Learning how rats escape from cats also reveals how a storm of electrical pulses sweeping across the brain is translated into information

SEEKING the

By Miguel A. L. Nicolelis and Sidarta Ribeiro

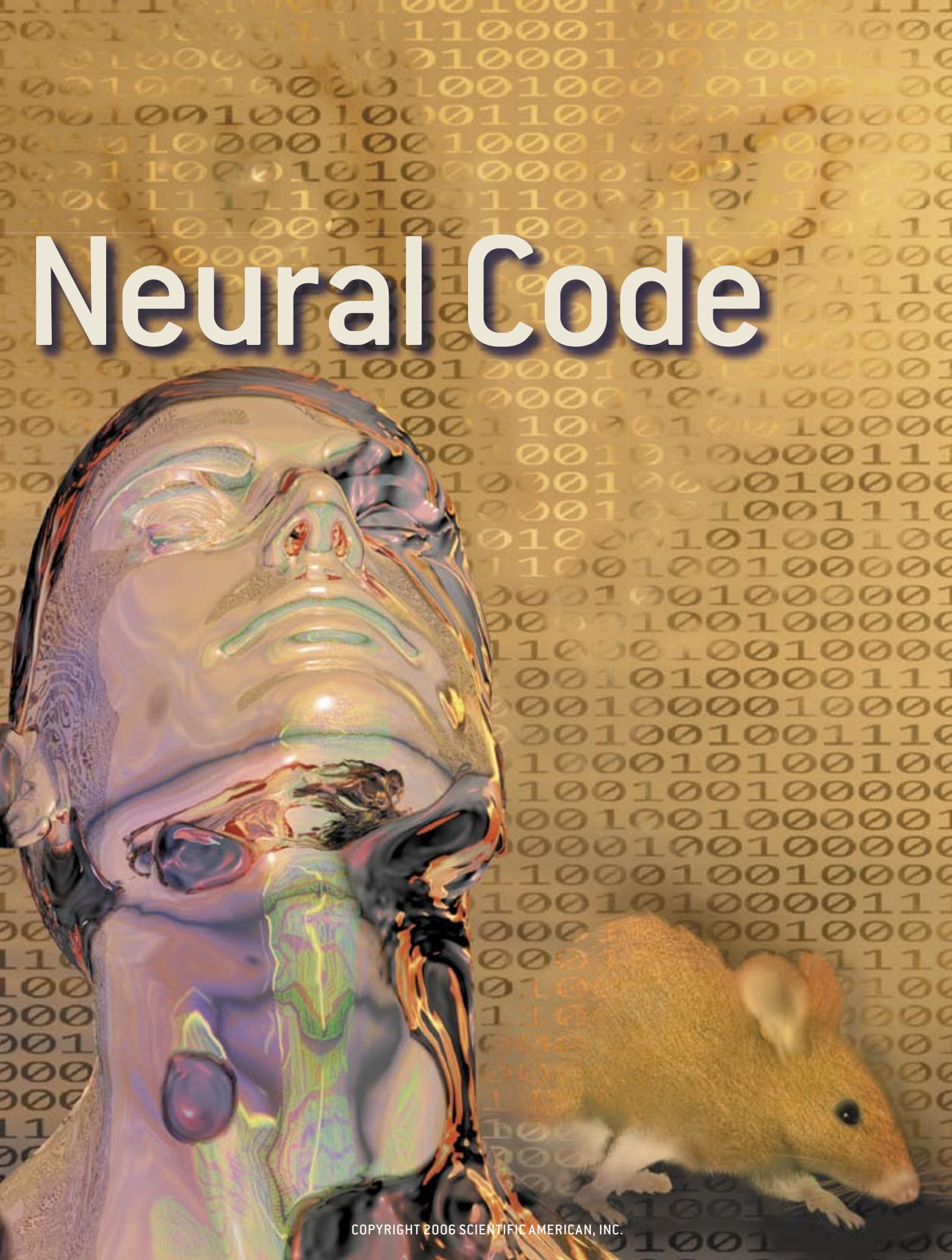
As the computer-controlled sliding doors suddenly opened, revealing a pitch-dark but already familiar chamber, Eshe did exactly what was expected of her after all those demanding weeks of training. Without hesitation—and most likely counting on the reward she was certain to receive given her superb performance of late—she lunged into the narrow room moving at full speed toward the opposite wall. She was ready to show off her skills.

