, crosses the disk membrane seven times; its odd shape is shared by the three receptor proteins in cone cells. Retinal (which absorbs light) is shown in purple. The other colored balls represent amino acids that make up the rhodopsin structure.

Nathans found that the DNA sequences of the genes for red and green receptors differ by only 2 percent—evidence of their common origin and recent divergence.

Nathans himself is not color-blind. Before using his own DNA, he thoroughly tested his color vision to ensure that it was normal. Nevertheless, one of his initial findings presented a puzzle: Lying head to tail along his X chromosome were not just the two genes for the red and green receptors, but also an extra copy of the green receptor gene.

Here was the explanation for the prevalence of color blindness, he realized. Because the DNA sequences of the red and green receptor genes are so similar, and because they lie head to tail, it is easy for mistakes to occur during the development of egg and sperm, as genetic material is replicated and exchanged between chromosomes. One X chromosome—like Nathans'—may receive an extra green receptor gene, for instance, or maybe even two. This does no

A Narrow Tunnel of Light

Inability to see well in the dark may be an ominous sign in a child. Though it could just signal a need to eat more carrots or take vitamin A supplements, for more than a million people around the world (one out of every 4,000), it is the first symptom of retinitis pigmentosa (RP), a genetic disorder that may leave them totally blind by the age of 40.

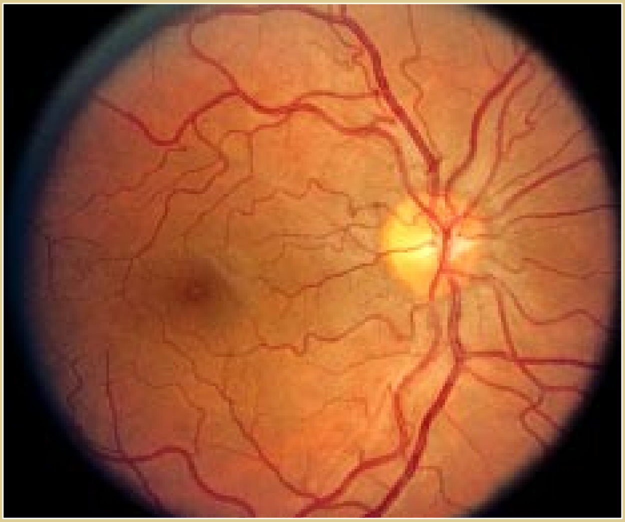
"Sometime between their teens and their thirties, depending on the family, their retinas begin to degenerate," says Jeremy Nathans, who has been studying the genetic errors that cause the disease. First, the rod cells die at the retina's periphery. Then these zones of cell death slowly expand, leaving only a small patch of functioning retinal cells near the center of vision. The patients' visible world contracts to a narrow tunnel of light. Finally, the dying tissue may take everything with it, including the precious cones in the central retina, which are responsible for high-acuity vision.

"The retina doesn't regenerate," explains Nathans. "If any part of it goes, you won't get it back. And so far, there is no effective therapy for RP."

Until five years ago, no one even knew the cause of RP. Thinking the disease might be related to a defect in rhodopsin, the receptor protein of rod cells (which are responsible for night vision), Nathans began to collect blood samples from patients so he could study their DNA. In 1989, Peter Humphries at Trinity College in Dublin, Ireland, found the location of a gene defect in a very large Irish family that had a dominant form of the disease (in which the inheritance of a mutated gene from just one parent causes the disease). Remarkably, Humphries mapped the defect to the same region of chromosome 3 in which Nathans had located the gene for rhodopsin.

Since then, two teams of scientists—Thaddeus Dryja at the Massachusetts Eye and Ear Infirmary, and Nathans and HHMI associate Ching-Hwa Sung at Johns Hopkins—have shown that about one-fourth of patients with the dominant form of the disease have mutations in their gene for rhodopsin. Other forms of RP result from mutations in different genes. Most of the errors in the rhodopsin gene cause the protein to be unstable,

A healthy retina (below), as seen through an ophthalmoscope, has a firm, regular structure. In the retina of a person with retinitis pigmentosa (right), cells die, starting at the periphery. Cells laden with the black pigment melatonin invade the dead retinal tissue, producing black deposits that are characteristic of the disease.

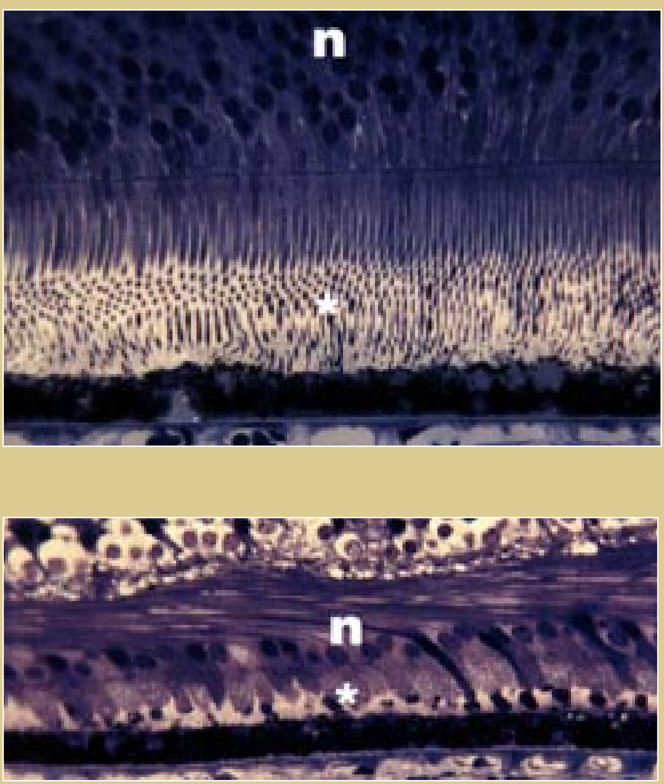


Laboratory of Samuel Jacobson, University of Miami (2)

Nathans says. "Either it doesn't fold correctly to start with, or once it folds, it falls apart." There seems to be a correlation between the kind of mutation and the severity of the disease.

To find out how these mutations damage the retina and what drugs might be designed to prevent this process, several groups of researchers recently inserted defective genes for rhodopsin into mice, where these mutant genes cause an RP-like disease. They hope to use this mouse model to develop new treatments.

Nathans points out that the retina normally consumes more energy per gram than any other tissue in the body. A rod cell that needs to dispose of mutant rhodopsin must expend further energy still, which may "push the cell over the edge, so that it runs out of energy and dies," taking along the adjoining cones. Nathans speculates that perhaps a drug that reduced energy consumption in the rod cells might minimize or delay the retina's degeneration—and thereby save the patient's cones. "RP is a slow disease," says Nathans. "It may take 30 years to develop, so if we can delay its progression by another 30 years, that's virtually a cure."



Laboratory of Ann Milam, University of Washington (2)

*Cones are tightly packed in the fovea, which is specialized for high-acuity vision. In a healthy retina (top), cones appear tall and straight. In the retinas of people with advanced retinitis pigmentosa, cones lose their light-sensitive outer segment (shown with an *) and then die.*

So many cones have died in the retina seen in the bottom picture that only one layer of cones remains (n indicates the cones' nuclei) and the whole area has shrunk. The two pictures were taken under a microscope at the same magnification.

harm. But then the other chromosome with which it is exchanging bits of genetic information is left with only a red receptor gene. The man who inherits this slightly truncated chromosome will be color-blind, bereft of the genetic information needed to make a green receptor.

More than 95 percent of all variations in human color vision involve the red and green receptors in men's eyes. It is very rare for anyone—male or female—to be "blind" to the blue end of the spectrum. Nathans provided a genetic explanation for this phenomenon. He showed that the gene coding for the blue receptor lies on chromosome 7, which is shared equally by men and women, and that this gene does not have any neighbor whose DNA sequence is similar. Blue color blindness is caused by a simple mutation in this gene.

But the eye is not a camera.

What Color Is It?

Seeing a color involves making comparisons. "All that a single cone can do is capture light and tell you something about its intensity," Nathans points out; "it tells you nothing about color." To see any color, the brain must compare the input from different kinds of cone cells—and then make many other comparisons as well.

The lightning-fast work of judging a color begins in the retina, which has three layers of cells. Signals from the red and green cones in the first layer, for instance, are compared by specialized red-green "opponent" cells in the second layer. These opponent cells compute the balance between red and green light coming from a particular part of the visual field. Other opponent cells then compare signals from blue cones with the combined signals from red and green cones.

On a broader scale, comparisons of neighboring portions of an image lead to our amazing ability to see colors as constants in an ever-changing world. Nathans vividly remembers demonstrations of this "color constancy" by the late Edwin Land, the inventor of instant photography and founder of the Polaroid Corporation. Land and his colleagues had made a large collage of multi-colored geometric shapes, called a "Mondrian" after its resemblance to the works of the Dutch painter Piet Mondrian. They used three projectors that beamed light matching the wavelength-sensitivity of the three human cone types. With these projectors, the wavelength composition reflected from any given patch on the Mondrian could be exactly controlled.

"Land pointed out a patch on the Mondrian that looked orange in the context of the surrounding colors," Nathans recalls. "Then he gave me a tube, like the tube inside a paper towel roll, and had me look at this patch in isolation. And it wasn't orange anymore. It was a perfect red."

The patch was in fact painted orange, but Land had beamed a high-intensity long-wave light from the red end of the spectrum on it so that it reflected a high proportion of red light. Under normal viewing conditions, however—when the patch was surrounded by other Mondrian colors—Nathans still saw the orange figure by its true color. Somehow, by comparing a patch of color with the surrounding colored region, the brain is able to discount the wavelength of the illuminating light and reconstruct the patch's color as it would be seen in daylight.

"Color constancy is the most important property of the color system," declares neurobiologist Semir Zeki of University College, London. Color would be a poor way of labeling objects if the perceived colors kept shifting under different conditions, he points out. But the eye is not a camera. Instead, the eye-brain pathway constitutes a kind of computer—vastly more complex and powerful than any that human engineers have built—designed to construct a stable visual representation of reality.

The key to color constancy is that we do not determine the color of an object in isolation; rather, the object's color derives from a comparison of the wavelengths reflected from the object and its surroundings. In the rosy light of dawn, for instance, a yellow lemon will reflect more long-wave light and therefore may appear orange; but its surrounding leaves also reflect more long-wave light. The brain compares the two and cancels out the increases.

Land's "Retinex" theory of color vision—a mathematical model of this comparison process—left open the question of where in the pathway between retina and cortex color constancy was achieved. This issue could only be addressed by studying the brain itself.

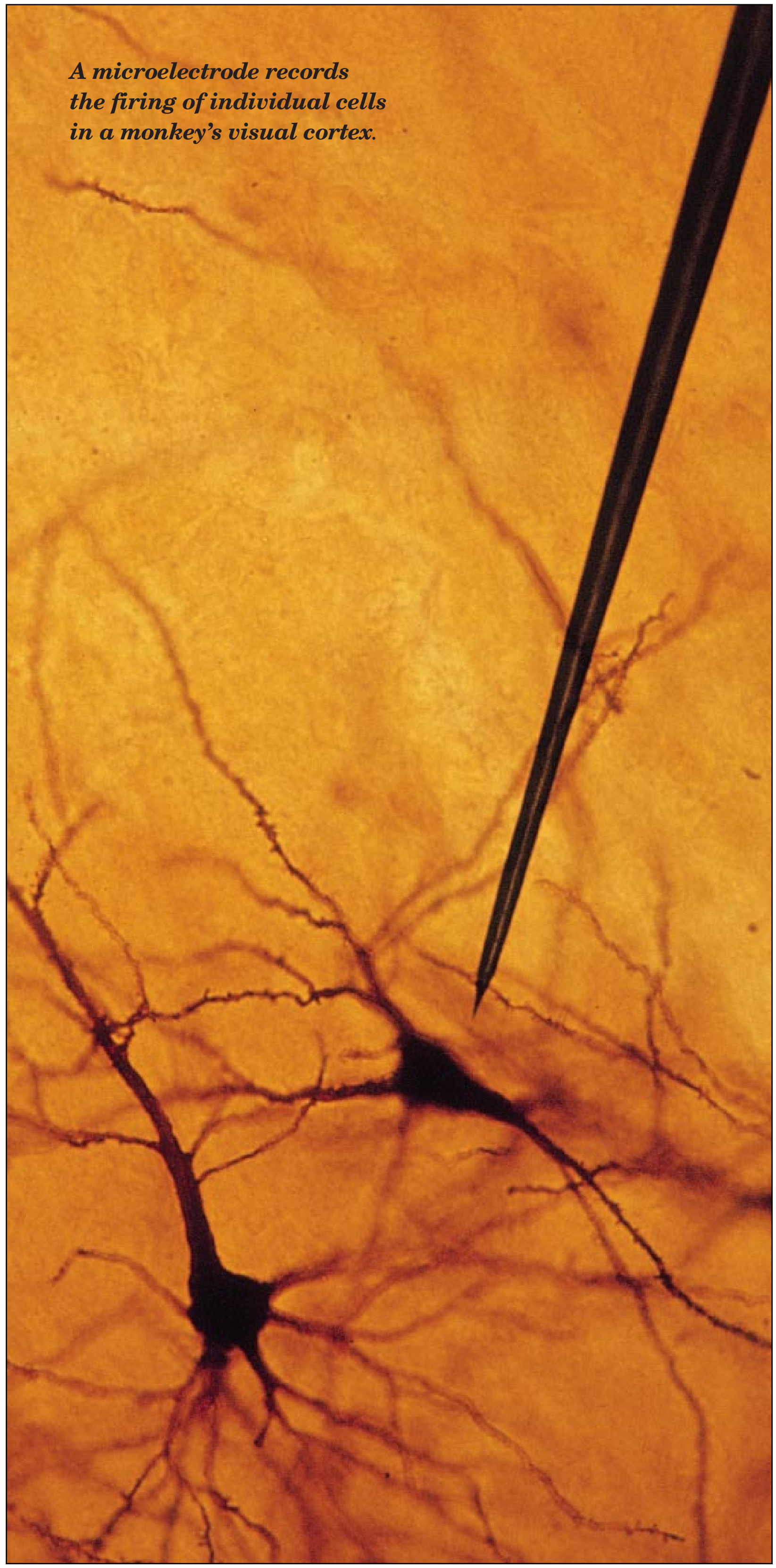
Working with anesthetized monkeys in the 1960s, David Hubel and Torsten Wiesel of Harvard Medical School had shown that the primary visual cortex or area V1, a credit-card-sized region at the back of the brain, possesses a highly organized system of neurons for analyzing the orientation of an object's outlines. But in their early studies they found relatively few color-sensitive cells. Then in 1973, Semir Zeki identified a separate area that he called V4, which was full of cells that crackled with activity when the monkeys' visual field was exposed to different colors.

A few years later, Edwin Land paid Zeki a visit in London. "He showed me his demonstration, and I was much taken by that," Zeki says. "I was converted, in fact. So I used his Mondrian display to study the single cells in area V4."

In this way, Zeki discovered that some of the cells in area V4 consistently respond to the actual surface color of a Mondrian patch, regardless of the lighting conditions. He believes these are the cells that perform color constancy. More recently, with the aid of PET scans, he found an area similar in location to the monkeys' V4 that is specifically activated in humans when they look at Mondrian color displays. The color displays also stimulate the primary visual area and an area that is adjacent to it, V2.

Much controversy exists about all aspects of the color pathway beyond the retina, however. Researchers disagree about the exact role of cells in human V1 and V2, about the importance of V4, about the similarities between monkey and human brains.

To resolve such issues, scientists await the results of further experiments on humans. The new, noninvasive imaging techniques that can show the brain at work (see p. 30) may supply key answers. Within a few years, researchers hope, these techniques will reveal the precise paths of the neural messages that make it possible for us to see the wealth of colors around us.



A microelectrode records the firing of individual cells in a monkey's visual cortex.

HOW WE SEE THINGS THAT MOVE

BY GEOFFREY MONTGOMERY

The patient had great difficulty pouring coffee into a cup. She could clearly see the cup's shape, color, and position on the table, she told her doctor. She was able to pour the coffee from the pot. But the column of fluid flowing from the spout appeared frozen, like a waterfall turned to ice. She could not see its motion. So the coffee would rise in the cup and spill over the sides.

