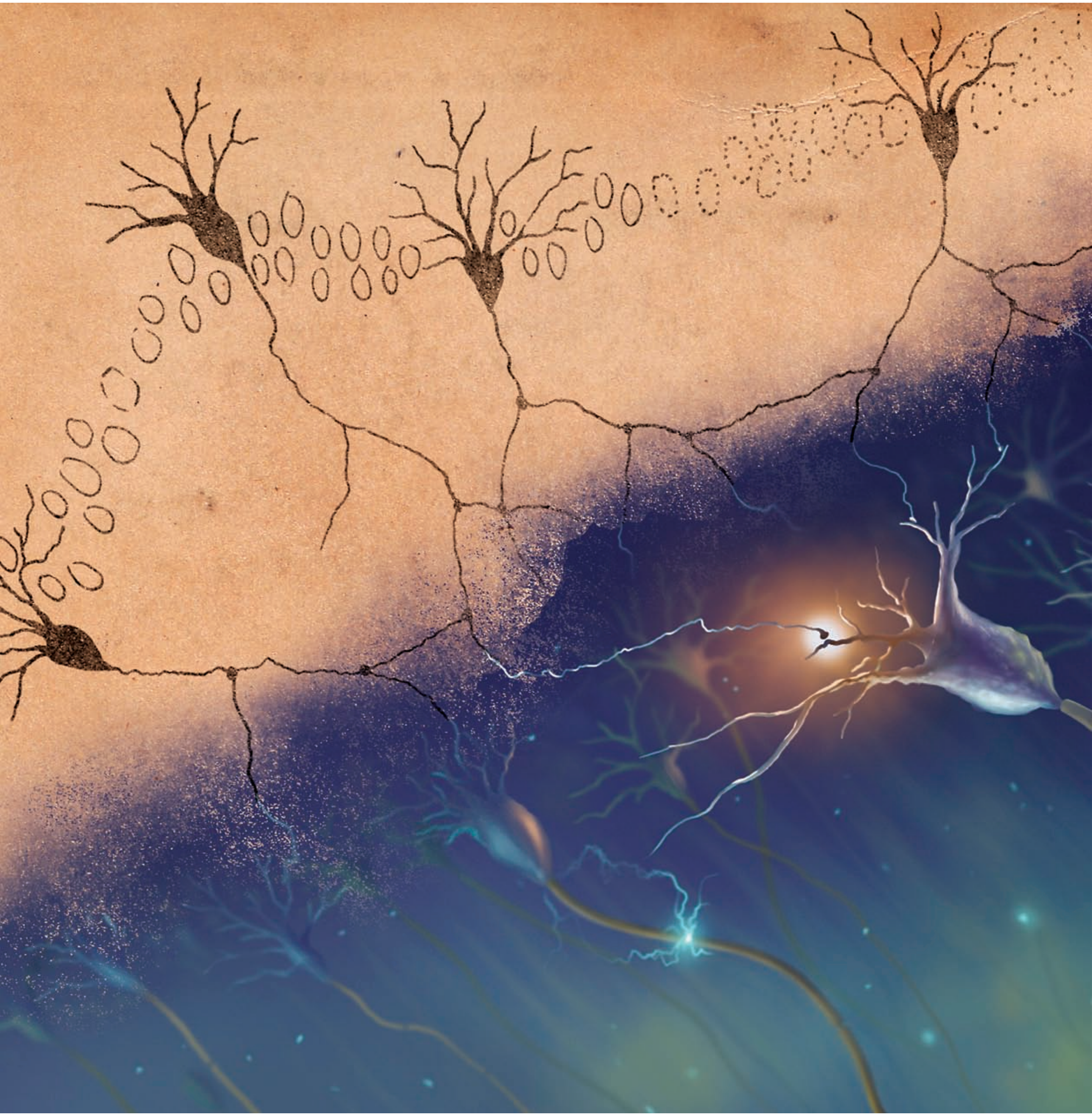
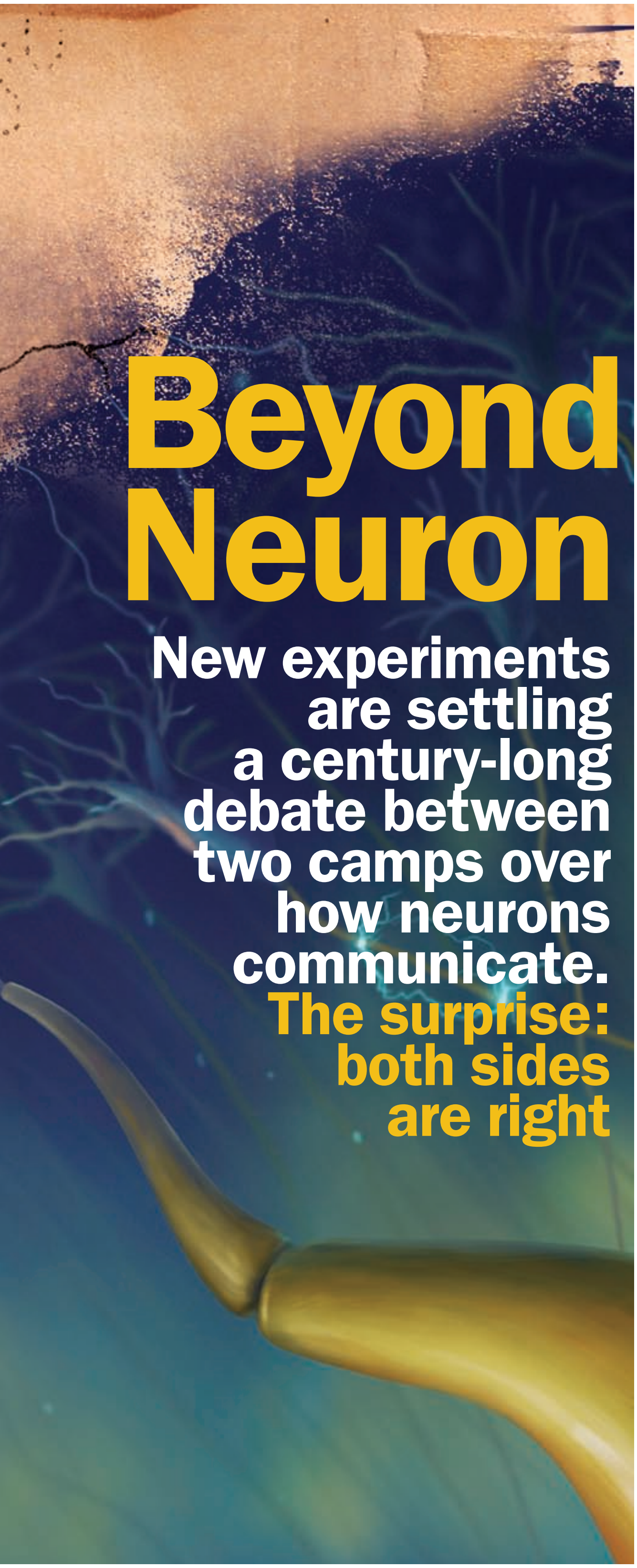