The trial started the moment Eshe crossed an infrared light beam in front of an aperture positioned directly in her running path. The opening, flanked by the small arms of two T-shaped metal bars protruding from each side of the chamber, defined a slot through which Eshe had to pass to reach the opposite wall. Her job was far from trivial: in total darkness she had to estimate, in a single attempt, the aperture's diameter as quickly as possible. To make things more complicated and interesting, the opening's size varied randomly from trial to trial. Without being able to see the bars, Eshe had only one way to achieve her goal—she had to rely entirely on her exquisite sense of touch.

Amazingly, even when the aperture's diameter varied by only a couple of millimeters, Eshe could correctly discriminate in 90 percent of trials whether it was narrower or wider than before. And she solved this tactile riddle in barely 150 milliseconds by touching the edges of both bars with only the tips of the prominent long hairs that sprouted from both sides of her face. From a human perspective, Eshe's trick was no small feat. Anyone trying to solve a similar task by applying a mustache or beard to the same aperture would have failed miserably.

But Eshe was a rat, and the base of each of her whiskers contained a very high density of specialized peripheral sensory organs, known as mechanoreceptors, which translate the main

Neural Code



attributes of tactile stimuli into a language that the brain can understand: electricity. In rats, as in people, such electrical signals are conveyed by a multitude of peripheral nerves throughout the body into multiple interconnected brain structures, forming a vast neural circuit known as the somatosensory system, which accounts for our broad repertoire of tactile sensations. This same vast circuit also contributes to the genesis of our most intimate perceptual experience: our own sense of self.

Yet exactly how the brain translates a language of electrical pulses into such fine and varied perceptions has long been a profound puzzle and one of the holy grails of brain research. To crack this neural code is to open the doors to comprehending the essence of who we are. Our abilities to speak, love, hate and perceive the world around us, as well as our memories, our dreams, even our species history, emerge from the combination of a multitude of tiny electrical signals that spread across our brains, just like a thunderstorm sweeps the sky on a summer night.

Deceptively Straight Lines

WITHOUT KNOWING IT, Eshe had been participating in an experiment designed to address this very central question. That she decided to use her facial hair to solve her task was only proper. When rats really need to escape from cats, dashing through an opening of uncertain size located somewhere in the wall of a dark, unfamiliar place, whiskers offer their best hope to succeed.

A rat's mechanoreceptors translate any minute mechanical deflection of the whiskers into fast sequences of small electrical discharges, known as action potentials, to signal the location, intensity and duration of tactile stimuli. These pulses are transmitted to the brain via the trigeminal system, a nerve network that is the part of the somatosensory system specializing in conveying and processing tactile signals from the face. Understanding how Eshe and other rats can so readily compute an aperture's diameter in a mere fraction of a second, using only tactile information gathered by their whiskers, therefore rests on elucidating how vast populations of neurons distributed across the trigeminal system interact to process this incoming sensory information.

Researching this question, of course, reveals a lot more than simply how anxious rats elude hungry cats. Indeed, since the early 1970s neurophysiologists have studied the rodent trigeminal system to try to answer fundamental questions about the nature of neural coding. The work of our laboratory and many others around the world toward deciphering the code illustrates just how dramatically hypotheses have evolved since that time, as well as how much more we have yet to learn.