More dangerous problems arose when she went outdoors. She could not cross a street, for instance, because the motion of cars was invisible to her: a car was up the street and then upon her, without ever seeming to occupy the intervening space.

Even people milling through a room made her feel very uneasy, she complained to Josef Zihl, a neuropsychologist who saw her at the Max Planck Institute for Psychiatry in Munich, Germany, in 1980, because "the people were suddenly here or there but I did not see them moving."

The woman's rare motion blindness resulted from a stroke that damaged selected areas of her brain. What she lost—the ability to see objects move through space—is a key aspect of vision. In animals, this ability is crucial to survival: Both predators and their prey depend upon being able to detect motion rapidly.

In fact, frogs and some other simple vertebrates may not even see an object unless it is moving. If a dead fly on a string is dangled motionlessly in front of a starving frog, the frog cannot sense this winged meal. The "bug-detecting" cells in its retina are wired to respond only to movement. The frog might starve to death, tongue firmly folded in its mouth, unaware that salvation lies suspended on a string in front of its eyes.

Unable to see motion, Gisela Leibold feels anxious as she rides down an escalator in Munich.

While the retina of frogs can detect movement, the retina of humans and other primates cannot. “The dumber the animal, the smarter its retina,” observes Denis Baylor of Stanford Medical School. The large, versatile brain of humans takes over the job, analyzing motion through a highly specialized pathway of neural connections.

This is the pathway that was damaged in the motion-blind patient from Munich. Compared with the complex ensemble of regions in the visual cortex that are devoted to perceiving color and form, this motion-perception pathway seems relatively streamlined and simple. More than any other part of the cortex, it has yielded to efforts to unveil “the precise relationship between perception and the activity of a sensory neuron somewhere in the brain,” says Anthony Movshon, an HHMI investigator at New York University. By studying the reactions of humans and monkeys to different moving stimuli and probing the parts of the visual cortex that are aroused at such times, researchers have begun to build a bridge between the objective world of electrically signaling neurons that can be observed in a laboratory and the subjective world of perception accessible only to an individual’s own consciousness.

One way to visualize the key challenges for the motion-perception system, suggests Thomas Albright of the Salk Institute, is to consider what happens when we watch a movie. Each of the 24 frames projected per second on the theater screen is a still photograph; nothing in a movie truly moves except the film. The illusion of movement is created by the motion-processing system in our brains, which automatically fuses, for instance, the images of legs that shift position slightly from frame to frame into the appearance of a walking actor. The Munich patient is unable to perform this fusion. In life or in the movie theater, she sees the world as a series of stills.

“The motion system must match up image elements from frame to frame, over space and time,” says Albright. “It has to detect which direction a hand is moving in, for instance, and not confuse that hand with a head when it waves in front of someone’s face.”

Researchers have now traced the path of neural connections that make up the motion pathway and tested the responses of cells at different steps along this path. This has revealed the basic stages by which the motion system senses which way a hand is waving.

Starting in the retina, large ganglion cells called magnocellular neurons, or M cells, are triggered into action when part of the image of a moving hand sweeps across their receptive field—the small area of the visual field to which each cell is sensitive. The M cells’ impulses travel along the optic nerve to a relay station in the thalamus, near the middle of the brain, called the lateral geniculate nucleus. Then they flash to the middle layer of neurons in the primary visual cortex. There, by pooling together the inputs from many M cells, certain neurons gain a new property: they become sensitive to the direction in which the hand is moving across their window of vision.

Such direction-sensitive cells were first discovered in the mammalian visual cortex by David Hubel and Torsten Wiesel, who projected moving bars of light across the receptive fields of cells in the primary visual cortex of anesthetized cats and monkeys. Electrodes very close to these cells picked up their response to different moving lines, and the pattern of activity could be heard as a crackling “pop-pop-pop” when the signals were amplified and fed into a loudspeaker.

“Listening to a strongly direction-selective cell responding,” Hubel has written, “the feeling you get is that the line moving in one direction grabs the cell and pulls it along and that the line moving in the other direction fails utterly to engage it, something like the feeling you get with a ratchet, in winding a watch.”

The keystone of the motion pathway is an area of the cortex that lies just beyond the primary and secondary visual areas (V1 and V2)—a largely unexplored wilderness that used to be known as the “sensory association cortex.” “It was thought that somewhere in this mishmash of association cortex visual forms were recognized and associated with information from other senses,” says John Allman of Caltech. But studies in the owl monkey by Allman and Jon Kaas

Nothing in a movie truly moves...

(who is now at Vanderbilt) and in the rhesus monkey by Semir Zeki revealed that the area was not a mishmash at all. Instead, much of it was made up of separate visual maps, each containing a distinct representation of the visual field. In 1971, Zeki showed that one of these visual maps was remarkably specialized. Though its cells did not respond to color or form, over 90 percent of them responded to movement in a particular direction. American scientists usually call this map MT (middle temporal area), but Zeki called it V5. He also nicknamed it "the motion area."

"This very striking finding of this little hot spot, this little pocket, in which almost all the cells are sensitive for the direction of movement," says Anthony Movshon, was the impetus for many vision researchers to turn their attention to motion. Nowhere else in the visual cortex was there an area that seemed so functionally specialized.

The cells of this motion area, MT, are directly connected to the layer of direction-sensitive cells in the primary visual area, V1. And the two areas have a remarkably similar architecture. Hubel and Wiesel had discovered that V1 is organized into a series of columns. The cells in one column may fire only when shown lines oriented like an hour hand pointing to one o'clock, for instance, while the cells in the next column fire most readily to lines oriented at two o'clock, and so on around the dial. Amazingly, MT has the same kind of orientation system as V1, but in addition the cells in its columns respond preferentially to the direction of movement.

"When you see that an area, like V1 or MT, has this highly organized columnar structure," says Wiesel, "you get a sense of uncovering something fundamental about the way the cells in the visual area work."

In perceiving motion, as in determining color, the brain constructs a view of the world from pieces of information that can themselves be mistaken or ambiguous. Suppose you paint an X on a piece of paper and then move that paper up and down in front of someone's eyes. Direction-selective cells in the motion-pathway layer of V1—each of which sees only a small part of the scene—will respond to the diagonal orientation of each of the lines making up the X but will not register the movement of the X as a whole. How, then, is this overall movement sensed?

There must be two stages of motion analysis in the cortex, suggested Movshon and Edward Adelson, who was then a postdoctoral fellow at New York University (he is now a professor at the Massachusetts Institute of Technology). At the second stage, certain cells must integrate the signals regarding the orientation of moving lines and produce an overall signal about the motion of the whole object.

When Movshon presented this idea at an annual meeting of vision researchers in 1981, William Newsome, then a postdoctoral fellow at the National Institutes of Health (he is now a professor of neurobiology at Stanford University School of Medicine), approached him. A lively three-hour dinner ensued and the two men resolved to collaborate. Together with Adelson, they would search for such cells in the motion area.

The researchers soon found that one-third of MT's cells could, in fact, signal the direction in which a hand waves through space. Later on, Albright's research group showed that MT cells can detect "transparent" motion, such as a shadow sweeping across the ground.

Then Allman and his colleagues discovered that many MT cells are able to integrate motion information from a large swath of the scene. "Even though an MT cell may respond directly to just one spot in the visual field," says Allman, "the cells have knowledge of what's going on in the region surrounding them." Using a computer display with a background texture that looks vaguely like a leafy forest, Allman showed that some MT cells will fire particularly furiously if the leafy background moves in a direction opposite to a moving object—the sort of visual pattern a cheetah would see when chasing an antelope along a stand of trees. If, however, the background moved in the same direction as the moving object, the cell's firing was suppressed. The cell acted as a large-scale

The Urgent Need To Use Both Eyes

When you look at yourself in the mirror, "you are looking into a predator's eyes," writes Diana Ackerman in *A Natural History of the Senses*. Predators generally have eyes set right on the front of their heads so they can use precise, binocular vision to track their prey, she explains, whereas prey have eyes at the sides of their heads so they can be aware of predators sneaking up on them.

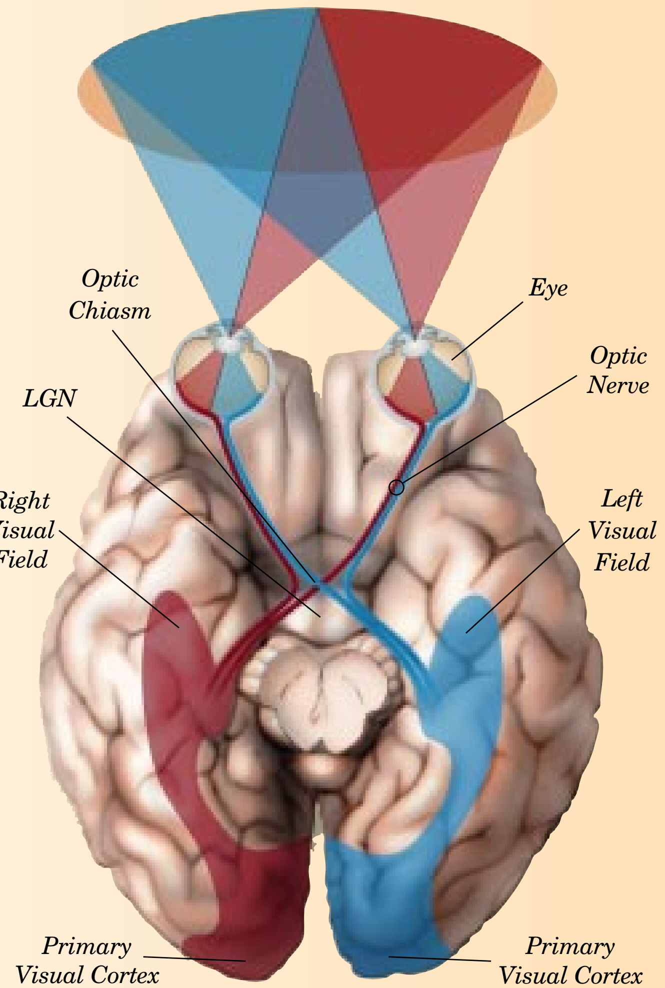
Binocular vision lets us see much more sharply—but only if our two eyes work smoothly together, starting early in life. Most of the time there is no problem. Each year, however, at least 30,000 babies in the United States develop strabismus, which means that their left and right eyes fail to align properly in the first few months after birth.

Until the 1970s, doctors did not realize the urgency of doing something about this condition. Treatment was generally delayed until the children were 4 or older—too late to do much good.

The need for earlier intervention became clear as a result of David Hubel and Torsten Wiesel's experiments with kittens. They showed that there is a critical period, shortly after birth, during which the visual cortex requires normal signals from both eyes in order to develop properly. In kittens, the critical period lasts for about a month or six weeks. In humans, it continues until the age of 5 or 6.

A curious feature of cells in the visual cortex is that those responding to information from the left and right eyes form separate "ocular dominance columns," one for each eye. Normally these columns are arranged in a series of alternating bands that can be labelled by injecting a marker into one eye. This produces a pattern that resembles the black-and-white stripes of a zebra. But the columns are not fully wired at birth; they take shape during the first months of life, in response to visual experience.

If vision through one eye is blocked during the critical period, the ocular dominance columns responding to the open eye expand in the cortex, while the columns that would normally respond to the blocked eye progressively shrink. An adult who loses his vision because of a cataract (a clouding of the lens of the eye) will generally see normally again if the opaque lens is removed and replaced with a clear artificial lens. But a child whose cataract is not removed until the age of 7 will be blind in the eye that was blocked by the cataract, even though the cataract is gone and the retina of



The two eyes provide slightly different views of the same scene. Information from the left visual field goes to the right side of the retina in both eyes. At the optic chiasm, half the nerve fibers from the left eye cross over to the right hemisphere and the rest stay uncrossed, so that all the information from the left visual field ends up in the right hemisphere. In this way, a given hemisphere gets information from the opposite half of the visual world—but each hemisphere gets input from both eyes.

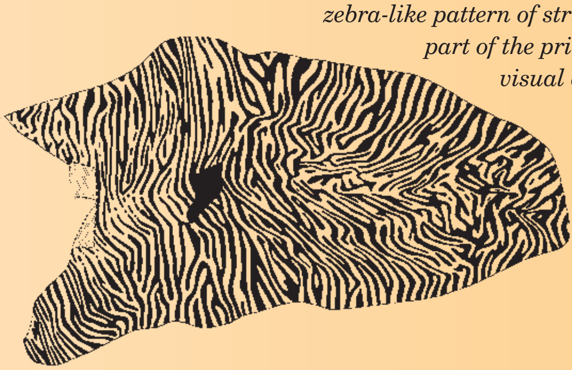
the eye is able to function normally.

The same kind of amblyopia—a loss of vision without any apparent defect of the eye—occurs in children whose eyes are misaligned, as well as in those whose eyes focus at different distances. To avoid double vision, such children generally favor one eye and stop using the other. The brain then suppresses the signals coming from the unfavored or "lazy" eye. The neurons in the ocular dominance columns that should receive signals from this eye become wired incorrectly, and the child loses his ability to see with the neglected eye. After a few years, neither surgery nor exercises nor a patch over the favored eye can restore the lost vision.

Armed with this information, ophthalmologists now treat infants who have visual defects as early as possible, with either spectacles or surgery, since normal vision can be restored if treatment begins before the age of 3 or 4.

Anthony Movshon and other researchers have studied monkeys with artificially produced amblyopia. They found that the cells in these monkeys' retinas and lateral geniculate nuclei (LGN), the visual system's relay stations in the center of the brain, are all normal. But in the case of the neglected eye, "the signals that go from the LGN to the primary visual cortex don't make it," Movshon says. The loss occurs because the connections between cells in the LGN and cells in the corresponding eye dominance columns fail to develop or be maintained.

Ocular dominance columns form a zebra-like pattern of stripes in part of the primary visual cortex.



detector of motion contrast, performing exactly the sort of operation an animal would need to sense a figure moving through the camouflage of the forest.

While MT cells do not respond to static forms and colors, Albright has found that they will detect a moving object much more easily if its form or color strongly contrasts with its background.

"Imagine you're looking down the concourse in Grand Central Station and you're supposed to find the woman in the red dress," says Albright. "There are hundreds of surrounding people moving in different directions. Yet there's no problem at all in detecting the woman in the red dress walking along. Your visual system uses the dress's color to filter out all the irrelevant noise around it and homes in on the moving object of interest."

Suppose scientists could record from the MT cells in a laboratory monkey that looked at the woman in the red dress crossing Grand Central Station. They could determine that a particular cell fired when the woman in the red dress passed through its receptive field. But how would they know that the firing of this specific MT cell—and not a network of thousands of other cells in the brain, of which this cell is only one node—actually causes the monkey to perceive the direction of the woman's movement? How could they ever get inside the monkey's mind and determine what it perceives?

Since Hubel and Wiesel's pioneering studies in the visual cortex, most visual scientists have assumed that the perception of form, color, depth, and motion corresponds to the firing of cells specialized to detect these visual qualities. In a spectacular series of experiments conducted since the mid-1980s, Newsome and his colleagues at Stanford have been directly testing this link between perception and the activity of specific neurons.

They use a device that was developed in Movshon's laboratory at NYU: a blizzard of white dots moving on a computer monitor. When all the white dots are moving randomly, the display looks like a TV tuned to a nonbroadcasting channel. However, the experimenters can gradually increase the percentage of dots moving in the same direction. When 10 percent of the dots move coherently together, their motion becomes apparent. By 25 percent, it is unmistakable.

Movshon had found that whenever a human subject could detect the dots' motion at all, he or she could also tell the direction in which the dots were moving. "This means that the part of the visual pathway carrying the information used for motion detection is also carrying a label that says what direction is being detected," says Movshon. This is precisely how one would expect MT, with its columns of direction-selective cells, to encode a moving target.

Next, Newsome began to teach rhesus monkeys to "tell" him what they saw on the computer screen. When they saw dots moving downward, for instance, the monkeys were supposed to move their eyes to a downward point on the screen. Correct responses were rewarded with fruit juice. Soon the monkeys could signal with eye movements that they saw the dots move in any of six directions around the clock. And after much training on low-percentage moving dot displays, the monkeys were able to perform nearly as well as Movshon's human subjects.

Everything was in place. Newsome, Movshon, and their colleagues were ready to study the relationship between the monkeys' perception of motion and the activity of cells in particular columns of MT.