Beyond the Neuron Doctrine

New experiments are settling a century-long debate between two camps over how neurons communicate. The surprise: both sides are right

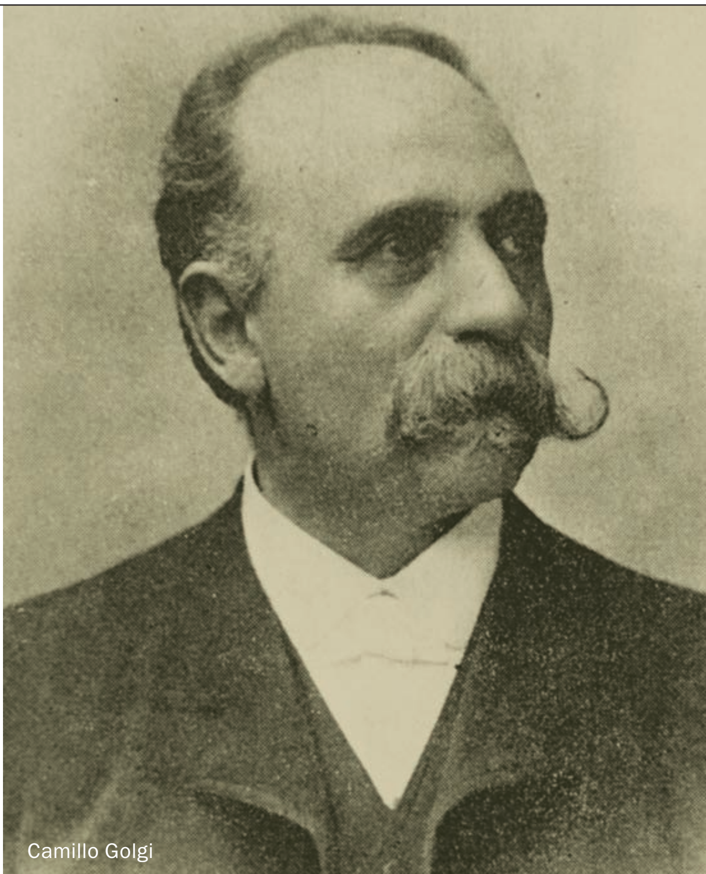
DON BLISS *NIH Medical Arts*, REPRINTED WITH PERMISSION FROM T. H. BULLOCK ET AL. IN *SCIENCE*, VOL. 310; NOVEMBER 4, 2005, © AAAS