Three decades ago the theory favored by most neuroscientists was known as the labeled-line model because it proposed that sensory information generated at the body's periphery is conveyed through multiple parallel neural pathways all the way to the brain's neocortex. In essence, the message would travel through a strict feedforward circuit connecting peripheral sensory receptors, such as facial whiskers, to higher-order structures in the brain.

That paradigm received a significant boost during the 1970s, when Tom Woolsey and Hendrik Van der Loos, neuroanatomists at the Johns Hopkins University School of Medicine, revealed what appeared to be the trigeminal system's physical lines of communication within the primary somatosensory cortex (S1) of the mouse brain. As in other mammals, the mouse cortex can be divided into six layers based on each one's distinctive texture and distribution of nerve cell types and numbered I to VI from the outermost brain surface to the innermost cortical layer. By extracting blocks of tissue containing the whole S1 cortex of a mouse, Woolsey and Van der Loos were able to produce thin tangential slices spanning the entire cortical width and then stain those tissue sections for the presence of cytochrome oxidase (CO), a mitochondrial enzyme associated with intensive cellular activity.

To their surprise, Woolsey and Van der Loos found that cortical layer IV contained multiple distinct clusters of CO-rich neurons in a well-delineated arrangement of rows and columns. Thousands of tightly packed neurons made up each barrel-shaped cluster, prompting the researchers to call a single cluster a barrel and the entire matrix the "barrel field." Most astonishingly, this barrel field defined a beautiful, if slightly distorted, map of the mouse's snout.

A similar barrel-field arrangement was soon found in the rat cortex [*see box on opposite page*], and further studies revealed such topographic maps in subcortical structures, including the brain stem and thalamus, where the clusters were dubbed barrelets and barreloids. Indeed, stacks of these topographic maps at each of the subcortical relays of the trigeminal system were shown by subsequent investigators to link the peripheral sensory receptors in the facial whiskers of rats all the way up to the S1 cortex.

Sensory neurophysiologists use the term "receptive field" to define the amount of skin that when stimulated causes a neuron to respond by producing action potentials. In the case of the