"We found, very much to our surprise," says Newsome, "that the average MT cell was as sensitive to the direction of motion as the monkey was." As more dots moved together and the monkey's ability to recognize their direction increased, so did the firing of the MT neuron surveying the dots.

If the monkeys were actually "listening" to the cells in a single MT column as they made their decision about the direction of movement of the dots on the screen, could the decision be altered by stimulating a different MT column, the researchers wondered. So they electrically stimulated an MT "up" column while the monkeys looked at a downward-moving display. This radically changed the monkeys' reports of what they saw.

"The tenth experiment was an unforgettable experience," remembers Newsome. "We got the first of what became known in the lab as 'whoppers'—when the effects of microstimulation were just massive. Fifty percent of the dots would move down, and yet if we'd stimulate an 'up' column, the monkey would signal 'up' with its eyes. That was really a remarkable day."

The monkeys' perceptual responses no longer seemed to be driven by the direction of dots on the screen. Instead, the animals' perceptual responses were being controlled by an electric stimulus applied to specific cells in the brain by an experimenter. "Intellectually," says Newsome, "it's like putting a novel gene into a bacterium and seeing a novel protein come out. We're putting a signal into this motion circuitry, and we're seeing a predictable behavior come out that corresponds to the signal we put in."

These experiments, says Movshon, "close a loop between what the cells are doing and what the monkey's doing." Allman calls the finding "the most direct link that's yet been established between visual perception and the behavior of neurons in the visual cortex."

It is still possible, however, that when the dots are moving down and the experimenters stimulate an MT "up" column, the stimulation changes what the monkey decides without actually changing what it sees.

"This is a key question," says Newsome. "We now know a lot about the first and last stages of this process. But we are almost totally ignorant about the decision process out there in the middle—the mechanism that links sensory input to the appropriate motor output. How does the decision get made?"

It is a burning question not only for research on the visual system, but for all of cognitive neuroscience, Newsome believes. The answer would provide a bridge from the study of the senses, where so much progress has been made, to the much more difficult study of human thought. At long last, Newsome says, "we're now poised to approach this question."

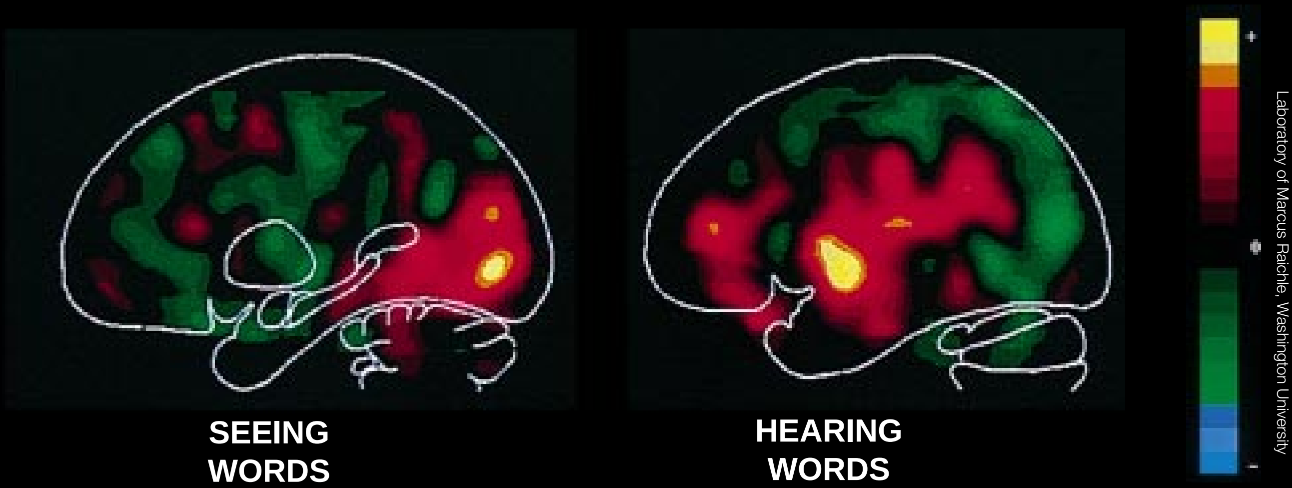
BRAIN SCANS THAT SPY ON THE SENSES

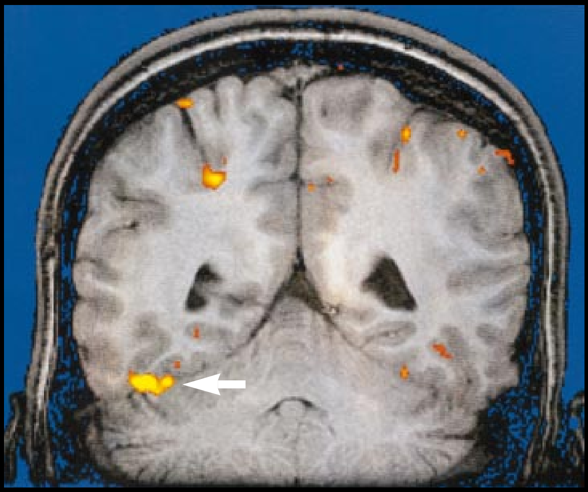
For centuries, scientists dreamed of being able to peer into a human brain as it performs various activities—for example, while a person is seeing, hearing, smelling, tasting, or touching something. Now several imaging techniques such as PET (positron emission tomography) and the newer fMRI (functional magnetic resonance imaging) make it possible to observe human brains at work.

The PET scan on the left shows two areas of the brain (red and yellow) that become particularly active when volunteers read words on a video screen: the primary visual cortex and an additional part of the visual system, both in the left hemisphere.

Other brain regions become especially active when subjects hear words through earphones, as seen in the PET scan on the right.

To create these images, researchers gave volunteers injections of radioactive water and then placed them, head first, into a doughnut-shaped PET scanner. Since brain activity involves an increase in blood flow, more blood—and radioactive water—streamed into the areas of the volunteers' brains that were most active while they saw or heard words. The radiation counts on the PET scanner went up accordingly. This enabled the scientists to build electronic images of brain activity along any desired "slice" of the subjects' brains. The images below were produced by averaging the results of tests on nine different volunteers.





Section on Functional Brain Imaging, NIMH

The volunteer's brain is particularly active in an area of her right hemisphere called the fusiform gyrus (arrow) as she matches one of the two faces at the bottom of the display with the face at the top. This "slice" of her brain is seen as though looking through her face.

A GIANT MAGNET REVEALS THE BRAIN'S ACTIVITY

Much excitement surrounds a newer technique, fMRI, that needs no radioactive materials and produces images at a higher resolution than PET. In this system, a giant magnet surrounds the subject's head. Changes in the direction of the magnetic field induce hydrogen atoms in the brain to emit radio signals. These signals increase when the level of blood oxygen goes up, indicating which parts of the brain are most active.

Since the method is non-invasive, researchers can do hundreds of scans on the same person and obtain very detailed information about a particular brain's activity, as well as its structure. They no longer need to average the resuts from tests on different subjects, whose brains are as individual as fingerprints.

Here a normal volunteer prepares for a fMRI study of face recognition. She will have to match one of the two faces at the bottom of the display with the face at the top. James Haxby, chief of the section on functional brain imaging at the National Institute of Mental Health in Bethesda, Maryland, adjusts the mirror that will allow her to see the display from inside the magnet.

David Corey and James Hudspeth, who played major roles in discovering how the ear's hair cells respond to sound, discuss the cells' conversion of vibrations into nerve signals. In the background, a slide shows hair cells in the inner ear.

THE QUIVERING BUNDLES THAT LET US HEAR

BY
JEFF GOLDBERG

An unusual dance recital was videotaped in David Corey's lab at Massachusetts General Hospital recently. The star of the performance, magnified many times under a high-powered microscope, was a sound-receptor cell from the ear of a bullfrog, called a hair cell because of the distinctive tuft of fine bristles sprouting from its top. The music ranged from the opening bars of Beethoven's Fifth Symphony and Richard Strauss's "Thus Spake Zarathustra" to David Byrne and the Beatles.

As the music rose and fell, an electronic amplifier translated it into vibrations of a tiny glass probe that stimulated the hair cell, mimicking its normal stimulation in the ear. The bristly bundle of "stereocilia" at the top of the cell quivered to the high-pitched tones of violins, swayed to the rumblings of kettle drums, and bowed and recoiled, like tiny trees in a hurricane, to the blasts of rock-and-roll.

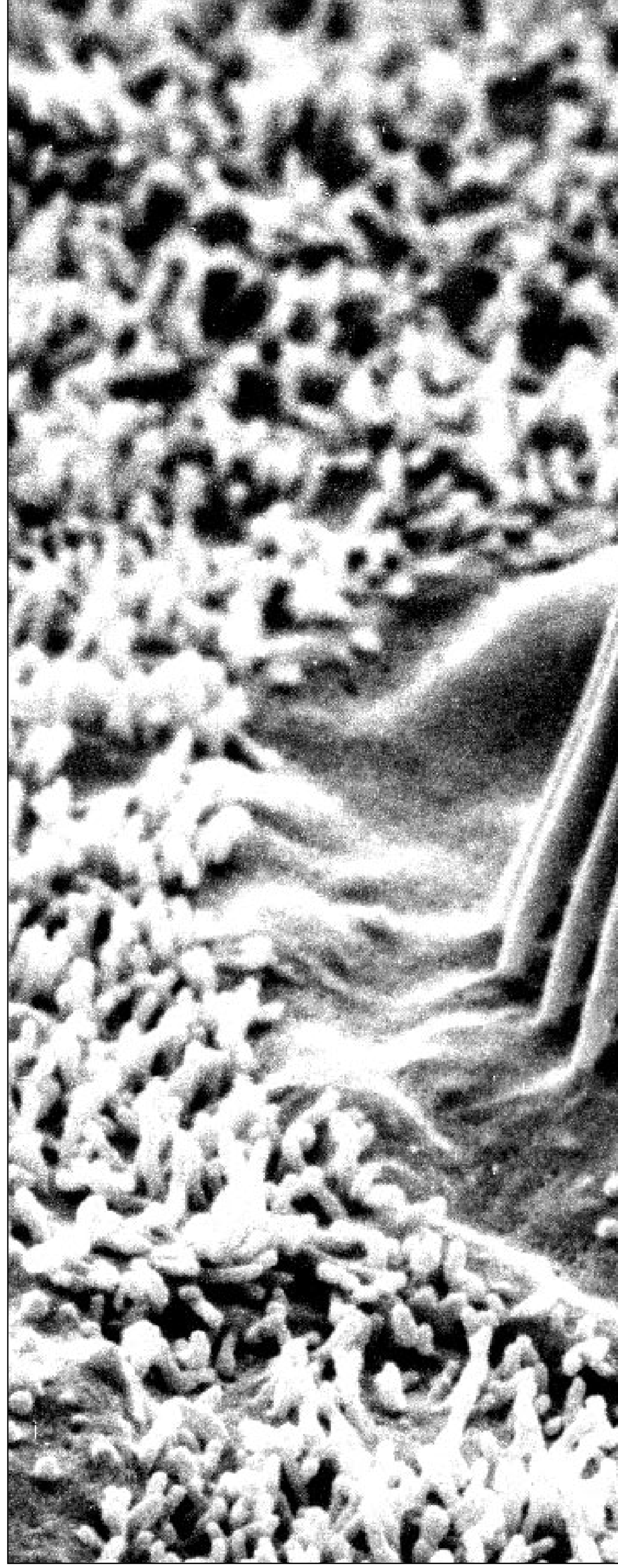
The dance of the hair cell's cilia plays a vital role in hearing, Corey explains. Now an HHMI investigator at MGH and Harvard Medical School, Corey was a graduate student at the California Institute of Technology when he began working with James Hudspeth, a leading authority on hair cells. Together, the two researchers have helped discover how movements of the cilia, which quiver with the mechanical vibrations of sound waves, cause the cell to produce a series of brief electrical signals that are conveyed to the brain as a burst of acoustic information.

In humans and other mammals, hair cell bundles are arranged in four long, parallel columns on a gauzy strip of tissue called the basilar membrane. This membrane, just over an inch long, coils within the cochlea, a bony, snail-shaped structure about the size of a pea that is located deep inside the inner ear.

Sound waves generated by mechanical forces, such as a bow being drawn across a string, water splashing on a hard surface, or air being expelled across the larynx, cause the eardrum—and, in turn, the three tiny bones of the middle ear—to vibrate. The last of these three bones (the stapes, or "stirrup") jiggles a flexible layer of tissue at the base of the cochlea. This pressure sends waves rippling along the basilar membrane, stimulating some of its hair cells. These cells then send out a rapid-fire code of electrical signals about the frequency, intensity, and duration of a sound. The messages travel through auditory nerve fibers that run from the base of the hair cells to the center of the cochlea, and from there to the brain. After several relays within the brain, the messages finally reach the auditory areas of the cerebral cortex, which processes and interprets these signals as a musical phrase, a dripping faucet, a human voice, or any of the myriad sounds in the world around us at any particular moment.

"The mechanics of the hair cell are fascinating—the fact that simply pushing a little bundle of cilia magically allows us to hear.

When this bundle of 50 to 60 cilia at the top of a hair cell vibrates in response to sound, the hair cell (from a bullfrog's inner ear) produces an electrical signal. Tiny tip links can be seen joining the tops of shorter cilia to the sides of taller ones (arrow).

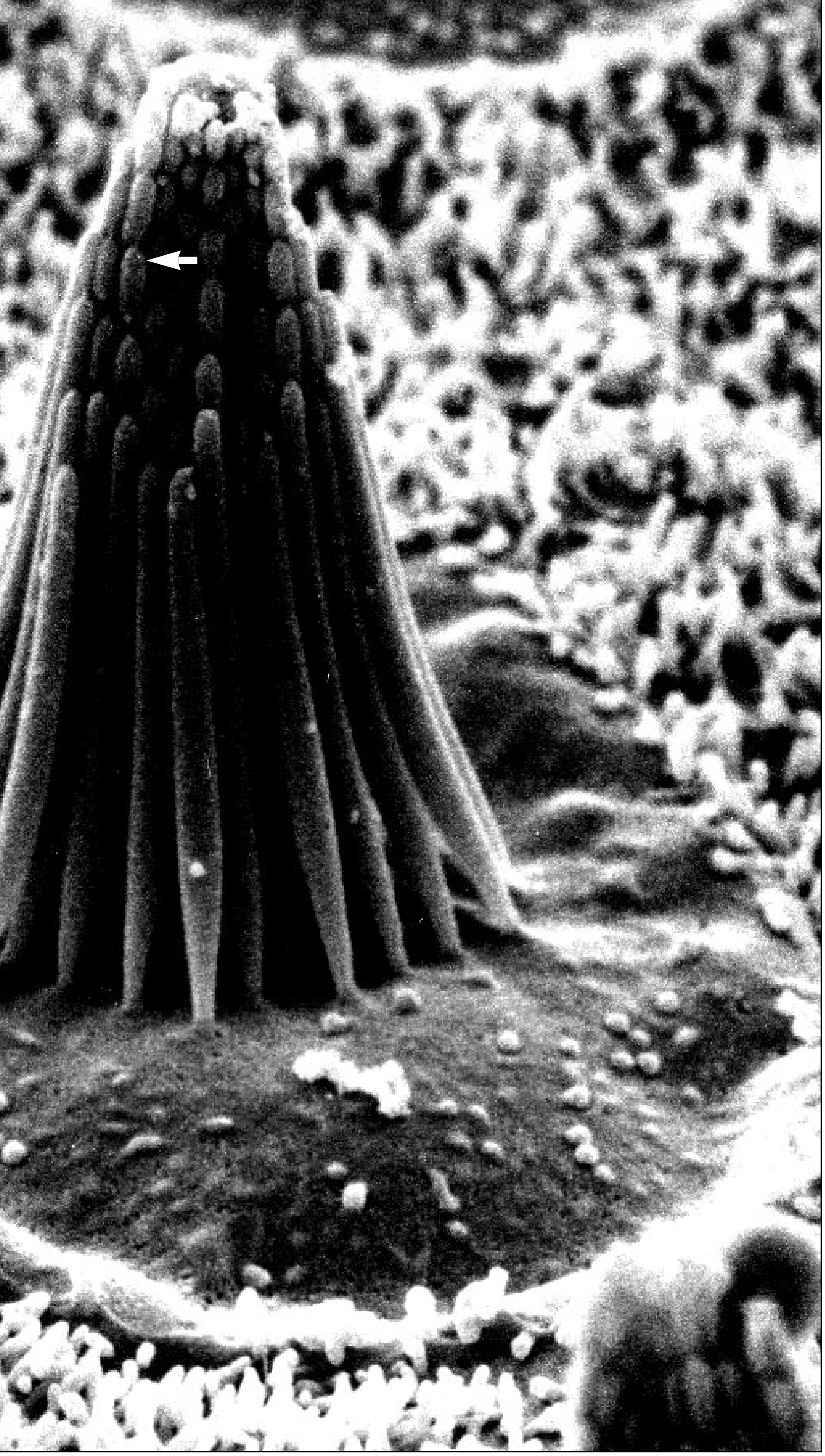


Laboratory of David Corey, Massachusetts General Hospital

And the cells are beautiful. I never get tired of looking at them," says Corey.