I sliced the heart in two with a big kitchen knife. All was revealed—the four chambers separated by moist, gristly valves that suck blood into auricles and squeeze it out ventricles. Eleven years old and fascinated, I asked my mother if, next time, she could bring me a brain. When she returned from the butcher shop with a calf brain, I beamed and cleaved the melon in two. But inside I saw nothing notable. Just a hollow cavity at the core of a fleshy mush.

How did it work? Books offered names for its bumps and folds but failed to provide a detailed explanation for how this supreme organ functioned. My parents, teachers—no one seemed to have the answer.

Today we know the brain's power comes from components so miniaturized they are invisible. But even though technology now allows us to see individual neurons, our models of how they function en masse are still inadequate. We like to think of each cell as a microprocessor linked to billions of others. But how sure can we be that this analogy is accurate? Are we held captive by our analogies just as tightly as the scientists who preceded us were bound by their own now obsolete ideas?

By R. Douglas Fields



Camillo Golgi

Santiago Ramón y Cajal

The answer is yes. The discoveries are convincing neuroscientists that our fundamental concept of how the brain works is naive. Yet ironically, the two prevailing models, which have been at odds since their founders were jointly awarded the Nobel Prize 100 years ago, are both relevant. Indeed, by joining the models and adding a third, yet unanswered piece to the puzzle raised by recent research—how brain cells give rise to brain waves—we can finally explain not only how the human brain works but also what makes it unique in the animal world.

Networked or Isolated

Analogies are helpful because they make complicated situations more accessible. But such simplification also encourages rigid thinking. As the 20th century approached, anatomists probed the brain with the most powerful instrument available: the newly perfected microscope. They peered into an invisible world of baffling complexity, a densely tangled mass of microscopic, interconnected fibers. Anatomists naturally presumed that these tiny tubes, called axons, were like pipes, plumbed into a Byzantine network that allowed sensations and commands to flow freely to wherever they were required. The neuron was simply a node in the interconnected network.

One man looking at this world saw something different, however. The great Spanish anatomist Santiago Ramón y Cajal was at heart an artist. As a boy, he sketched cadavers dissected by his physician father before he, too, became a doctor. With an artist's ability to see motion in the curve of a line, Ramón y Cajal began to see a logic in the tangle of cells and pipes. His vision, hotly contested for the next 50 years, became known as the neuron doctrine.

Ramón y Cajal observed that a single, long axon running from one neuron tended to end in a field of dendrites—other, short tubes attached to another neuron. He maintained, however, that the tubes were not interconnected everywhere. In a brilliant deduction, Ramón y Cajal concluded that each neuron was an island unto itself, not a node in a network. Moreover, he surmised that information flowed in one direction: into dendrites, then through a neuron cell body, and out its axon.

Furthermore, the axon did not connect with the dendrites. It remained separated by a minuscule gap, or synapse. This gap functioned as a switch that allowed information to pass to the next neuron—or not. The space of separation was so small it was beyond the resolution of the best microscopes. Scientists would not get their

PHOTO RESEARCHERS, INC. (*left*); THE NOBEL FOUNDATION (*right*) first fuzzy glimpse of the synapse until the 1950s, when focused electron beams replaced light beams in microscopes.