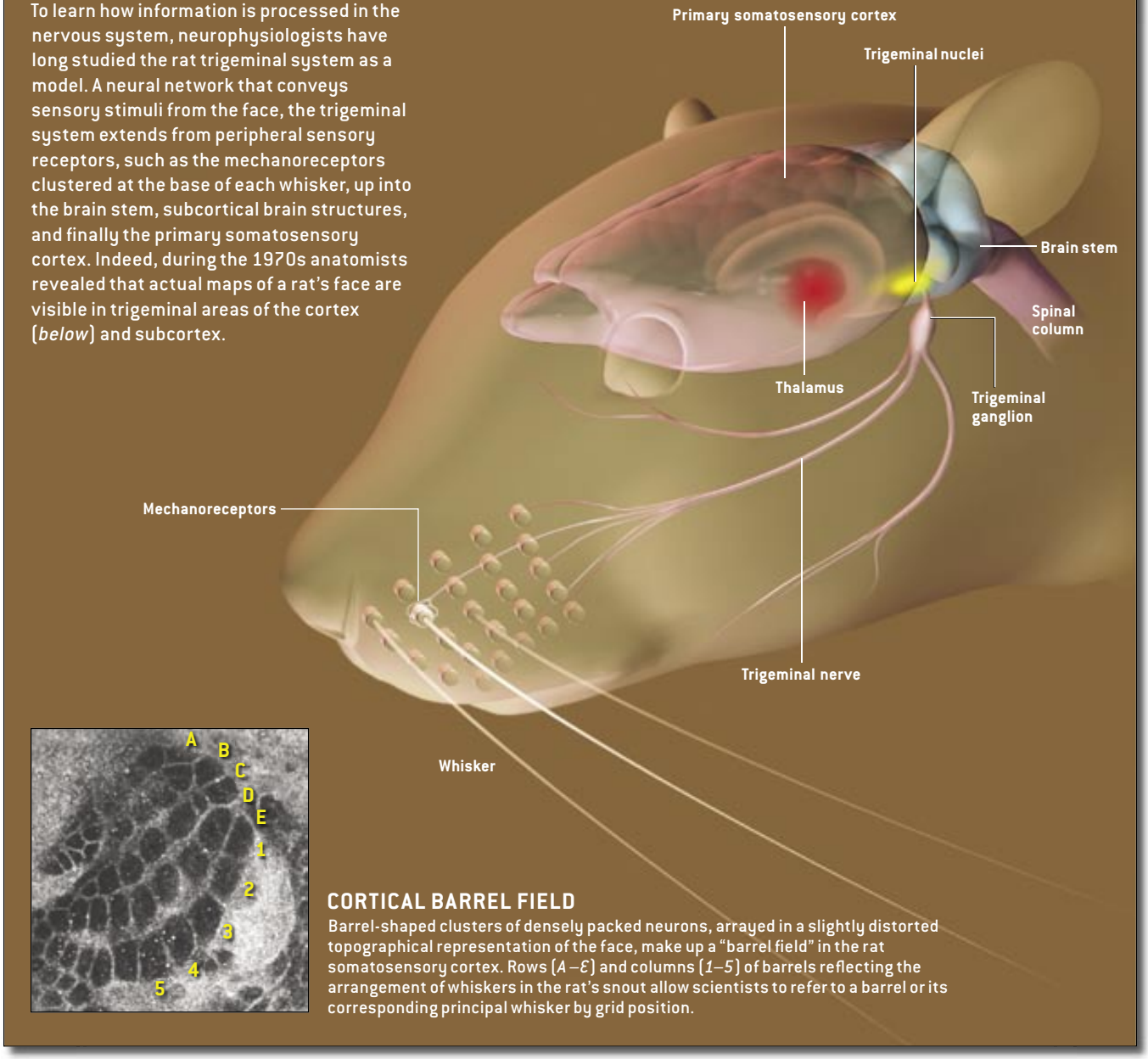
Overview/*An Emerging Code*

- Storms of electrical pulses sweeping through the central nervous system somehow translate into thoughts, emotions and sensations. Neuroscientists have spent decades trying decipher this neural language.
- Early hypotheses about sensory perception envisioned strictly linear transmission of signals along discrete neural routes between stimulus receptors and higher processing centers in the brain.
- Monitoring large populations of neurons in sensory pathways has revealed instead that information is encoded in the spatiotemporal activity patterns of entire neural ensembles.

PHIL SAUNDERS *Space Channel Ltd.* (*preceding pages*)

A SENSORY NETWORK

To learn how information is processed in the nervous system, neurophysiologists have long studied the rat trigeminal system as a model. A neural network that conveys sensory stimuli from the face, the trigeminal system extends from peripheral sensory receptors, such as the mechanoreceptors clustered at the base of each whisker, up into the brain stem, subcortical brain structures, and finally the primary somatosensory cortex. Indeed, during the 1970s anatomists revealed that actual maps of a rat's face are visible in trigeminal areas of the cortex (*below*) and subcortex.



CORTICAL BARREL FIELD

Barrel-shaped clusters of densely packed neurons, arrayed in a slightly distorted topographical representation of the face, make up a "barrel field" in the rat somatosensory cortex. Rows (*A*–*E*) and columns (*1*–*5*) of barrels reflecting the arrangement of whiskers in the rat's snout allow scientists to refer to a barrel or its corresponding principal whisker by grid position.

GARY CARLSON (*illustration*); COURTESY OF HERB KILLACKEY, FROM "ORGANIZATION OF CORTICOCORTICAL CONNECTIONS IN THE PARIETAL CORTEX OF THE RAT" IN *JOURNAL OF COMPARATIVE NEUROLOGY*, VOL. 181, NO. 3; 1978 (*micrograph*)

rodent somatosensory system, therefore, the most important prediction of the labeled-line model was that the receptive field, or spatial domain, of a single neuron located in one of these trigeminal barrels would be restricted to a single principal whisker.