Corey and Hudspeth have explored the microscopic inner workings of hair cells in finer and finer detail over the past 20 years, gaining a solid understanding of how the cells work. Some pieces of the puzzle have fallen into place recently with the discovery of a unique mechanism that endows hair cells with their two most distinctive properties—

Jeff Goldberg, the author of Anatomy of a Scientific Discovery*, is writing a book about fetal tissue transplantation.*



extreme sensitivity and extreme speed.

This success has attracted a large number of scientists to the study of the auditory system. But until the early 1970s, when Hudspeth set out to determine precisely what hair cells did and how they did it, research into the basic biology of the auditory system lagged so far behind the exciting advances being made in vision that it was dubbed the "Cinderella sense" by some researchers.

Because of the hair cell bundles' uncanny resemblance to little antennae and their location in the inner ear, the cells had long been suspected of playing an important role in hearing. This view was bolstered by clinical evidence that the majority of hearing impairments—which affect some 30 million Americans—involve damage to hair cells.

There are only 16,000 hair cells in a human cochlea, compared to 100 million photoreceptors in the retina of the eye, and they are extremely vulnerable. Life in a high-decibel society of pounding jackhammers, screeching subway cars, and heavy metal rock music can take a devastating toll on them. But whatever the cause—overexposure to loud noises, disease, heredity, or aging—people tend to lose 40 percent of their hair cells by the age of 65. And once destroyed, these cells do not regenerate.

Hudspeth's investigation of these cells was initially a solitary, frustrating effort. "I was struck by the fact that so little was known about them," recalls Hudspeth, who is now an HHMI investigator at the University of Texas Southwestern Medical Center. "So I decided to apply myself to solving this one basic problem." He wanted to see whether movements of the ciliabundle on top of the cell could convert mechanical vibrations into electrical signals to the brain, a process known as transduction.

Together with Corey, who joined him in 1975, Hudspeth began a series of experiments that focused on transduction in hair cells. Such experiments, now routine in their labs, are tricky. Protected deep inside the skull, hair cells cannot easily be studied in living creatures—and once removed from laboratory animals, these cells quickly die. Even now, Corey acknowledges, "a good experiment would be to study three or four cells for maybe 15 minutes each."

The measurements are so delicate that they are usually carried out on a table mounted on air-cushioned legs, to reduce any external movements or vibrations; otherwise, the building's own vibration would deafen a hair cell in seconds. Hudspeth found that an unused swimming pool built on bedrock in a basement at the University of California, San Francisco, where he worked previously, made the perfect laboratory for hair cell experiments—especially after he had it filled with 30 truckloads of concrete for more stability.

Hair cells from bullfrogs were exposed by removing the sacculus, a part of the inner ear, and pinning the pinhead-sized tissue to a microscope slide. Working under a microscope, Hudspeth and Corey were then able to manipulate an individual hair cell's bundle of cilia with a thin glass tube. They slipped the tube over the bundle's 50 to 60 stereocilia, which are arranged like a tepee on the top of each hair cell, and moved the tube back and forth, deflecting the bundle less than a ten-thousandth of an inch. The hair cell's response was detected by a microelectrode inserted through the cell membrane.

Corey and Hudspeth found that the bundle of stereocilia operated like a light switch. When the bundle was prodded in one direction—from the shortest cilia to the tallest—it turned the cell on; when the bundle moved in the opposite direction, it turned the cell off.

Based on data from thousands of experiments in which they wiggled the bundle back and forth, the researchers calculated that hair cells are so sensitive that deflecting the tip of a bundle by the width of an atom is enough to make the cell respond. This infinitesimal movement, which might be caused by a very low, quiet sound at the threshold of hearing, is equivalent to displacing the top of the Eiffel Tower by only half an inch.

At the same time, the investigators reasoned that the hair cells' response had to be amazingly rapid. "In order to be able to process sounds at the highest frequency range of human hearing, hair cells must be able to turn current on and off 20,000 times per second. They are capable of even more astonishing speeds in bats and whales, which can distinguish sounds at frequencies as high as 200,000 cycles per second," says Hudspeth.

Photoreceptors in the eye are much slower, he points out. "The visual system is so slow that when you look at a movie at 24 frames per second, it seems continuous,

On the Trail of a "Deafness" Gene

Being able to hear speech is taken for granted—"Is it possible for a hearing person to comprehend the enormity of its absence in someone else?" asks Hannah Merker in her poignant book *Listening*. "The silence around me is invisible...."

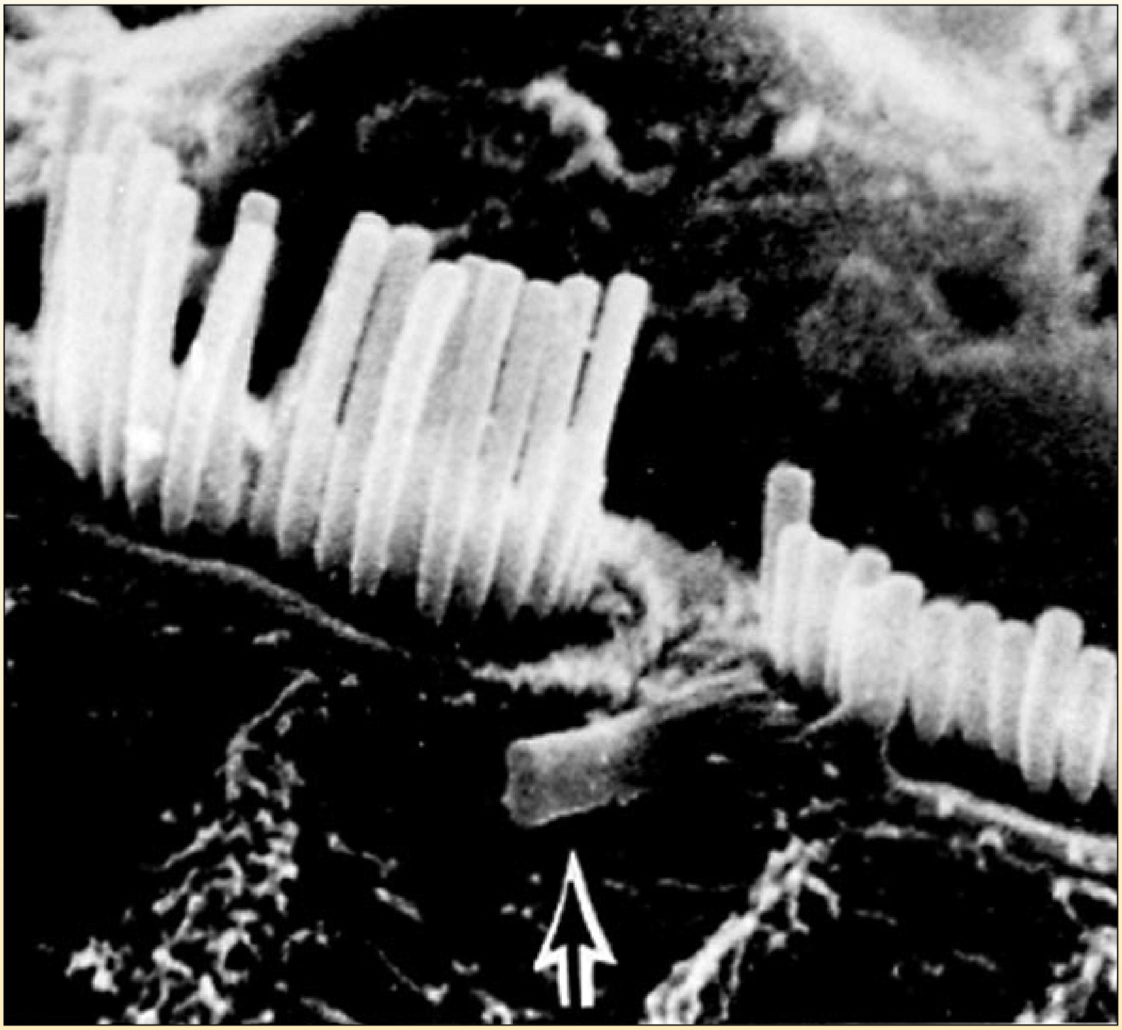
Most of the 28 million deaf or hearing-impaired people in the United States were born with normal hearing, as was Merker, who became deaf after a skiing accident in her twenties. Deafness generally results from overexposure to loud noise, disease, or old age. But genetic factors are also an important cause of hearing loss, especially in children.

It has been estimated that 1 in every 1,000 newborns is profoundly deaf, while nearly 1 in 20 has significant hearing impairment. In more than half of these cases, the cause is genetic.

Large families in which a single type of deafness is clearly inherited are rare, however. Geoffrey Duyk, until recently an HHMI investigator at Harvard Medical School, often spends hours on the telephone with health care workers and geneticists in the United States and abroad, trying to track down leads on families with similar hearing disorders so he can search for the genetic error leading to their deafness. He recently found an unusually large family in Worcester, Massachusetts, whose DNA he can analyze, as well as an entire tribe of Bedouin Arabs in Northern Israel.

Hearing loss has taken an extremely high toll among the members of both families. Thirteen of fifty members of the Worcester family, recently examined by a team of specialists coordinated by Duyk, were going through the same disastrous sequence of events: although they could hear well at birth, they would start losing their hearing in their teens; by their early forties they were profoundly deaf. The Bedouin tribe suffered from an even more damaging kind of hereditary disorder: about a quarter of their children were born deaf.

Duyk took samples of blood from both families and set out to find mutations in their DNA that could account for their hearing loss. The task was formidable. "Deafness is associated with over 100 different genetic disorders, and there are upwards of 30 forms of hereditary hearing loss alone, each caused by a different mutation," Duyk says. Yet he



Laboratory of Charles Liberman, Massachusetts Eye and Ear Infirmary

A two-hour exposure to loud noise—such as that of loud rock bands—is enough to seriously damage cilia bundles on the hair cells of a cat's inner ear. Normal mammalian hair cell bundles have two or three parallel rows of cilia, one taller than the next. The tall cilia are most vulnerable to noise. After exposure to loud noise, all the tall cilia on the right of this picture have disappeared or fused together and fallen over.

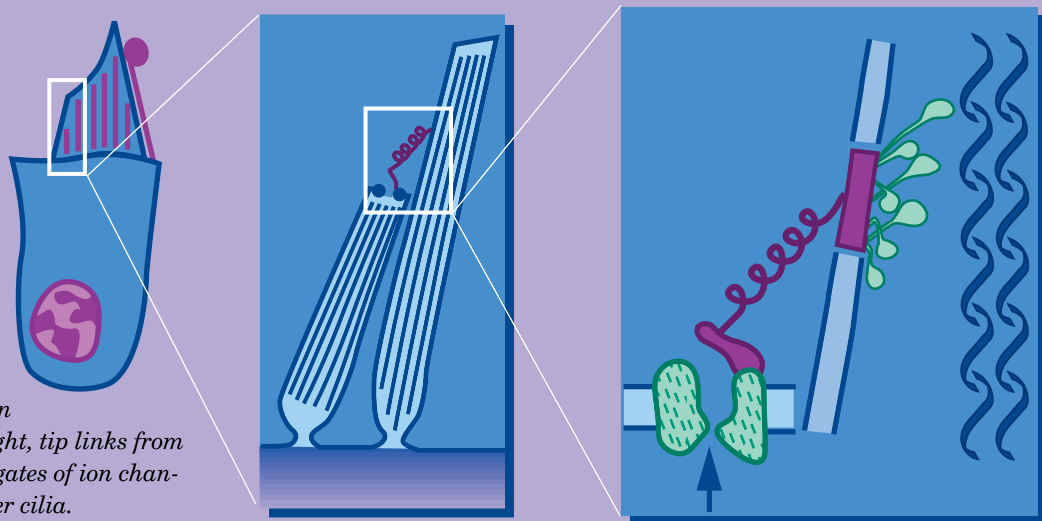
is hot on the trail of a genetic error that appears to be responsible for the Bedouin tribe's deafness.

Meanwhile, David Corey and his colleague Xandra Breakefield at Massachusetts General Hospital are examining the gene that is defective in Norrie disease, a different disorder that causes not only a progressive loss of hearing similar to that of the Worcester family but, in addition, blindness at birth. The scientists are now analyzing the protein made by normal copies of this gene and trying to understand its function, which might lead to ways of preventing the disorder.

For a deeper understanding of such disorders, scientists need to work with animal models. Duyk's research group is presently studying two strains of mice, called "jerkers" and "shakers," that have been found to suffer from inherited forms of progressive hearing loss (as well as the peculiar movement disorders that give them their names.) The researchers are looking for fragments of DNA from these mice that might be similar to pieces of DNA from families with genetic deafness.

"We would like to develop new kinds of treatment for hearing loss," Duyk explains. "But first we need to identify the proteins and genes that are essential to hearing."

A Tip Link Pulls Up the Gate of a Channel.



In this sketch, James Hudspeth suggests how the movement of a hair cell's cilia bundle (top) opens ion channels at the tips of the cilia. When the bundle tilts to the right, tip links from higher cilia pull up the gates of ion channels on adjoining, shorter cilia.

A close-up shows how a tip link between two cilia opens an ion channel on the shorter cilium.

Even more highly magnified (right), the open channel allows ions into the cell. A cluster of myosin molecules in the taller cilium is shown in green and some actin filaments are shown in blue.

without any flicker. Contrast 24 frames per second with 20,000 cycles per second. The auditory system is a thousand times faster."

How do hair cells do this?

Unlike other types of sensory receptor cells, hair cells do not rely on a cascade of chemical reactions to generate a signal. Photoreceptor cells in the eye, for instance, require a series of intricate interactions with a G protein and a second messenger before their ion channels close, sending a signal to the brain. This process would be much too slow to deal with sounds. Hair cells have to possess a mechanism that allows their ion channels to open and close more rapidly than those of any other sensory receptor cells.

The answer is that hair cells use something very much like a spring to open their channels when the cilia bend, without the need for a time-consuming chemical exchange.

Corey and Hudspeth first theorized that such a "gating spring" mechanism existed in the early 1980s. They proposed that hair cells had a previously unknown type of ion channel—a channel directly activated by mechanical force. They also developed a biophysical theory to account for the hair cells' rapid response. But their theory didn't tell them where the channels were or what the spring was.

By painstakingly measuring the electrical field around the cilia with an electrode, Hudspeth detected a tiny drop in voltage at the cilia's tips, as if the current were being sucked into a minute whirlpool. This led him to conclude that the channels through which charged particles move into the cell, changing its electrical potential, were located at the cilia's tips. He then reasoned that the gating springs that opened these channels should be there as well.

The springs themselves were first observed in 1984, in electron microscope images taken by James Pickles and his colleagues in England. Called tip links, these minute filaments join each stereocilium to its tallest neighbor. Pickles pointed out that the geometry of the cilia bundle would cause the bundle to stretch the links when it was deflected in one direction and relax them when it was moved in the other. If the tip links were the hypothetical gating springs, it would explain everything.

"This was a completely new kind of mechanism, unlike anything ever observed before," says Corey, who provided compelling evidence two years ago that the tip links pull on the channels. By "cutting" the tip links with a chemical, Corey could stop the cell's response cold. "Within less than a second, as the tip links became unstable, the whole mechanical sensitivity of the cell was destroyed," Corey observed.