In 1906 the Nobel Prize in Physiology or Medicine was awarded jointly to Ramón y Cajal and his rival, Italian physician Camillo Golgi. The unusual pairing sparked a standoff in modeling how the brain works that is only being settled today, on the award's centennial. Like many others, Golgi assailed the validity of the neuron doctrine and vigorously defended the free-flowing network view of the brain. The great irony was that Ramón y Cajal used an ingenious lab technique Golgi had invented to provide evidence for his neuron doctrine. Golgi had devised a way to stain nerve cells with silver nitrate, making their features visible against background tissue. For reasons that are still not understood, the Golgi method stains only a fraction of neurons in a sample, but the neurons that absorb the stain are revealed in exquisite detail. Ramón y Cajal's pen-and-ink drawings of Golgi-stained neurons were the basis of his theory. Golgi was backed into the uncomfortable predicament of arguing that his marvelous Nobel Prize–winning procedure was merely producing an artifact when it showed neurons as individual cells.

Welded Together

The debate between doctrinaires who supported Ramón y Cajal's neuron doctrine and reticularists (from the Latin for "network") who supported Golgi's scheme raged for decades because every new tool turned up evidence fueling both arguments. For example, electrophysiologists, using electrodes and electronic amplifiers to study the transmission of electrical signals from axon to dendrite, proved in fine detail that when an impulse reached the end of an axon, the axon released chemical substances called neurotransmitters. This event was followed by a delay of about 1/1,000 of a second, as the substances diffused across the tiny synapse and stimulated an electrical response in the neighboring dendrite. Yet in some cases, the recordings showed that an electrical signal swept from axon to dendrite with no delay at all, as if the two nerve cells were fused. No neurotransmitters were involved, and the connection appeared to be direct and electrical.

When the electron microscope finally revealed the synapse in 1955, scientists again were faced with evidence for both sides. There was no longer any doubt that neurons were stand-alone entities or that they communicated across the gap using chemical messengers. But some images showed individual neurons to be connected to one another, as though spot-welded. Researchers soon determined that protein channels, called gap junctions, formed these welds—like a short coupling that joins two hoses. Ions and organic molecules passed freely, allowing impulses to speed directly from one neuron to the next.

(Neurons can release neurotransmitters far away from synapses, an overlooked form of communication.)

Transmission of signals across "chemical" synapses—the basis for learning and memory—could be regulated by the release or uptake of neurotransmitters, so they drew most of the attention from neuroscientists. In contrast, "electrical" synapses appeared static, and their role in brain function was much less interesting. Electrical synapses seemed peculiar, relevant only when very rapid communication was necessary or when a bunch of neurons needed to be tethered to a group.

Yet recent work by neuroscientist Michael V. L. Bennett of Albert Einstein College of Medicine and others shows that simple view to be wrong: conduction through gap junctions can be regulated by changes in the voltage of cell membranes and by biochemical reactions that control the size of the channel through the junction. There are even cases where chemical and electrical synapses form together at the same junction. One thing is certain: Golgi was right. Neurons can be networked together.

A Changing Tide

Whether signals travel one way down a chain of neurons or back and forth across a network, using chemical or electrical messengers, even more fundamental questions remain: What do the signals mean? How do traveling impulses translate into a visual image, a feeling, a thought? What's the code? Neither model has provided answers, yet proponents have generated surprising insights that undermine the exclusivity of each theory.

One of the great discoveries made in examining the neuron doctrine is that neural impulses (called action potentials) carry information in one direction, from the cell body to the axon tip. Every morsel we taste, every idea we have, is described by a pattern of impulses firing through axons. Neuroscientists were eager to decipher this code, and they did. They found that the codebook changes constantly depending on the prior history of stimulation. The same frequency of impulses might signify very bright light when we are outside during the day and relatively dim light when we are inside at night. That is because the impulse code is concerned with reporting *changes* of state, rather than slavishly transcribing our every sensation. This phenomenon explains why when you pop your head through a fresh cotton T-shirt in the morning, you are flooded with sensations about the soft fabric, but soon afterward you are not aware of feeling the cloth at all.

Action potential coding explains a great deal, but it only goes so far. The same rules for impulses are used by animals down to the lowly earthworm. There must be more. American Theodore H. Bullock, one of the grand men of 20th-century neuroscience, fleshed out the code more than any other individual. The electrophysiologist and comparative neuroanatomist was interested in how information is coded in the nervous system in all types of animals, from snails to whales. He traveled from the Amazon rain forest to tidal pools everywhere with his electrodes and microscope. In 1959 Bullock published a paper in *Science* stating that in addition to high-speed nerve impulses firing through axons, many other electrical events were playing out in the background, deviating from the neuron doctrine. In particular, he observed slow surges and wanes in the voltage on nerve cell membranes. These potentials strongly influenced how many impulses an axon would fire in a burst and the probability that an axon would fire at all.