By the late 1980s, however, contradictory results began to challenge this neat linear view. For instance, neurophysiologist Michael Armstrong-James, then at the University of London, recorded the activity of individual neurons located in multiple cortical barrels of anesthetized rats. Although he could identify the principal whisker of most of these cortical neurons, he also showed that an individual neuron was able to respond to deflection of whiskers surrounding that principal whisker.

In an almost heretical conclusion for the time, Armstrong-James suggested that the receptive fields of single neurons in the rat barrel cortex were not confined to single primary whiskers. Instead the neurons' spatial domains included a few surrounding whiskers, which, when deflected, drove neurons to produce weaker and slower—but still highly significant—tactile responses. This idea was enough to trigger a major controversy in the field, yet it was just the beginning of what would be a transformative decade for scientists' understanding of neural coding.

Distributed Computing

THE TECHNIQUE employed by Armstrong-James to record the activity of

single neurons, one at a time, in anesthetized rats was more or less state of the art in 1989 when one of us (Nicolelis) and John K. Chapin, now at the State University of New York Downstate Medical Center, decided to apply a new method for listening to the electrical activity of multiple individual neurons simultaneously.

We focused initially on neurons located in the barreloids of the ventral posterior medial (VPM) nucleus, a structure within the thalamus that is the main source of ascending nerve connections to the barrel fields of the primary somatosensory cortex. Our first studies showed that those VPM neurons exhibited very large, multiwhisker receptive fields. Much as Armstrong-James had found in the cortex, the VPM neurons' strongest and fastest responses resulted from deflection of each one's principal whisker, defining the center of its receptive field, while weaker and slower responses were triggered by stimulation of surrounding whiskers.

In fact, as rats became less and less anesthetized and finally fully awake, the size of individual VPM neurons' receptive fields increased significantly, sometimes including most of the facial whiskers on the same side of the rat's face. Moreover, because the VPM neurons responded with different latencies, or delays, to stimulation of different whiskers, the spatial domain of each neuron's receptive field shifted as a function of poststimulus time. In other words, we literally could not define the center and boundaries of a given neuron's receptive field *unless* we specified a particular moment in time.

This dynamic spatiotemporal aspect of the neurons' responses also allowed the cells to quickly reorganize their reactions immediately after any change in the flow of tactile information from the periphery. By simply anesthetizing small patches of skin in the rat's face, for example, we were able to see within a few seconds a complete reorganization of the receptive fields of VPM neurons to accommodate the new pattern of incoming tactile information.

We followed these findings with even more technically challenging experiments involving simultaneous monitoring of the activity of larger samples of individual neurons in multiple brain stem, thalamic and cortical relays of the rat trigeminal system. Our concurrent multisite, multielectrode recordings yielded simultaneous samples of up to 48 single neurons per animal, distributed across up to five different neural structures.

This was the first time such a comprehensive spatial sampling of an animal's sensory pathway had ever been performed. And the result was as clear as it was shocking: single whisker deflections in awake animals triggered complex

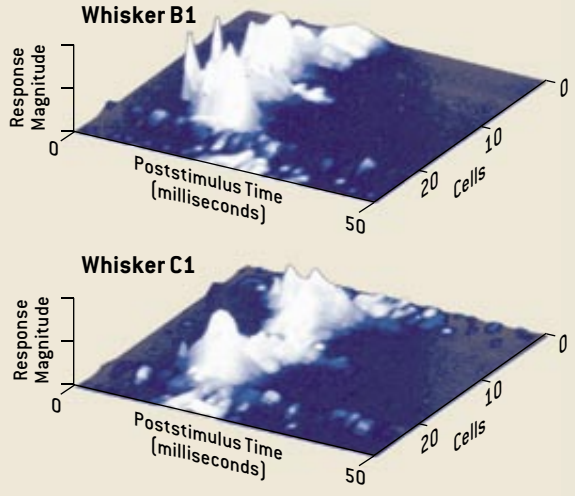
COURTESY OF *NEURON*, VOL. 18; APRIL 1997 (*left and center*); MELISSA THOMAS (*bottom right*)