Recently, both he and Hudspeth have been independently investigating another property of hair cells: their ability to adapt to being deflected. At first, when a hair cell bundle is deflected, the ion channels open. But if the bundle remains deflected for a tenth of a second, the channels close spontaneously. It appears from electron microscope images and physiological evidence that the channels close when the tip links relax. This is related to the activity of the tip links' attachment points, which can move up and down along the cilia to fine-tune the tension on the channels. When the attachment points move down, the tip links are relaxed and the ion channels close.

While the researchers are still trying to figure out what enables the attachment points to move, they strongly suspect that myosin plays a role. Myosin is the protein that gives muscle cells their ability to contract and relax, and Hudspeth's group has found evidence of myosin in cilia bundles. Both labs have now cloned and sequenced the gene for a myosin molecule in hair cells. A cluster of such molecules in each stereocilium could provide the force to move the attachment point up or down.

Slight movements of the attachment points allow the hair cell to set just the right amount of tension on each channel so it is maximally sensitive. They also permit the cell to avoid being overloaded when it is barraged by sound.

A second type of hair cell in the highly specialized cochlea of mammals may enable us to distinguish the quietest sounds. These outer hair cells, which are shaped like tiny hot dogs, look distinctly different from inner hair cells. The outer hair cells also have a peculiar ability to become shorter or longer within microseconds when stimulated, doing so with a flamboyant, bouncy, up-and-down motion not found in any other cell type. They outnumber inner hair cells 3 to 1. However, the 4,000 inner hair cells are connected to most of the auditory nerve fibers leading to the brain and are clearly the main transmitters of sound.

The precise function of the outer hair cells is still unclear. Auditory researchers speculate that these cells may serve as an amplification mechanism for tuning up low-frequency sound waves, possibly by accelerating the motion of the basilar membrane.

Hudspeth is also intrigued by the possibility that outer hair cells may be responsible for something that has puzzled researchers for years: the fact that our ears not only receive sounds, but emit them as well. When sensitive microphones are placed in the ear and a tone is played, a faint echo can be detected resonating back out. Such otoacoustic emissions are considered normal; in fact, their presence in screening exams of newborn babies is thought to be indicative of healthy hearing. However, in certain cases, otoacoustic emissions can be spontaneous and so intense that they are audible without the aid of special equipment. "In some people, you can actually hear them. The loudest ones ever recorded were in a dog in Minnesota, whose owner noticed the sound coming out of the animal's ear and took the dog to a specialist, who did recordings and analysis," says Hudspeth.

"What may be happening is that the amplification system driven by the movements of outer hair cells is generating feedback, like a public address system that's tuned up too high," he speculates, adding that such otoacoustic emissions gone awry may account for certain unusual forms of tinnitus, or ringing in the ear.

Hudspeth and Corey's research is providing such a detailed picture of the hair cell that it is now possible to begin to identify the individual proteins making up the tip links, ion channels, and motor mechanisms involved, as well as the genes that produce them. Malfunctions in those genes, resulting in defects in these important structures, may be the cause of inherited forms of deafness (see p. 36)

...our ears not only receive sounds, but emit them as well.

Locating a Mouse by Its Sound

by Jeff Goldberg

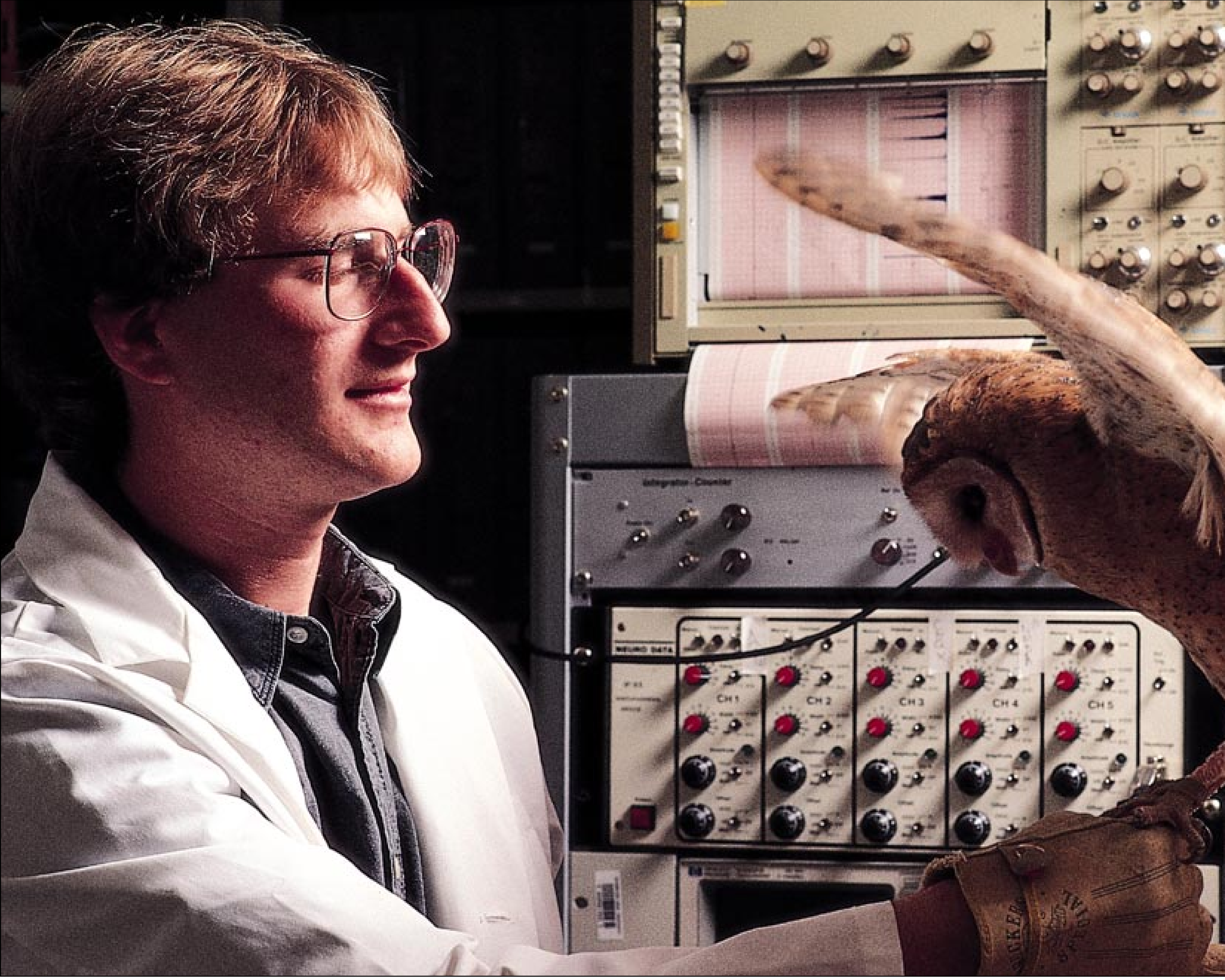
While some scientists investigate the mystery of how we hear from the bottom up, beginning with the ear's sound receptors, others search for answers from the top down, mapping networks of auditory neurons in the brain in an effort to understand how the brain processes sounds.

At Caltech in the mid-1970s, Masakazu ("Mark") Konishi began studying the auditory system of barn owls in an effort to resolve a seemingly simple question: Why do we have two ears?

While most sounds can be distinguished quite well with one ear alone, the task of pinpointing where sounds are coming from in space requires a complex process called binaural fusion, in which the brain must compare information received from each ear, then translate subtle differences into a unified perception of a single sound—say a dog's bark—coming from a particular location.

Konishi, a zoologist and expert on the nervous system of birds, chose to study this process in owls. The ability to identify where sounds are coming from based on auditory cues alone is common to all hearing creatures, but owls—especially barn owls—excel at the task. These birds exhibit such extraordinary sound localization abilities that they are able to hunt in total darkness.

In total darkness, a barn owl swoops down on a mouse.



Dan Feldman, an HHMI predoctoral fellow in Eric Knudsen's lab, wears protective gloves as he prepares an owl for an experiment that will record the owl's head-turning movements in response to sounds.

Working with Eric Knudsen, who is now conducting his own research on owls at Stanford University, Konishi undertook a series of experiments on owls in 1977 to identify networks of neurons that could distinguish sounds coming from different locations. He used a technique pioneered by vision researchers, probing the brains of anesthetized owls with fine electrodes. With the electrodes in place, a remote-controlled sound speaker was moved to different locations around the owl's head along an imaginary sphere. As the speaker moved, imitating sounds the owl would hear in the wild, the investigators recorded the firing of neurons in the vicinity of the electrodes.

Over the course of several months, Konishi and Knudsen were able to identify an area in the midbrain of the birds containing cells called space-specific neurons—about 10,000 in all—which would fire only when sounds were presented in a particular location. Astonishingly, the cells were organized in a precise topographic array, similar to maps of cells in the visual cortex of the brain. Aggregates of space-specific neurons, corresponding to the precise vertical and horizontal coordinates of the speaker, fired when a tone was played at that location.

"Regardless of the level of the sound or the content of the sound, these cells always responded to the sources at the same place in space. Each group of cells across the circuit was sensitive to sound coming from a different place in space, so when the sound moved, the pattern of firing shifted across the map of cells," Knudsen recalls.

The discovery of auditory brain cells that could identify the location of sounds in space quickly produced a new mystery. "The

lens of the eye projects visual space onto receptors on a 2-dimensional sheet, the retina, and the optic nerve fibers project the same spatial relationships to the brain," says Konishi. "But in the auditory system, only the frequency of sound waves is mapped on the receptor layer, and the auditory nerve fibers project this map of frequency to the brain. How can the brain create a map of auditory space, based only on frequency cues?"

The answer, Konishi believes, may shed light on how the brain and the auditory system process all sounds.

To enable the brain to process efficiently the rapid stream of impulses emanating from the hair cells in the ear, the auditory system must first filter out simple, discrete aspects of complex sounds. Information about how high- or low-pitched a sound is, how loud it is, and how often it is heard is then channeled along separate nerve pathways to higher-order processing centers in the brain, where millions of auditory neurons can compute the raw data into a recognizable sound pattern.

This filtering process begins with the hair cells, which respond to different frequencies at different locations along the basilar membrane. Hair cells at the bottom of the basilar membrane respond more readily when they detect high-frequency sound waves, while those at the top are more sensitive to low-frequency sounds. David Corey compares the arrangement to the strings of a grand piano, with the high notes at the base of the cochlea, where the basilar membrane is narrow and stiff, and the bass notes at the apex, where the membrane is wider and more flexible.

Hair cells also convey basic information about the intensity and duration of sounds. The louder a sound is at any particular frequency, the more vigorously hair cells tuned to that frequency respond, while their signaling pattern provides information about the timing and rhythm of a sound.

Konishi hypothesized that such timing and intensity information was vital for sound localization. So he placed microphones in the ears of owls to measure precisely what they were hearing as the portable loudspeaker rotated around their head. He then recorded the differences in time and intensity as sounds reached each of the owl's ears. The differences are very slight. A sound that originates at the extreme left of the animal will arrive at the left ear about 200 microseconds (millionths of a second) before it reaches the right ear. (In humans, whose sound localization abilities are keen but not on a par with those of owls, the difference between a similar sound's time of arrival in each ear would be about three times greater.)

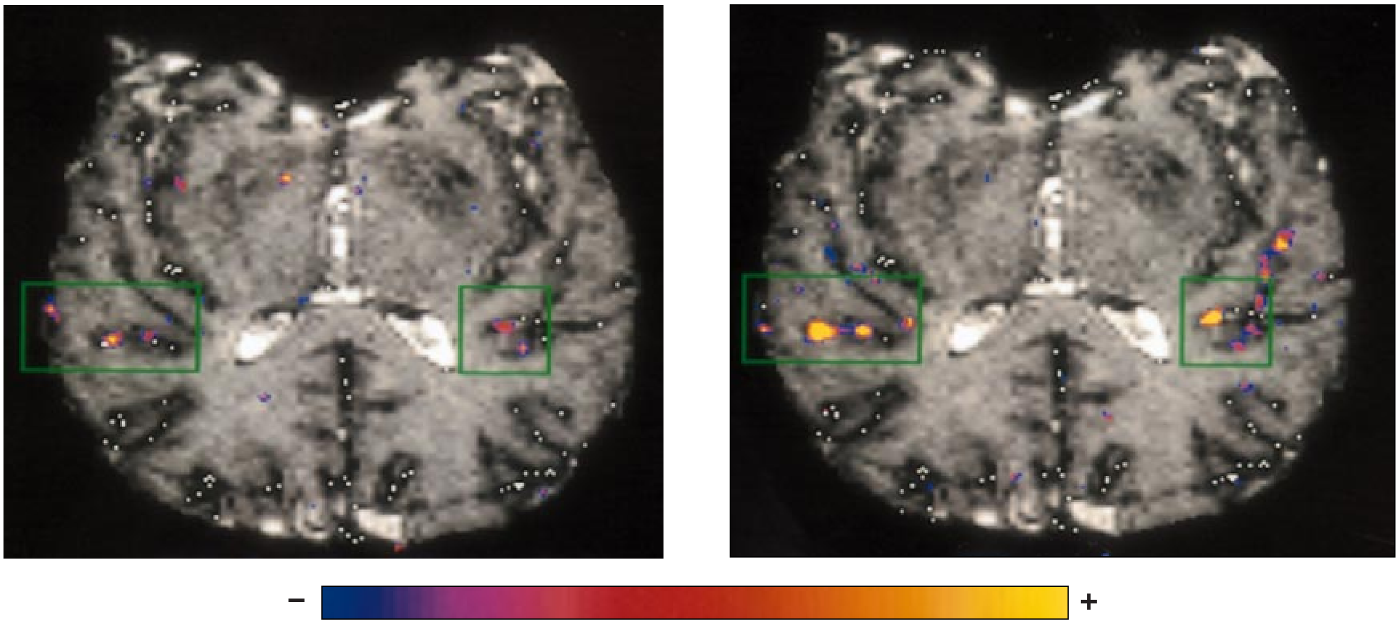
As the sound source was moved toward the center of the owl's head, these interaural time differences diminished, Konishi observed. Differences in the intensity of sounds entering the two ears occurred as the speaker was moved up and down, mostly because the owl's ears are asymmetrical—the left ear is higher than eye level and points downward, while the right ear is lower and points upward.

Based on his findings, Konishi delivered signals separated by various time intervals and volume differences through tiny earphones inserted into the owls' ear canals. Then he observed how the animals responded. Because owls' eyes are fixed in their sockets and cannot rotate, the animals turn quickly in the direction of a sound, a characteristic movement. By electronically monitoring these head-turning

After wearing prism spectacles for a few months, this owl began to miss auditory targets because the sound localization system in its brain tried to harmonize with the visual system, which received erroneous cues.

Laboratory of Eric Knudsen, Stanford University

CAN FUNCTIONAL MRI TELL WHETHER A PERSON IS HEARING MUSIC OR JUST MEANINGLESS CLICKS?



NMR Center, Massachusetts General Hospital

Parts of a volunteer's brain were activated (white box on left of first picture) when he heard a series of sharp but meaningless clicks while inside a fMRI magnet at Massachusetts General Hospital. Some of the same areas became much more active and several new areas were activated as well (square box on right of second picture) when he listened to instrumental music, reflecting the richer meaning of the sounds.

movements, Konishi and his assistants showed that the owls would turn toward a precise location in space corresponding to the interaural time and intensity differences in the signals. This suggested that owls fuse the two sounds that are delivered to their two ears into an image of a single source—in this case, a phantom source.

"When the sound in one ear preceded that in the other ear, the head turned in the direction of the leading ear. The longer we delayed delivering the sound to the second ear, the further the head turned," Konishi recalls.

Next, Konishi tried the same experiment on anesthetized owls to learn how their brains carry out binaural fusion. Years earlier, he and Knudsen had identified space-specific neurons in the auditory area of the owl's midbrain that fire only in response to sounds coming from specific areas in space. Now Konishi and his associates found that these space-specific neurons react to specific combinations of signals, corresponding to the exact direction in which the animal turned its head when phantom sounds were played. "Each neuron was set to a particular combination of interaural time and intensity difference," Konishi recalls.