Moreover, a sharp impulse was needed only to transmit information over long distances. The slow voltage waves could easily spread in all directions across small, closely spaced neurons, and Bullock's electrophysiological records confirmed that many such neurons did not emit sharp, spiked impulses at all.

These small, tightly packed "interneurons" process information within internal circuits of the brain, rather than communicating directly with the body or environment as motor and sensory neurons do. Interneurons are concerned with the fundamental, internal workings of the brain rather than with transmitting commands or sensations, and the neuron doctrine did not fit well for many of these internal processors. Roughly 100 billion interneurons in the human brain control information processing in learning and memory and are implicated in diseases such as epilepsy and Parkinson's.

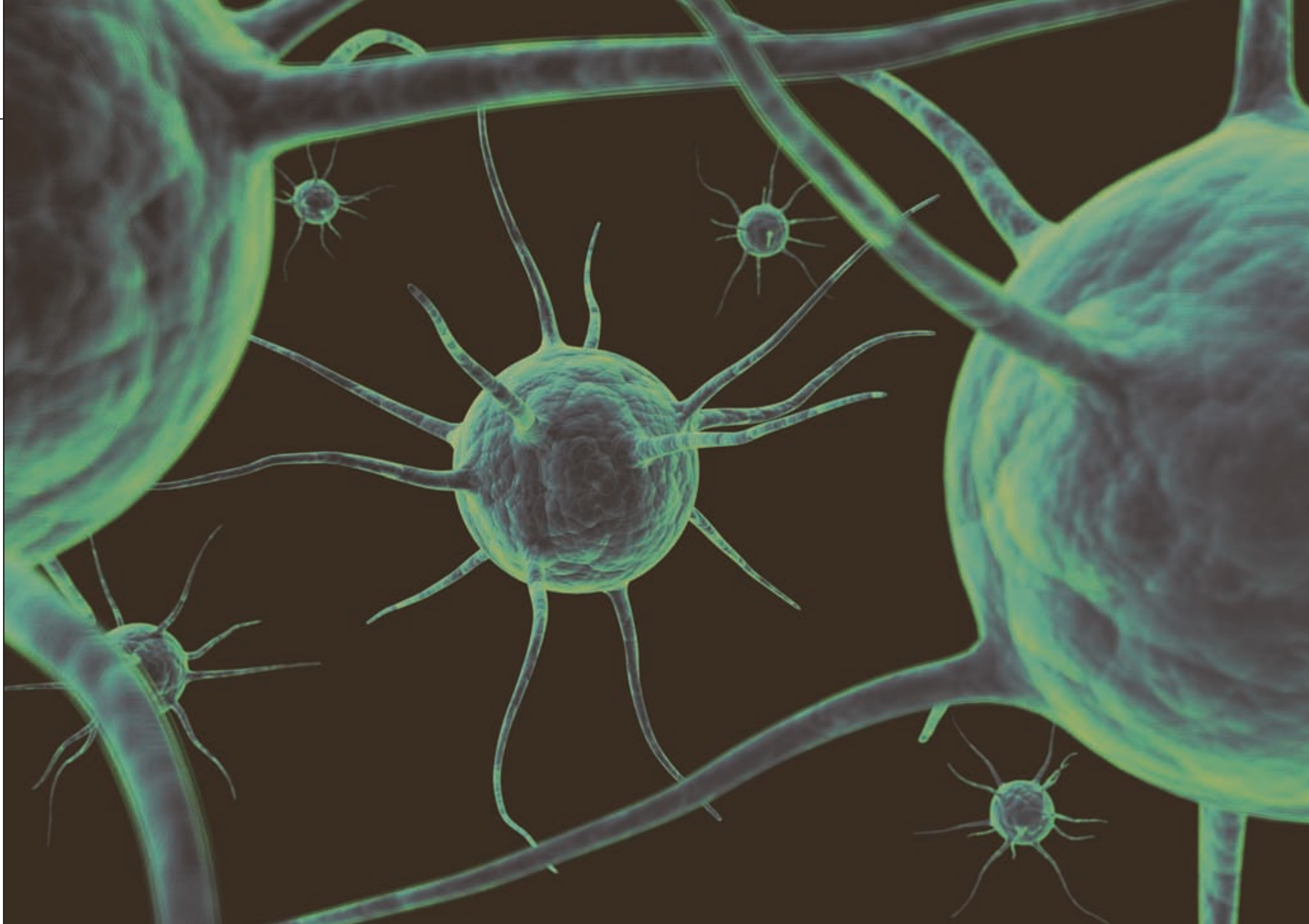
> (Glia **broadcast signals** across hardwired neurons, coupling them together into functional groups.)