CONVERGING SIGNALS

Stimulating individual whiskers on the face of a rat reveals a complex network of reactions distributed across populations of neurons and over time. Sensory information from a single whisker is thus encoded in the spatiotemporal pattern of responses by a multitude of cells throughout the animal's trigeminal system.

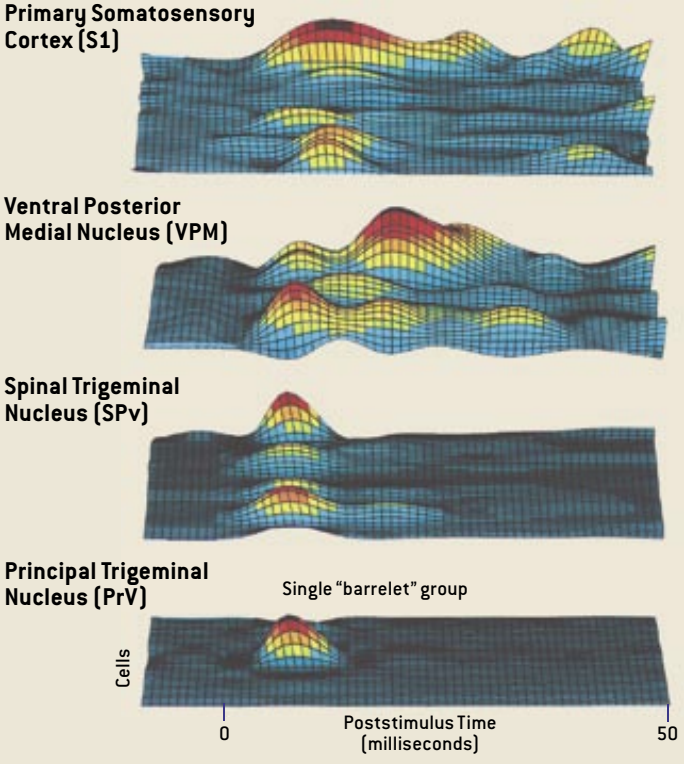
NEURON POPULATION RESPONSES

Instead of responding only to one principal whisker, 25 neurons in various cortical barrel columns react to the stimulation of different whiskers with distinct response profiles (*below*). Each row depicts a single cell's electrical activity after whisker stimulation.

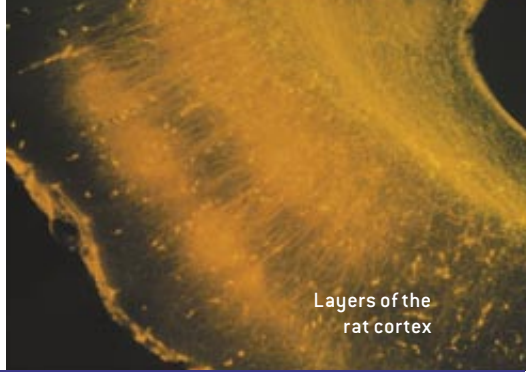


CELL GROUP RESPONSES

Stimulation of a single whisker produces waves of electrical activity in barrel-shaped cell clusters within the brain stem (SPv and PrV), thalamus (VPM) and cortex (S1).



Only by combining the activity of neuron populations would the brain extract meaningful information.


Layers of the rat cortex

MIGUEL A. L. NICOLELIS

waves of electrical activity that spread across multiple barrel-shaped clusters within each of the neural structures along the trigeminal system [*see box on opposite page*]. What we were observing was not at all consistent with information traveling along static, segregated, labeled lines. Instead our findings suggested an alternative model known as a distributed representation or a population neural code: only by combining the activity of large populations of single neurons would the rat brain be capable of extracting precise and meaningful tactile information about the animal's immediate surrounding environment.