Konishi then decided to trace the pathways of neurons that carry successively more refined information about the timing and intensity of sounds to the owl's midbrain. Such information is first processed in the cochlear nuclei, two bundles of neurons projecting from the inner ear. Working with Terry Takahashi, who is now at the University of Oregon, Konishi showed that one of the nuclei in this first way station signals only the timing of each frequency band, while the other records intensity. The signals are then transmitted to two higher-order processing stations before reaching the space-specific neurons in the owl's midbrain.

One more experiment proved conclusively that the timing and intensity of sounds are processed along separate pathways. When the researchers injected a minute amount of local anesthetic into one of the cochlear nuclei (the magnocellular nucleus), the space-specific neurons higher in the brain stopped responding to differences in interaural time, though their response to differences in intensity was unchanged. The converse occurred when neurons carrying intensity information were blocked.

"I think we are dealing with basic principles of how an auditory stimulus is processed and analyzed in the brain. Different features are processed along parallel, almost independent pathways to higher stations, which create more and more refined neural codes for the stimulus," says Konishi. "Our knowledge is not complete, but we know a great deal. We are very lucky. The problem with taking a top-down approach is that often you find nothing."

Konishi has been able to express the mechanical principles of the owl's sound localization process as a step-by-step sequence. He has collaborated with computer scientists at Caltech in developing an "owl chip" that harnesses the speed and accuracy of the owl's neural networks for possible use in computers.

At Stanford University, Eric Knudsen has recently been conducting experiments on owls fitted with prism spectacles to determine whether distortions in their vision affect their sound localization abilities. Despite their exceptionally acute hearing, he has found, the owls trust their vision even more. When they wear distorting prisms, their hunting skills deteriorate over a period of weeks as their auditory systems try to adapt to the optical displacement of the prisms. "The visual system has ultimate control and basically dictates how the brain will interpret auditory localization cues," Knudsen says.

He is also examining a particular network of neurons in the animals' brains where he believes auditory and visual system signals converge. "This network makes it possible for the owls to direct their eyes and attention to a sound once it's heard," Knudsen explains. His research is part of a new wave of studies that focus not just on single sensory pathways, but on how the brain combines information it receives from many different sources. ●

Help from a Bat

Perhaps the finest achievement in sound processing is the ability to understand speech. Since this is a uniquely human trait, it would seem difficult to study in animals. Yet a researcher at Washington University in St. Louis believes it can be examined—by working with bats.

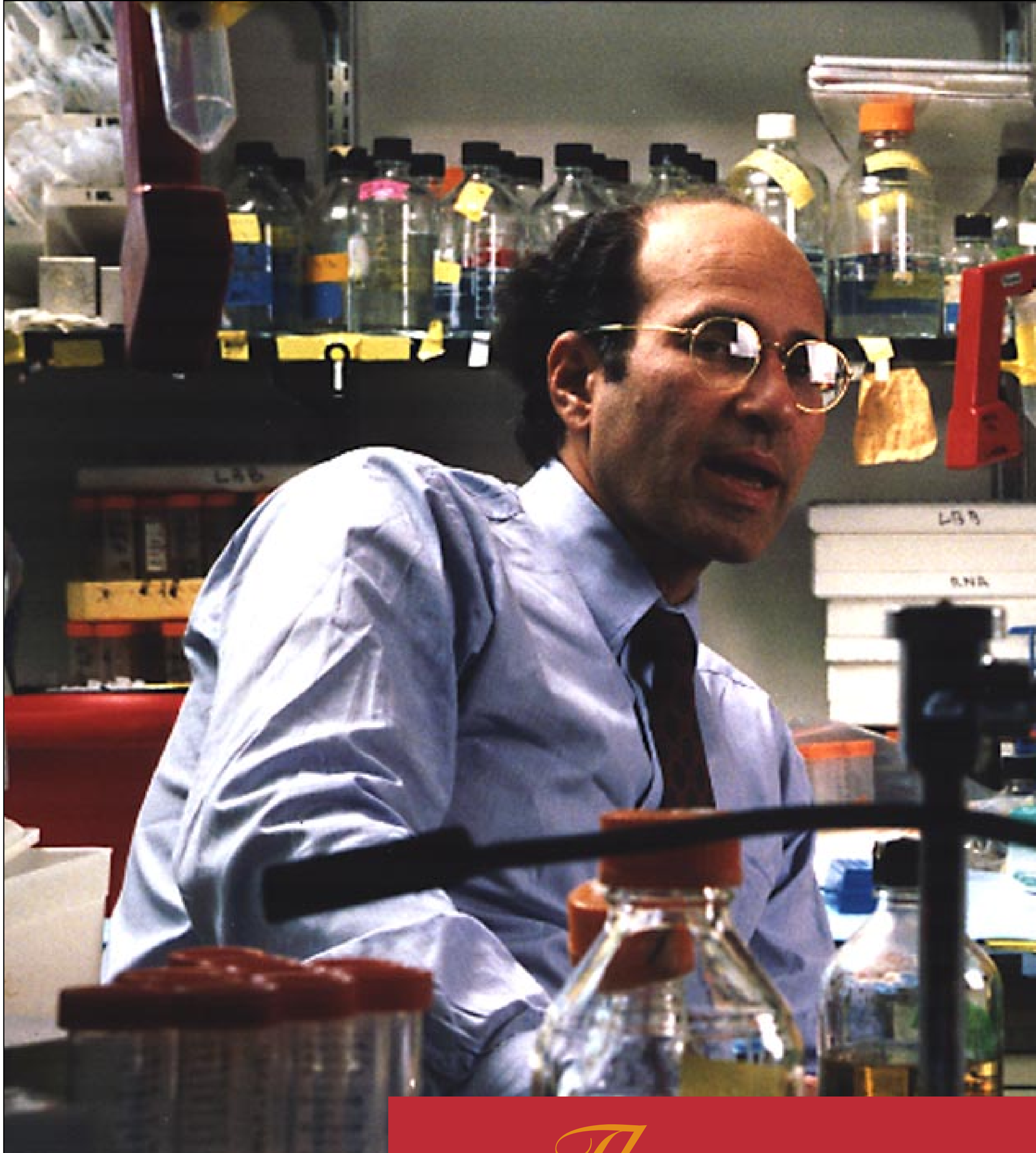
Bats navigate and locate prey by echolocation, a form of sonar in which they emit sound signals of their own and then analyze the reflected sounds. Nobuo Suga, who has spent nearly 20 years investigating the neural mechanisms used by bats to process the reflected signals, is convinced that such research can shed light on the understanding of human speech.

When Suga slowed down recordings of the high-frequency, short-duration sounds that bats hear, he found that the sounds' acoustic components were surprisingly similar to those of mammalian communication, including human speech. There were some constant frequencies and noise bursts, not unlike vowel and consonant sounds, as well as frequency-modulated components that were similar to those in combinations of phonemes such as "papa."

Each of these acoustic elements is processed along a distinct pathway to higher-order neurons, which combine and refine different aspects of the sonar pattern in much the same way that space-specific neurons combine the timing and intensity cues of sound signals.

Suga also identified maps of neurons in the bats' auditory cortex which register slight variations in these components of sound. Humans may use similar maps to process the basic acoustic patterns of speech, though speech requires additional, higher-level mechanisms, he points out.

"The ability to recognize variations in sound is what enables us to understand each other. No two people pronounce vowels and consonants in exactly the same way, but we are able to recognize the similarities," says Suga. He believes that neuronal maps may also play a role in human voice recognition—the ability to recognize who is speaking as well as what is being said.



The MYSTERY

After taking a mixture of mind-altering drugs one night, Stephen D., a 22-year-old medical student, dreamed that he had become a dog and was surrounded by extraordinarily rich, meaningful smells. The dream seemed to continue after he woke up—his world was suddenly filled with pungent odors.

Walking into the hospital clinic that morning, "I sniffed like a dog. And in that sniff I recognized, before seeing them, the twenty patients who were there," he later told neurologist Oliver Sacks. "Each had his own smell-face, far more vivid and evocative than any sight-face." He also recognized local streets and shops by their smell. Some smells gave him pleasure and others disgusted him, but all were so compelling that he could hardly think about anything else.

The strange symptoms disappeared after a few weeks. Stephen D. was greatly relieved to be normal again, but he felt "a tremendous loss, too," Sacks reported in his book *The Man Who Mistook His Wife for a Hat and Other Clinical Tales*. Years later, as a successful physician, Stephen D. still remembered "that smell-world—so vivid, so real! It was like a visit to another world, a world of pure perception, rich, alive, self-sufficient, and full...I see now what we give

Linda Buck (right) sniffs an odorant used to study the sense of smell. She and Richard Axel (left) discovered what appear to be the long-sought odorant receptor proteins.

of SMELL

BY MAYA PINES

up in being civilized and human."

Being civilized and human means, for one thing, that our lives are not ruled by smells. The social behavior of most animals is controlled by smells and other chemical signals. Dogs and mice rely on odors to locate food, recognize trails and territory, identify kin, find a receptive mate. Social insects such as ants send and receive intricate chemical signals that tell them precisely where to go and how to behave at all times of day. But humans "see" the world largely through eyes and ears. We neglect the sense of smell—and often suppress our awareness of what our nose tells us. Many of us have been taught that there is something shameful about odors.

Yet mothers can recognize their babies by smell, and newborns recognize their mothers in the same way. The smells that surround us affect our well-being throughout our lives. Smells also retain an uncanny power to move us. A whiff of pipe tobacco, a particular perfume, or a long-forgotten scent can instantly conjure up scenes and emotions from the past. Many writers and artists have marveled at the haunting quality of such memories.

In *Remembrance of Things Past*, French novelist Marcel Proust described what happened to him after drinking a spoonful of tea in which he had soaked a piece of a madeleine, a type of cake: "No sooner had the warm liquid mixed with the crumbs touched my palate than a shudder ran through my whole body, and I stopped, intent upon the extraordinary thing that was happening to me," he wrote. "An exquisite pleasure had invaded my senses...with no suggestion of its origin....

"Suddenly the memory revealed itself. The taste was of a little piece of madeleine which on Sunday mornings...my Aunt Leonie used to give me, dipping it first in her own cup of tea....Immediately the old gray house on the street, where her room was, rose up like a stage set...and the entire town, with its people and houses, gardens, church, and surroundings, taking shape and solidity, sprang into being from my cup of tea."

"Suddenly the memory revealed itself…"

Just seeing the madeleine had not brought back these memories, Proust noted. He needed to taste and smell it. "When nothing else subsists from the past," he wrote, "after the people are dead, after the things are broken and scattered...the smell and taste of things remain poised a long time, like souls...bearing resiliently, on impalpable droplets, the immense edifice of memory."

Proust referred to both taste and smell—and rightly so, because most of the flavor of food comes from its aroma, which wafts up the nostrils to sensory cells in the nose and also reaches these cells through a passageway in the back of the mouth. Our taste buds provide only four distinct sensations: sweet, salty, sour, and bitter. Other flavors come from smell, and when the nose is blocked, as by a cold, most foods seem bland or tasteless.

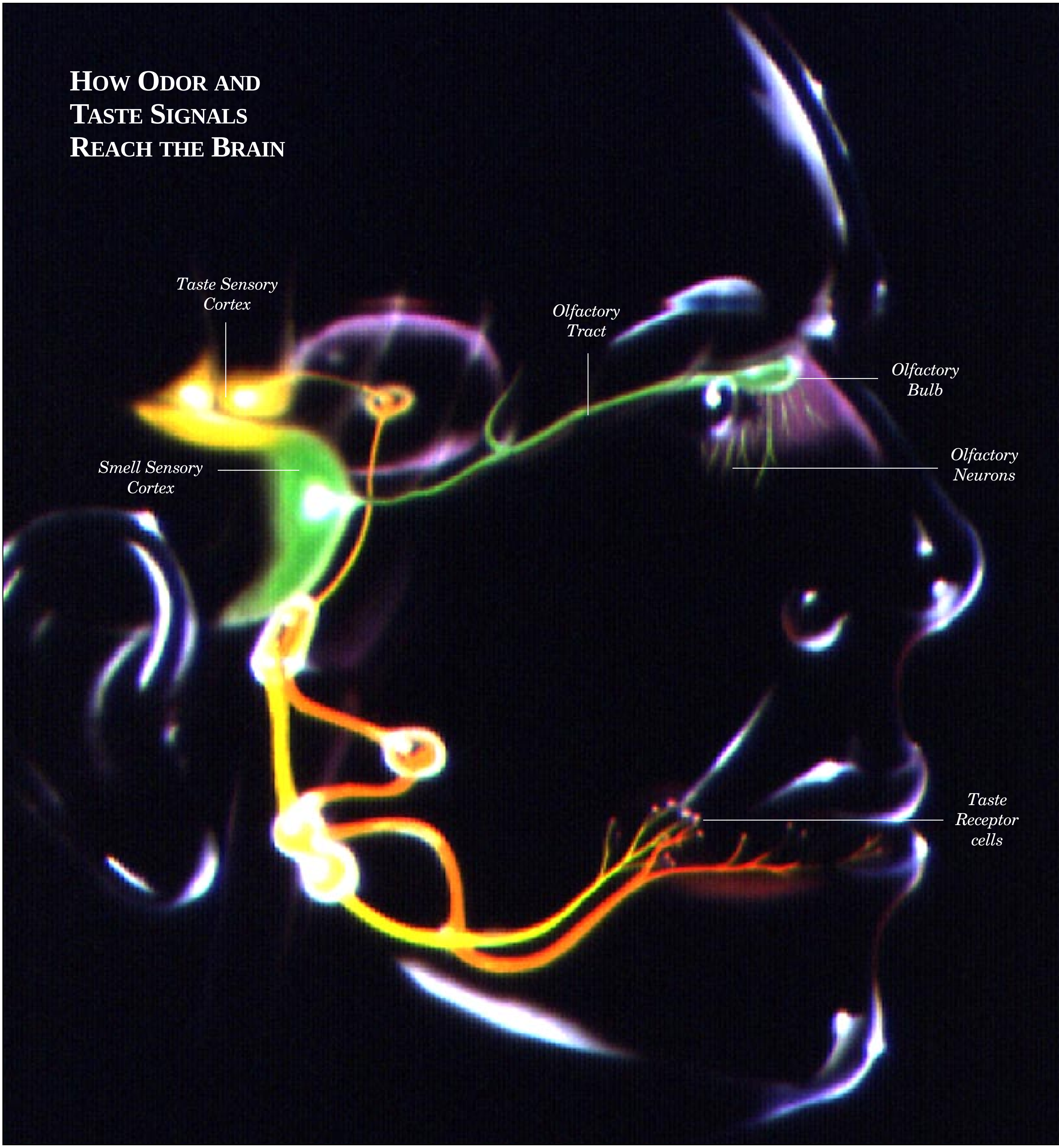
Both smell and taste require us to incorporate—to breathe in or swallow—chemical substances that attach themselves to receptors on our sensory cells. Early in evolution, the two senses had the same precursor, a common chemical sense that enabled bacteria and other single-celled organisms to locate food or be aware of harmful substances.

How we perceive such chemical substances as odors is a mystery that, until recently, defeated most attempts to solve it. Anatomical studies showed that signals from the olfactory cells in the nose reach the olfactory area of the cortex after only a single relay in the olfactory bulb. The olfactory cortex, in turn, connects directly with a key structure called the hypothalamus, which controls sexual and maternal behavior. When scientists tried to explore the details of this system, however, they hit a blank wall. None of the methods that had proved fruitful in the study of vision seemed to work.

To make matters worse, very little was known about the substances to which the olfactory system responds. The average human being, it is said, can recognize some 10,000 separate odors. We are surrounded by odorant molecules that emanate from trees, flowers, earth, animals, food, industrial activity, bacterial decomposition, other humans. Yet when we want to describe these myriad odors, we often resort to crude analogies: something smells like a rose, like sweat, or like ammonia. Our culture places such low value on olfaction that we have never developed a proper vocabulary for it. In *A Natural History of the Senses*, poet and essayist Diane Ackerman notes that it is almost impossible to explain how something smells to someone who hasn't smelled it. There are names for all the pastels in a hue, she writes—but none for the tones and tints of a smell.

Nor can odors be measured on the kind of linear scale that scientists use to measure the wavelength of light or the frequency of sounds. "It would be nice if one smell corresponded to a short wavelength and another to a long wavelength, such as rose versus skunk, and you could place every smell on this linear scale," says Randall Reed, an HHMI investigator at the Johns Hopkins University School of Medicine who has long been interested in olfaction. "But there is no smell scale," since odorous molecules vary widely in chemical composition and three-dimensional shape.