Leaks and Backflow

As Bullock further defined the workings of interneurons, other researchers exposed additional shortcomings of the neuron doctrine. Neuroscientist Daniel Johnston of the University of Texas at Austin inserted microelectrodes inside dendrites in the rat hippocampus and found two events that would have surprised Ramón y Cajal. In some circumstances, action potentials traveled not only down the axon but also "backward" into the cell body and down the dendrites. Moreover, dendrites did not simply collect incoming signals; in some instances, they fired impulses of their own. We now know it is likely that dendritic processing is part of the mechanism for learning and memory. Dendrites are more than passive conductors; they integrate and transmit information.

A recent surprise is that dendrites can also release hormones and peptides that influence the slow voltage changes on neuronal membranes, which affect whether a neuron fires a single impulse or bursts of impulses. Eve Marder of Brandeis University has found that these neuromodulators work when applied to axons, the neuron cell body, or dendrites, scrambling the orderly one-way information flow Ramón y Cajal perceived. Neuromodulators can even cause neurons to fire in rhythmic burst patterns; this firing forces ensembles of neurons to work in synchrony, like musicians playing in tempo.

Even the synapse proved less simple to under-



ROBERT BROCKSMITH *Photo Researchers, Inc.*

stand than originally suspected. Synapses did not form just between an axon and surrounding dendrites. Refined electron microscopes showed that synapses often appeared on the cell body of a neuron, on its dendrites, and from axon to axon and dendrite to dendrite. Neurons, it seemed, might indeed be connected in multidirectional networks much the way Golgi and the reticularists had imagined.