To test this observation further, Asif Ghazanfar, a graduate student in our lab in the mid-1990s, attempted to "read" the coded messages sent by trigeminal neuron populations in a rat. He did this by feeding the activity of many cortical neurons, obtained during mechanical stimulation of multiple individual whiskers, to a series of artificial pattern-recognition algorithms known as artificial neural networks (ANNs). First Ghazanfar trained an algorithm to use the spatiotemporal firing patterns of entire populations of cortical neurons to correctly classify the location of single-whisker stimuli. Once the ANN reached a high level of accuracy, he introduced a new data set, then measured how well the algorithms could predict the location of a stimulated whisker. When the ANNs were fed the activity of single neurons in isolation, the accuracy of their predictions was extremely low. But when they had the combined responses of populations of individual neurons, the algorithms could easily predict the correct location of a whisker stimulus in a single trial.

By this time, other laboratories using a variety of methods were also obtaining data that supported our electrophysiological findings. And Ghazanfar, along with postdoctoral fellow David Krupa, went on to demonstrate for the first time that blocking neuron activity in the S1 cortex affected the responses of VPM neurons in the thalamus, suggesting that descending or feedback signals from the cortex to the VPM could also play a major role in modulating the ascending information from the brain stem. These and similar results together led our group to propose that the highly dynamic multiwhisker tactile responses seen in both S1 and VPM neurons were determined by a multitude of ascending, descending, lateral and modulatory signals that converge at each of these neurons at a different moment in time.

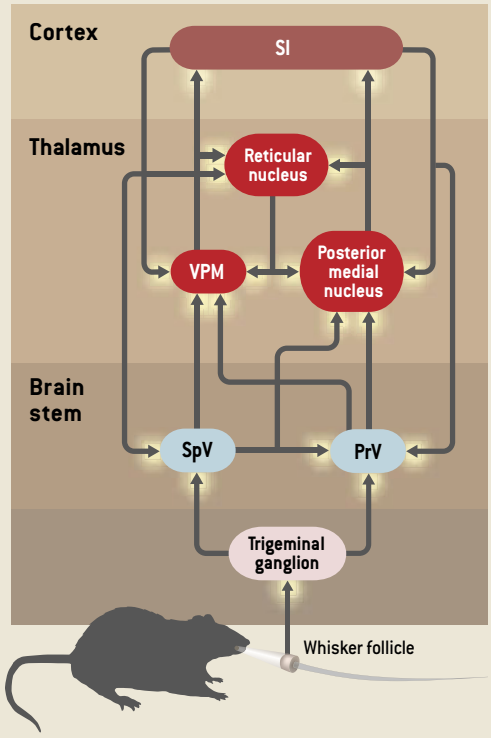
Our findings were already a far cry from the strict feedforward, labeled-line theory. But many predictions derived from our asynchronous convergence model still required extensive experimental testing, which led us into yet another decade-long journey of stimulating rat whiskers in a variety of ways that had never been tried before.

Context Counts

IN 1998 a graduate student in our laboratory, Erika Fanselow, designed a clever technique to measure how the S1 and VPM neurons would respond to similar tactile stimuli received under different conditions in freely moving rats. By im-

TRIGEMINAL SIGNAL PATHWAYS

Incoming tactile signals from a whisker are modulated by neural signals traveling along lateral and descending connections between brain structures.