To find out how these diverse odorants trigger our perception of smell, researchers needed to examine the olfactory cells and identify the receptor proteins that actually bind with the odorants. This task was made more difficult by the awkward location of


HOW ODOR AND TASTE SIGNALS REACH THE BRAIN
Taste Sensory Cortex
Olfactory Tract
Olfactory Bulb
Smell Sensory Cortex
Olfactory Neurons
Taste Receptor cells

the olfactory cells.

"We think that we smell with our noses, [but] this is a little like saying that we hear with our ear lobes," writes Gordon Shepherd, professor of neuroscience at Yale University. "In fact, the part of the nose we can see from the outside serves only to take in and channel the air containing odorous molecules." The neurons that sense these molecules lie high up in the nose, in a patch of cells called the olfactory epithelium.

Perched behind a sort of hairpin turn at the very top of the nasal cavity, the olfactory epithelium is only a few centimeters square. It contains some five million olfactory neurons, plus their supporting cells and stem cells. Actually, there are two such patches—one on each side of the nose—lying in a horizontal line just below the level of the eye. Each olfactory neuron in the epithelium is topped by at least ten hairlike cilia that protrude into a thin bath of mucus at the cell surface. Somewhere on these cilia, scientists were convinced, there must be receptor proteins that recognize and bind odorant molecules, thereby stimulating the cell to send signals to the brain.

The receptor proteins would be the key to answering two basic questions about olfaction, explains Richard Axel, an HHMI investigator at Columbia University. First, how does the system respond to the thousands of molecules of different shapes and sizes that we call odorants? "Does it use a restricted number of promiscuous receptors, or a large number of relatively specific receptors?" And second, how does the brain make use of these responses to discriminate between odors?

In the mid-1980s, Solomon Snyder of the Johns Hopkins University School of Medicine, whose research team had identified many other receptor proteins, took up the challenge. He decided to use the same approach that had worked so well for him before. He would attach radioactive tags to a set of molecules (in this case, odorant molecules that smelled of bell pepper), mix the tagged molecules with cells (in this case, olfactory neurons) in a test tube, and examine where the molecules bound to the cells. The cell's binding site would be the odorant receptor.

Snyder and his colleagues, including Jonathan Pevsner, Randall Reed, and Paul Feinstein, did find a receptor protein in this way, but it was not the one they were looking for. "It turned out we had discovered a protein that seems to function as a kind of amplifying device for odorants," Snyder says. This protein, an abundant constituent of mucus, is "made in a gland in the nose and sprayed, as by an atomizer, into the inhaled air, where it can bind to the maximum number of odorant molecules," he explains. Snyder believes this odorant-binding protein gathers the molecules into large enough concentrations to activate the sense of smell and then deposits them at the back of the nose, where the olfactory neurons are. If so, it might explain why we are able to perceive a few molecules of odorants in a trillion molecules of air, even though olfactory neurons are not activated until the concentration of odorant is 1,000 times greater.

This did not lead to the long-sought odorant receptor protein, however. The big break came in 1991. "In the past five years, we have gone from an era in which we knew nothing about the biochemical process involved in perceiving odors to knowing nearly all the biochemical steps involved—as well as how they generate electrical signals to the brain," says Reed. During this period, Reed himself discovered a protein, Golf (the olfactory G protein), that is activated early in the cascade of biochemical events leading to electrical signals. Then he cloned one of the genes for the ion channel that opens in the cell membrane in response to this cascade, generating an electrical signal. He is now trying to reconstruct the entire signaling pathway in lines of cultured cells.

The string of discoveries that totally changed the study of olfaction resulted from a new emphasis on genetics. Instead of hunting for the receptor proteins directly, Richard Axel and Linda Buck, who was then a postdoctoral fellow in Axel's group and is now an HHMI investigator at Harvard Medical School, looked for genes that contained instructions for proteins found only in the olfactory epithelium. Their efforts produced nothing at first. "Now we know why our initial schemes failed," says Axel. "It's because there are a large number of odorant receptors, and each was expressed only at a very low level."

Finally, Buck came up with what Axel calls "an extremely clever twist." She made three assumptions that drastically narrowed the field, allowing her to zero in on a group of genes that appear to code for the odorant receptor proteins, though the final proof is not yet in.

Her first assumption—based on bits of evidence from various labs—was that the odorant receptors look a lot like rhodopsin, the receptor protein in rod cells of the eye. Rhodopsin and at least 40 other receptor proteins criss-cross the cell surface seven times, which gives them a characteristic, snake-like shape. They also function in similar ways, by interacting with G proteins (see p.11) to transmit signals to the cell's interior. Since many receptors of this type share certain DNA sequences, Buck designed probes that would recognize these sequences.

Next, she assumed that the odorant receptors are members of a large family of related proteins. So she looked for groups of genes that had certain similarities. Third, the genes had to be expressed only in a rat's olfactory epithelium.

"Had we employed only one of these criteria, we would have had to sort through thousands more genes," says Axel. "This saved several years of drudgery."

Buck recalls that "I had tried so many things and had been working so hard for three and a half years, with nothing to show for it. So when I finally found the genes, I couldn't believe it! None of them had ever been seen before. They were all different but all related to each other. That was very satisfying."

The discovery made it possible to study the sense of smell with the techniques of modern molecular and cell biology and to explore how the brain discriminates among odors. It also allowed researchers to "pull out" the genes for similar receptor proteins in other species by searching through libraries of DNA from these species. Odorant receptors of humans, mice, catfish, dogs, and salamanders have been identified in this way.

The team's most surprising finding was that there are so many olfactory receptors. The 100 different genes the researchers identified first are just the tip of the iceberg, according to Axel. He thinks there must be a total of "about 1,000 separate receptor proteins" on rat—and probably human—olfactory neurons.

"That's really a lot of genes," Axel says. "It's 1 percent of the genome! This means that, at least in the rat, 1 out of every 100 genes is likely to be engaged in the detection of odors." This staggering number of genes reflects the crucial importance of smell to animals.

Large as the number of receptors may be, however, it is probably smaller than the number of odors we can recognize. "Most likely, the number of odorants far exceeds the number of receptor proteins—by a ratio of at least 10 to 1," Axel says. "In that case, how does the brain know what the nose is smelling?"

The visual system needs only three kinds of receptors to distinguish among all the colors that we can perceive, he points out. These receptors all respond to the same thing—light. Light of different wavelengths makes the three kinds of receptors react with different intensity, and then the brain compares their signals to determine color. But the olfactory system must use a different strategy in dealing with the wide variety of molecules that produce odors.

To figure out this strategy, Axel began by asking how many kinds of receptor proteins are made by a single olfactory neuron. "If a single neuron expresses only one or a small number of receptors, then the problem of determining which receptors have been activated reduces to determining which neurons have been activated," he says.

He thought he would make more rapid progress by working with simpler organisms than rats. So he turned to fish, which respond to fewer odorants and were likely to have fewer receptors. From studies with catfish, whose odorant receptors proved very similar to those of rats, Axel and his associates soon concluded that a given

"When I finally found the genes, I couldn't believe it!"

Separate zones of the olfactory epithelium of mice are shown in red, blue, and yellow. A different set of odorant receptor genes is expressed in each zone

Laboratory of Linda Buck, Harvard Medical School

olfactory neuron can make only one or, at most, a few odorant receptors. (Buck and her colleagues have come to the same conclusion from their work with mice.)

The next step was to find out how these odorant receptors—and the neurons that make them—are distributed in the nose. Also, what parts of the brain do these neurons connect with? "We want to learn the nature of the olfactory code," Axel says. "Will neurons that respond to jasmine relay to a different station in the brain than those responding to basil?" If so, he suggests, the brain might rely on the position of activated neurons to define the quality of odors.

Each olfactory neuron in the nose has a long fiber, or axon, that pokes through a tiny opening in the bone above it, the cribriform plate, to make a connection, or synapse, with other neurons in the olfactory bulb, which is a part of the brain. A round, knob-like structure, the olfactory bulb is quite large in animals that have an acute sense of smell. It decreases in relative size as this ability wanes. Thus, bloodhounds, which can follow the scent of a person's tracks for long distances over varied terrain, have larger olfactory bulbs than humans do—even though humans are more than twice the total size of these dogs and have brains that are several times as large.

In the olfactory epithelium of the nose, Axel's group found, neurons that have a given odorant receptor do not cluster together. Instead, these neurons are distributed randomly within certain broad regions of the nasal epithelium. Then their axons converge on the same place in the olfactory bulb, Axel believes.

"The brain is essentially saying something like, 'I'm seeing activity in positions 1, 15, and 54 of the olfactory bulb, which correspond to odorant receptors 1, 15, and 54, so that must be jasmine," Axel suggests. Most odors consist of mixtures of odorant molecules, so other odors would be identified by different combinations.

Buck, who has been trying to solve the same problem at Harvard, recently found that the olfactory epithelium of mice is divided into regions that she calls expression zones, each of which contains a different set of odorant receptors. These zones are symmetrical on the two sides of the animals' nasal cavities (see p.52). "This suggests that there may be an initial broad organization of sensory information that occurs in the nose, even before the information is sent on to the brain," she declares.

Earlier researchers had traced the anatomical connections between neurons in the olfactory epithelium and the olfactory bulb, using radioactive labels. When Buck and her associates examined this older work recently, "we were amazed," she says, "because their patterns [of connections] looked just like our zones." Putting the two sets of findings together, she has produced a tentative map of the connections between expression zones in the olfactory epithelium of mice and certain parts of the olfactory bulb. She believes the initial organization of information about smells is maintained as it reaches the bulb. She has preliminary evidence that once the axons get to the bulb, they reassort themselves so that all those that express the same receptor converge at a specific site.

And so the first stages of olfaction are beginning to yield to researchers. But many mysteries remain.

One riddle is how we manage to remember smells despite the fact that each olfactory neuron in the epithelium only survives for about 60 days, and is then replaced by a new cell. "The olfactory neurons are the only neurons in the body that are continually replaced in adults," points out Randall Reed. Other neurons die without any successors, and it is thought that we lose increasing numbers of brain cells as we age. The olfactory neurons are far more exposed and vulnerable than other neurons, since they come into direct contact with the outside environment. But as they die, a layer of stem cells beneath them constantly generates new olfactory neurons to maintain a steady supply.

"Then how can we remember smells?" asks Buck. "How do we maintain perceptual fidelity when these neurons are constantly dying and being replaced, and new synapses are being formed? You'd have to recreate the same kind of connections between olfactory epithelium and bulb over and over again, throughout life, or you wouldn't be able to remember smells in the same way."

An even deeper mystery is what happens to information about smells after it has made its way from the olfactory epithelium to the olfactory bulb. How is it processed there, as well as in the olfactory cortex? How does it go into long-term memory? How does it reach the higher brain centers, in which information about smells is linked to behavior?

Some researchers believe that such questions can best be answered by studying the salamander, in which the nasal cavity is a flattened sac. "You can open it up more or less like a book" to examine how its olfactory neurons respond to odors, says John Kauer, a neuroscientist at Tufts Medical School and New England Medical Center in Boston, Massachusetts, who has been working on olfaction since the mid-1970s.

Salamanders will make it possible to analyze the entire olfactory system—from odorant receptors to cells in the olfactory bulb, to higher levels of the brain, and even to behavior, Kauer thinks. His research group has already trained salamanders to change their skin potential—the type of behavioral response that is measured in lie detector tests—whenever they perceive a particular odor. To study the entire system non-invasively, Kauer uses arrays of photodetectors that record from many sites at once. He applies special dyes that reveal voltage changes in the membranes of cells. Then he turns on a videocamera that provides an image of activity in many parts of the system.

"We think this optical recording will give us a global view of what all the components do when they operate together," says Kauer. He hopes that "maybe 10 years from now, or 20 years from now, we'll be able to make a very careful description of each step in the process."

This would be amazing progress for a sensory system that was relatively neglected until five years ago. Axel and Buck's discoveries have galvanized the study of olfaction, and scientists now flock to this field, aroused by the possibility of success, at last, in solving its mysteries.

"How can we remember smells… when these neurons are constantly dying and being replaced?"

A SECRE

In addition to our sense of smell, do we have the ability to sense certain chemical signals emitted by people around us—without being aware of it? Many other mammals use a separate set of sensory receptor cells in their nose to receive social and sexual information from members of their species, and there is growing suspicion that we do, too.

A whiff of airborne chemicals from a female mouse, for instance, may spur a male mouse to mate immediately. Certain chemical messages from other males may make him aggressive. Other messages may produce changes in his physiology—as well as in that of the responding female.

The effects of such messages would be far less obvious in humans. If we do receive chemical signals from people in our vicinity, these signals must compete with many other factors that influence our behavior. Yet our physiology may be just as responsive to chemical messages as that of other mammals. It is known that certain chemical messages from other mice lead to the onset of puberty in young males, while a different set of signals brings young female mice into estrus. Similarly, there are some suggestions that women may alter their hormonal cycles when exposed to chemical signals from other people.

In the past five years, scientists have become extremely interested in these signals, as well as in the "accessory olfactory system" that responds to them in many animals. This system starts with nerve cells in a pair of tiny, cigar-shaped sacs called the vomeronasal organs (VNOs), where the signals are first picked up.

"The VNO appears to be a much more primitive structure that uses a different set of molecular machinery than the main olfactory system," says Richard Axel, who recently became intrigued with this system. "It seems to work in a different way—and we don't know how."

The VNOs are located just behind the nostrils, in the nose's dividing wall (they take their name from the vomer bone, where the nasal septum meets the hard palate). In rodents, at least, signals travel from the VNO to the accessory olfactory bulb (rather than the main olfactory bulb) and then, as Sally Winans of the University of Michigan showed in 1970, to parts of the brain that control reproduction and maternal behavior.

"It's an alternate route to the brain," explains Rochelle Small, who runs the chemical senses program at the National Institute

T SENSE

BY MAYA PINES

IN THE HUMAN NOSE?

on Deafness and Other Communicative Disorders in Bethesda, Maryland. If the accessory olfactory system functions in humans as it does in rodents, bypassing the cerebral cortex, there is likely to be no conscious awareness of it at all.

This system is particularly important to animals that are inexperienced sexually. Experiments by Michael Meredith, a neuroscientist at Florida State University in Tallahassee, Charles Wysocki, of the Monell Chemical Senses Center in Philadelphia, and others have shown that the VNOs play a key role in triggering sexual behavior in naive hamsters, mice, and rats.

A virgin male hamster or mouse whose vomeronasal organs are removed generally will not mate with a receptive female, even if the male's main olfactory nerves are undamaged. Apparently, the VNOs are needed to start certain chains of behavior that are already programmed in the brain.

Losing the VNOs has a much less drastic effect on experienced animals, says Wysocki, who has been studying the VNOs for nearly 20 years. When male mice have begun to associate sexual activity with other cues from females, including smells, they become less dependent on the VNOs. Sexually experienced males whose VNOs are removed mate almost as frequently as intact males.

Do human beings have VNOs? In the early 1800s, L. Jacobson, a Danish physician, detected likely structures in a patient's nose, but he assumed they were non-sensory organs. Others thought that although VNOs exist in human embryos, they disappear during development or remain "vestigial"—imperfectly developed.

Recently, both VNOs and vomeronasal pits—tiny openings to the VNO in the nasal septum—have been found in nearly all patients examined by Bruce Jafek, an otolaryngologist at the University of Colorado at Denver, and David Moran, who is now at the University of Pennsylvania's Smell and Taste Center in Philadelphia. Last year Thomas and Marilyn Getchell of the University of Kentucky College of Medicine in Lexington and their colleagues found that the cells lining these organs have several molecular markers in common with the olfactory neurons that respond to odors.

"This has opened up the possibility of a new sensory system in humans," says Rochelle Small. "We were often told that the VNO does not exist in adults, so we have taken a big step just to show that the structure is there." She cautions that we still don't know whether this organ actually has connections to the brain, however. "The question now," she says, "is what its function might be."