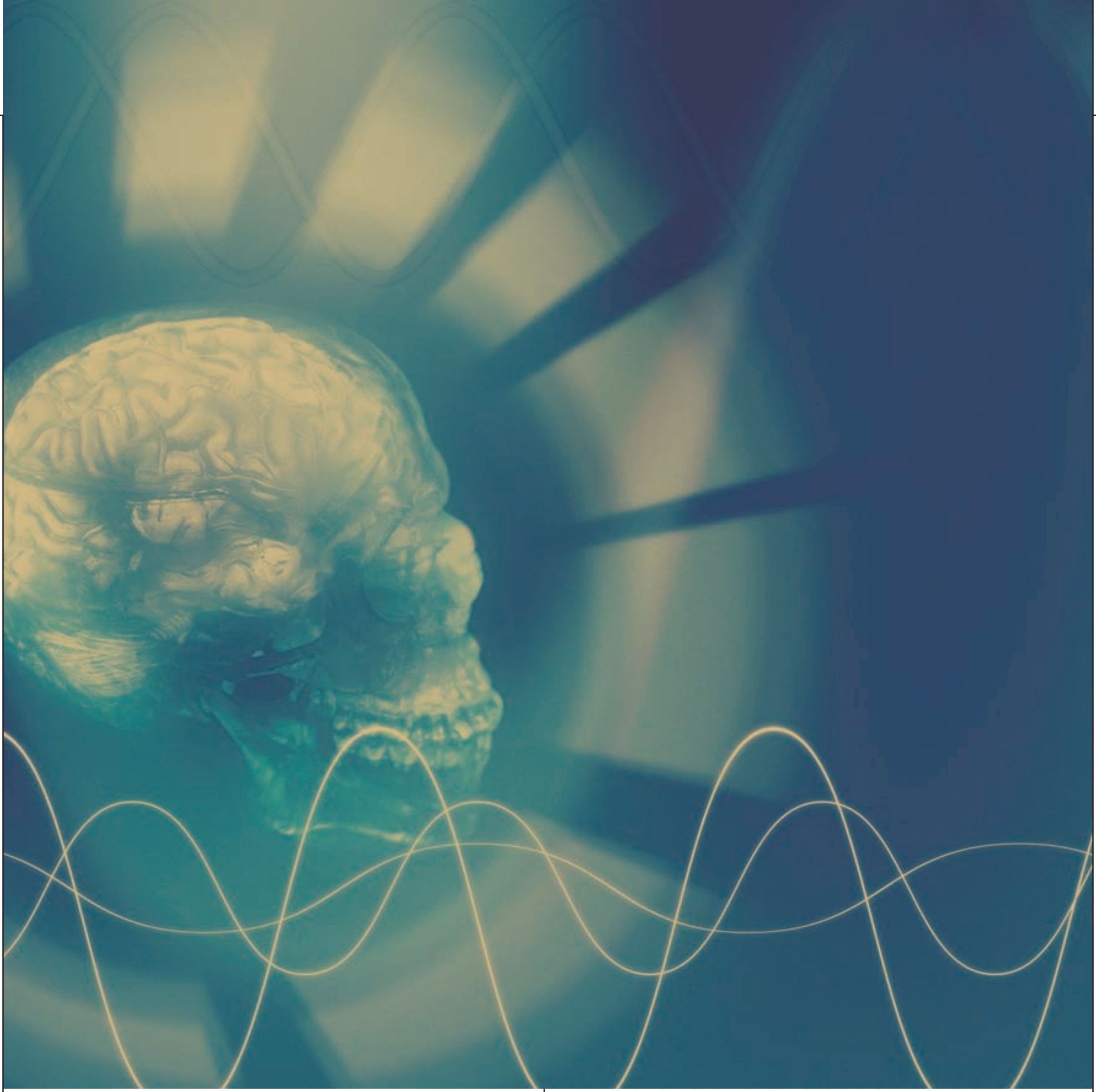
What is more, molecular neurobiologist Craig Jahr of the Vollum Institute at the Oregon Health & Science University recently proved that fast transmission using neurotransmitters can occur without any need for a synapse. At first, Jahr presumed that the neurotransmitters had seeped out of a nearby synapse, but his measurements indicated that neurons released the neurotransmitters through their cell membranes, far away from synapses. In 2005 computational neuroscientist Terrence J. Sejnowski of the Salk Institute for Biological Studies in La Jolla, Calif., and electron microscopist Mark H. Ellisman of the University of California, San Diego, concluded that this "ectopic" release of neurotransmitters outside synapses was an important and overlooked means of communication. If a neuron releases a single packet of neurotransmitters anywhere from its membrane, an adjacent neuron can detect it if it has neurotransmitter receptors in the vicinity. Today's best electron microscopes show neurons with thousands of these packets throughout their cell bodies. Suddenly, the model of how the brain processes information has become much more complicated.

The Glia Factor

Neuroscientists may be more willing to accept such heresy because of a startling expansion in thinking beyond the neuron doctrine in the early 1990s: most of the cells in the human brain are not neurons. Nearly 10 times as many cells, called glia, fill the space between neurons, and the ratio of glia to neurons increases in animals "higher" on the evolutionary tree. The very label "neuron doctrine" implies that neither Golgi nor Ramón y Cajal imagined that these cells had any information-processing function. For most of the 20th century, scientists believed glia provided only physical and nutritional support for neurons. But closer examination during the past decade has shown that glia have been listening in on conversations among neurons all along. Also astonishing has been the discovery that glia can communicate

(The Author)

R. DOUGLAS FIELDS is adjunct professor at the University of Maryland and wrote about the Dalai Lama's interest in neuroscience in the *Scientific American Mind* February/March issue. He dedicates this article to the memory of Theodore H. Bullock.



CHAD BAKER AND RYAN McVAY *Getty Images*

among themselves using chemical signaling (and no synapses are involved).

In addition, as glia eavesdrop they can control the flow of information among neurons. They perform this function by releasing or absorbing neurotransmitters or by controlling the concentration of ions surrounding neurons. Glia can also make and break connections between individual neurons.

Glia's habits violate the neuron doctrine in two ways. First, information flows through cells in the brain that are not neurons. Second, unlike neurons, which communicate through a series of links akin to telephone wires, glia communicate by broadcasting signals, the way cell phones do. Glia make shapeless connections that flow across the hardwired connections among neurons. In this way, glia can couple neurons together into functional groups. They communicate much more slowly than neurons do, but the speed may be adequate for many cognitive processes that do not require lightning-quick messages, such as the mechanisms that regulate mood and behavior.