Just what do the VNOs of rodents—or, perhaps, humans—respond to? Probably pheromones, a kind of chemical signal originally studied in insects. The first pheromone ever identified (in 1956) was a powerful sex attractant for silkworm moths. A team of German researchers worked 20 years to isolate it. After removing certain glands at the tip of the abdomen of 500,000 female moths, they extracted a curious compound. The minutest amount of it made male moths beat their wings madly in a "flutter dance." This clear sign that the males had sensed the attractant enabled the scientists to purify the pheromone. Step by step, they removed extraneous matter and sharply reduced the amount of attractant needed to provoke the flutter dance.

When at last they obtained a chemically pure pheromone, they named it "bombykol" for the silkworm moth, *Bombyx mori*, from which it was extracted. It signaled, "come to me!" from great distances. "It has been soberly calculated that if a single female moth were to release all the bombykol in her sac in a single spray, all at once, she could theoretically attract a trillion males in the instant," wrote Lewis Thomas in *The Lives of a Cell*.

In dealing with mammals, however, scientists faced an entirely different problem. Compared to insects, whose behavior is stereotyped and highly predictable, mammals are independent, ornery, complex creatures. Their behavior varies greatly, and its meaning is not always clear.

What scientists need is "a behavioral assay that is really specific, that leaves no doubt," explains Alan Singer of the Monell Chemical Senses Center. A few years ago, Singer and Foteos Macrides of the Worcester Foundation for Experimental Biology in Massachusetts did find an assay that worked with hamsters—but the experiment would be hard to repeat with larger mammals. It went as follows: First the researchers anesthetized a male golden hamster and placed it in a cage. Then they let a normal male hamster into the same cage. The normal hamster either ignored the anesthetized stranger or bit its ears and dragged it around the cage. Next the researchers repeated the procedure with an anesthetized male hamster on which they had rubbed some vaginal secretions from a female hamster. This time the normal male hamster's reaction was quite different: instead of rejecting the anesthetized male, the hamster tried to mate with it.

Eventually Singer isolated the protein that triggered this clear-cut response. "Aphrodisin," as the researchers called it, appears to be a carrier protein for a smaller molecule that is tightly bound to it and may be the real pheromone. The substance seems to work through the VNO, since male hamsters do not respond to it when their VNOs have been removed.

Many other substances have powerful effects on lower mammals, but the pheromones involved have not been precisely identified and it is not clear whether they activate the VNO or the main olfactory system, or both.

Humans are "the hardest of all" mammals to work with, Singer says. Yet some studies suggest that humans may also respond to some chemical signals from other people. In 1971, Martha McClintock, a researcher who is now at the University of Chicago (she was then at Harvard University), noted that college women who lived in the same dormitory and spent a lot of time together gradually developed closer menstrual cycles. Though the women's cycles were randomly scattered when they arrived, after a while their timing became more synchronized.

…some evidence of real, measurable sexual chemistry.

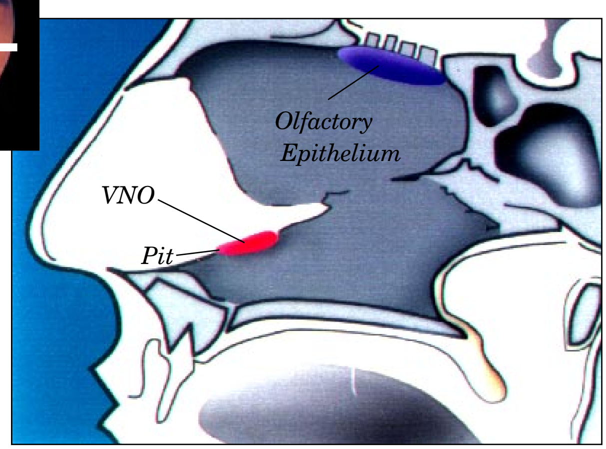
McClintock is now doing a new study of women's menstrual cycles, based on her findings from an experiment with rats. When she exposed a group of female rats—let's call them the "A" rats—to airborne "chemosignals" taken from various phases of other rats' estrous cycles, she discovered that one set of signals significantly shortened the A rats' cycles, while another set lengthened them. Now she wants to know whether the same is true for humans—whether there are two opposing pheromones that can either delay or advance women's cycles. In this study, she is focusing on the exact time of ovulation rather than on synchrony.

The opening of an adult woman's VNO is seen as a small pit (arrow) in the picture below, which was taken with an angled telescope. The VNOs are narrow sacs, only a few millimeters long. They lie on either side of the nasal septum, quite far from the olfactory epithelium.

Pit

The most direct scientific route to understanding pheromones and the VNO may, once again, be through genetics. Many researchers, including Axel, Buck, and Reed, are now racing to find the genes for the receptor proteins that actually bind to pheromones in the VNOs of rodents. These genes would lead them to the first receptors for pheromones ever identified in mammals—a prize tool for studying the mechanism and function of the VNO.

Once the genes for such receptors are identified, it should be easy to find out whether equivalent genes exist in humans. Scientists could then determine, once and for all, whether such genes are expressed in the human nose. If they are, the receptors may provide a new scientific clue to the mystery of attraction between men and women—some evidence of real, measurable sexual chemistry. ●



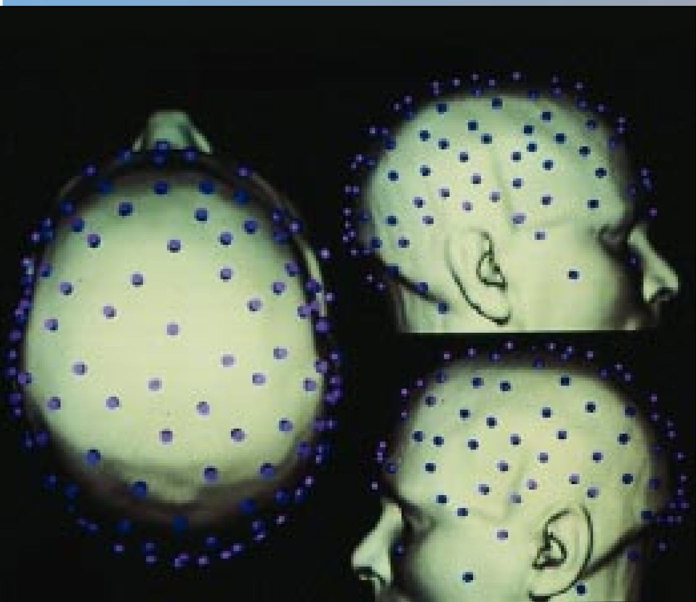
THE NEXT GENERATION

Messages from the senses travel so swiftly through the brain that imaging machines such as PET and fMRI cannot keep up with them. To track these messages in real time, scientists now use faster methods—electrical recording techniques such as MEG (magnetoencephalography) or EEG (electroencephalography). These techniques rely on large arrays of sensors or electrodes that are placed harmlessly on the scalp to record the firing of brain cells almost instantaneously. Their data are then combined with anatomical information obtained by structural MRI scans.

One of the first experiments in which structural MRI was used jointly with MEG produced a three-dimensional map of the areas of the brain that are activated by touching the five fingers of one hand (below). A New York University research team headed by Rodolfo Llinás found this map to be distorted in the brain of a patient who had two webbed fingers since birth. A few weeks after the man's fingers were separated by surgery, however, parts of his brain reorganized and the map became almost normal.

Laboratory of Rodolfo Llinas, New York University

Each of the color-coded areas in this combined MRI/MEG image of the brain responds to the touch of a different finger of the right hand.



EEG Systems Laboratory, San Francisco

In this high-tech version of EEG, the positions of 124 recording electrodes (attached to a soft helmet) are carefully plotted on an MRI model of the head.

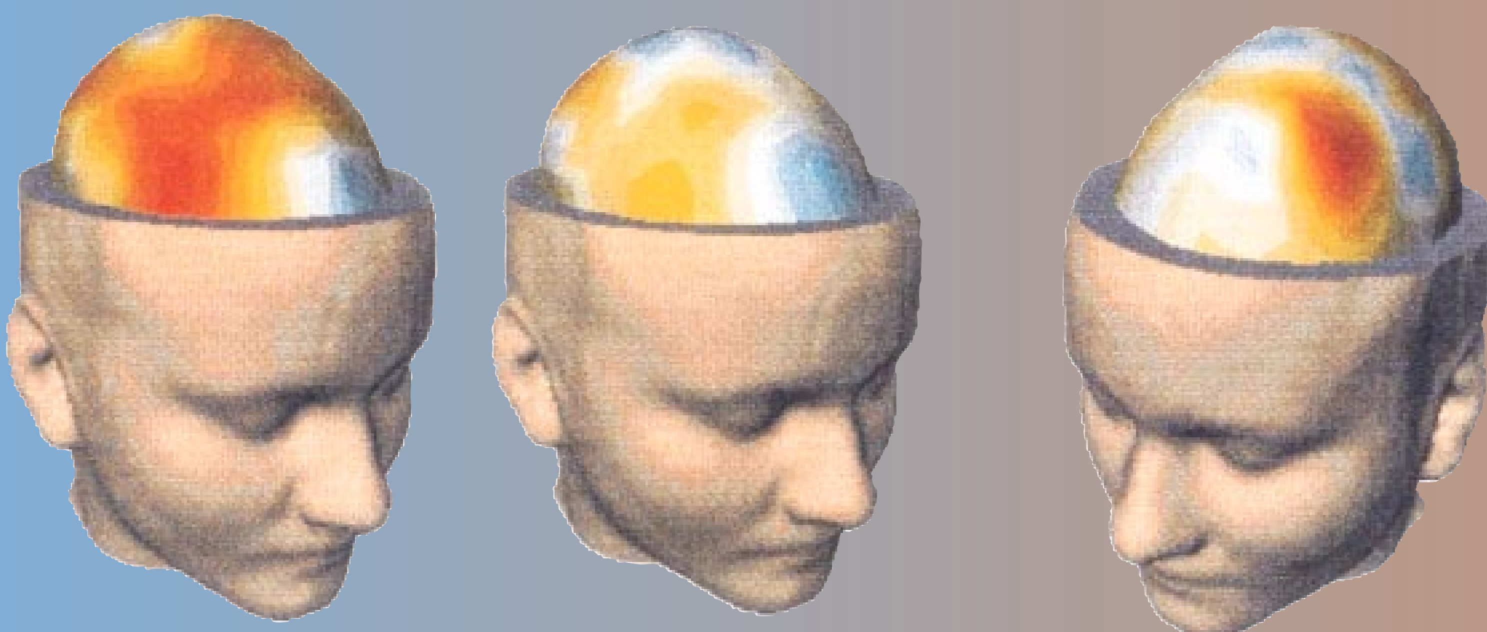
The rapidly-shifting patterns of activity in the six images below reflect what goes on in the brain of a woman who is looking at a letter on a screen during a test at the EEG Systems Laboratory, a private research center directed by Alan Gevins in San Francisco. The woman's task is to decide whether the letter is located in the same place as a letter she has seen before.

In the "low load" test she compares the new letter's location to a previous one. In the "high load" test she compares the new location to three previous ones, and the brighter colors reflect a higher degree of brain activation.

The images are based on data from 124 recording electrodes positioned in a soft helmet that covered the woman's head. The scientists used an MRI-derived model

MATCHING A LOCATION

EEG Systems Laboratory, San Francisco



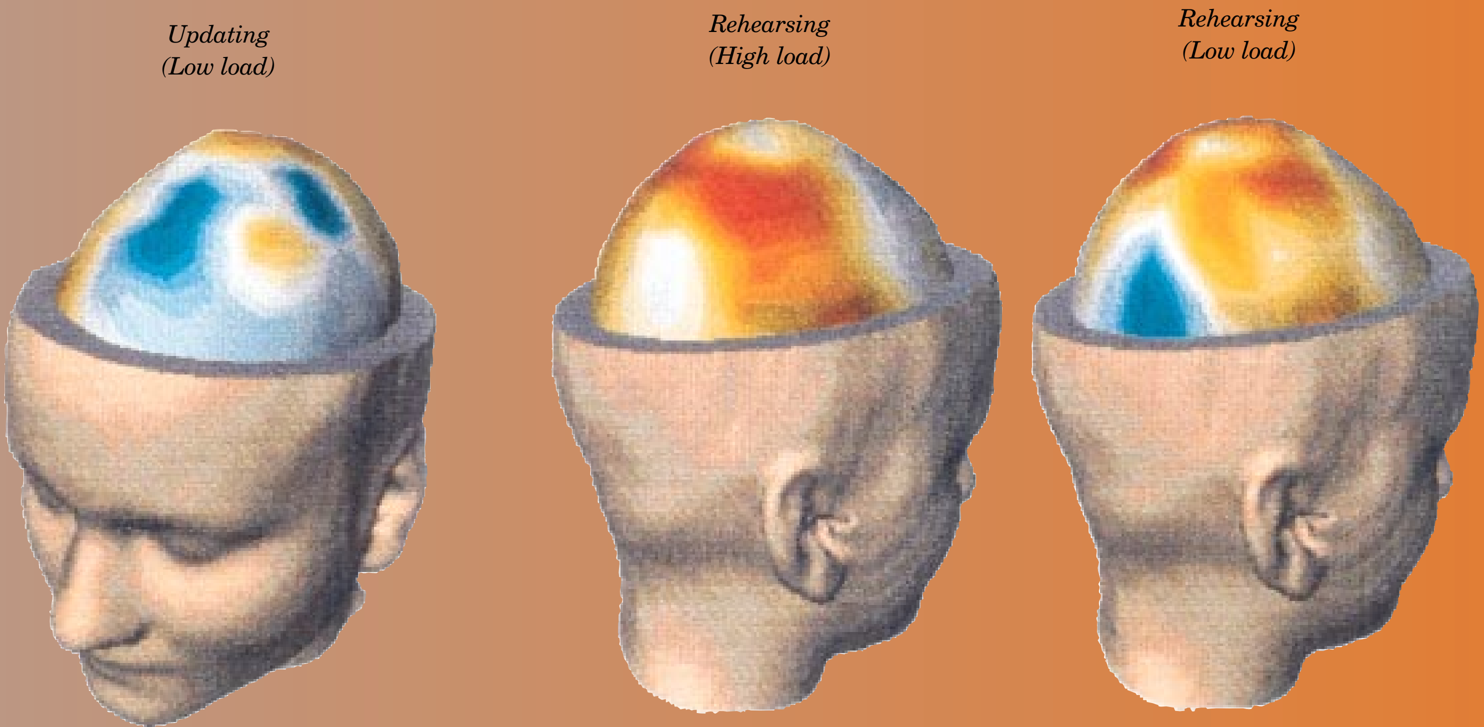
These computer-generated images recreate the electrical signals that flash across the brain of a volunteer during the matching test. A strong electrical signal (first image) sweeps across the frontal cortex of her right hemisphere 320 milliseconds after a new letter has appeared on the screen, as she compares the letter's location to three locations that she has seen before. The same areas of her brain are activated—but less intensively—in the second image, as she compares a new letter's location to only one location that she has seen before.

Only 140 milliseconds later, a different set of electrical signals is recorded from the volunteer's brain and recreated in these images. This time the frontal cortex of her left of her head to correct for any distortions in electrical transmission that might be caused by variations in the thickness of her skull.

The resulting images clearly show that various areas of the woman's brain are activated in turn. However, these images are limited to the brain's surface.

The next generation of imaging technology will use functional MRI in various combinations with MEG and EEG, predicts John Belliveau, director of cognitive neuroimaging at the Massachusetts General Hospital in Cambridge. Functional MRI shows activity deep in the brain with high spatial resolution, but is relatively slow since it is based on the blood-flow response, which takes about 450 milliseconds. "If you do a visual stimulation experiment, four to five different areas may have turned on within that time," Belliveau says. "We know where those areas are, but we don't know which one turned on first." By contrast, EEG's spatial resolution is relatively poor, but because of its speed it may reveal the sequence of events. His group has already done some EEG recordings right inside the magnet of an fMRI machine, to get simultaneous measurements.

Together, such techniques will offer scientists a glimpse of how information from the senses is processed in different parts of the brain. Building on the studies shown here, the new hybrids may then begin to tackle neural networks. They may help researchers examine how various parts of the brain exchange information and—most intriguing—how sensory information leads to thought. ●



hemisphere is activated as she enters the location of the new letter into her working memory. The signals are more intense in the high load than in the low load condition.

After the screen goes blank, the volunteer rehearses the new memory. As the next two images show, this activity produces yet another electrical signal over her right hemisphere. The signal is stronger in the high load than in the low load condition, but in both cases it is maintained until a new letter appears on the screen.

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