To the neuron doctrine we now must add the glia doctrine: glia are equal partners in information processing. Glia intervene not only at synapses but also along axons by sensing impulses flowing through them. When axons fire bursts of action potentials, they release adenosine triphosphate (ATP) molecules, which are detected by receptors on all four types of glia. This information turns on and off genes in glia, affecting how they form layers of insulation around axons, which in turn affects how fast axons can conduct impulses. All of this communication moves along without synapses—a completely different channel of information flow in the brain.

Beyond Doctrine

Neuroscience has drifted well beyond the limits of the neuron doctrine. So where will this new course lead? In 2005, 46 years after his *Science* paper shot the first hole in the doctrine, Bullock raised an intriguing question in another article in the same magazine: Why are the capabilities of the human brain so superior to those of all other animals? The neurons in animals' brains are not all that different; even the fly exploits the same neurotransmitters. Careful anatomical study does not support the notion that bigger brains or more neurons are the answer either. Bullock (who passed away in December 2005 at age 90) suggested that the answer lay in some property that allows neurons to operate as a network. Golgi would be proud.

Bullock had begun to explore brain waves in a variety of animals as simple as crabs and as complex as dolphins. He determined that patterns of brain waves in humans differed markedly from those in simpler animals. Brain waves arise from the collective activity of thousands of neurons working together, much like the din of a crowd at a baseball stadium. When Bullock examined the power spectrum of brain waves, he saw that waves belonging to animals that appeared earlier on the evolutionary ladder tended to have more high-frequency components, whereas mammalian brain waves were shifted toward lower frequencies.

Work by Bullock and others also showed that the electrical activity in different groups of neurons is often coupled, even though the neurons are not physically connected. It is as though people in different parts of a stadium are carrying on a single, coherent conversation. This coherence of activity in brain waves increases in animals with more powerful brains. Perhaps, Bullock suggested, the unparalleled abilities of the human mind arise not as a unique property of our neurons or brain circuitry but as an emergent property of the way its billions of neurons operate cooperatively.

But how is activity in different neurons coordinated? Part of the answer may lie in a phenomenon we are all familiar with from listening to the radio. Sometimes frequencies from one radio station bleed over to the frequencies of another. Similarly, electrical signals transmitted through nearby axons are sometimes picked up as weak signals in adjacent axons. This unruly behavior, called ephaptic transmission, may be simply an unavoidable characteristic of electricity. And the brain may tap into it to coordinate brain waves. The voltages from the intruding electrical signals heighten the probability that neurons will fire at the same time.

John J. Greer and his colleagues at the University of Alberta in Edmonton reported this past February that when they bathed a fetal rat in a solution that stopped all synaptic transmission, neural circuits in its spinal cord and developing brain continued to fire rhythmically and in concert. Somehow, without any neurotransmitters in motion, neurons found a way to fire coherently. Using similar methods over the past 20 years, F. Edward Dudek, now at the University of Utah, has found that electrical coupling synchronizes impulse firing during brain seizures and that ephaptic transmission couples firing of neurons in the hippocampus, a part of the brain essential for memory. Ephaptic transmission, gap junctions, neuromodulators and glia are all ways of making neurons work together in groups. This cooperation increases coherent activity in the brain, and all these processes operate outside the neuron doctrine.

(The unparalleled abilities of the human mind arise not from neurons but from the coherence of **brain waves**.)

So both Golgi and Ramón y Cajal were right, yet neither they nor their followers succeeded in explaining the entire universe inside our heads. Furthermore, the point of the century-long debate between the doctrinaires and reticularists is not to crown a victor but to hone our thinking and inspire new experiments to explore one of nature's greatest mysteries: how the human mind functions.

The question for the future is: How are brain waves so well coordinated in the brain? To many neuroscientists, the answer lies just over the horizon, just beyond the concept of neurons acting as single functional units. Perhaps our present instruments are inadequate to provide the essential data. Or perhaps, recalling Ramón y Cajal, the answer is already here waiting for someone to see it. M

(Further Reading)

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- **The Other Half of the Brain.** R. Douglas Fields in *Scientific American,* Vol. 290, No. 4, pages 54–61; April 2004.
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