THE AUTHORS

MIGUEL A. L. NICOLELIS and *SIDARTA RIBEIRO* investigated neural coding together when Ribeiro was a postdoctoral fellow in Nicolelis's laboratory at Duke University. As co-director of Duke's Center for Neuroengineering and Anne W. Deane Professor of Neuroscience, Nicolelis has pioneered the use of multielectrode brain implants to eavesdrop on the activity of large numbers of neurons and the development of computational methods to interpret and apply the results. Both Brazilian-born and avid soccer fans, Nicolelis and Ribeiro also share a passion for disseminating the benefits and resources of cutting-edge neuroscience. They are co-founders of the International Institute of Neuroscience of Natal in northeastern Brazil. Ribeiro is scientific director of the César Timo-Iaria Research and Education Center, a division of the institute, which ultimately plans to combine a world-class neuroscience research and training facility with a school, mental health and athletic facilities, a science museum and a conservation park to foster social and economic development in the remote region.

planting a tiny cuff electrode around the infraorbital nerve, the trigeminal nerve branch leading from the facial whiskers, Fanselow could deliver precise sequences of electrical pulses to the nerve while simultaneously measuring the responses of neurons in S1 and the VPM. She then measured how those neuronal responses varied during different behaviors exhibited by rats going about their daily routines. These experiments revealed that when rats were moving their whiskers, their cortical and thalamic neurons responded to tactile stimuli in a very different way than when the same animals were quietly awake or anesthetized.

In quiet rats, these neurons classically responded to stimulation with a brief sequence of action potentials, followed by a long-lasting period when their firing was inhibited by changes in their cell membranes. Fanselow found, however, that when the rats produced whisker movements of any kind, their cortical and thalamic neurons fired more steadily in response to a single electrical nerve pulse, without any periods of inhibition.

This observation prompted her to try delivering sequences of two electrical pulses to the nerve instead of just one, and the result was astounding. When rats were awake but immobile and not moving their whiskers, their cortical and thalamic neurons could respond only to the first stimulus of a pair; the second was masked by postexcitatory inhibition. But when rats were actively moving their whiskers, their S1 and VPM neurons could respond very well to both electrical pulses, even when separated by as little as 25 microseconds. Engaging in the whisking behavior clearly changed properties of the neurons, allowing both the cortex and the thalamus to faithfully represent a sequence of tactile stimuli.

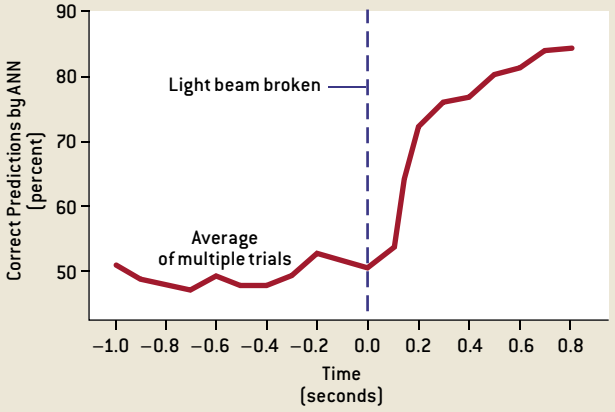
Around this time, Krupa was starting to succeed in training rats to perform the same task that Eshe would master so well a few years later. This method offered a new way to test whether neuron responses would also differ when the animal's active tactile discrimination task was more meaningful and demanding—more like real life—such as using its facial hair to judge the ever changing diameter of a hole.

His results confirmed and expanded on Fanselow's earlier observations: when animals actively used their whiskers to judge the diameter of the aperture, a large percentage of their S1 and VPM neurons exhibited intense, long-lasting responses without inhibition. Moreover, several neurons in the cortex clearly started to modulate their firing rates well before the rats' whiskers touched the edges of the bars, suggesting that the animals' behavioral state was already influencing properties of the neurons, priming them for the crucial task ahead.

As a final demonstration that these effects were also part of the encoded information feeding forward and backward within the animal's sensory system, Krupa fed the spatiotemporal firing patterns of neuron populations recorded during the execution of this task to an artificial neural network. With the combined activity of up to 50 cortical neurons, the ANN could predict with great

READING THE MIND OF A RAT

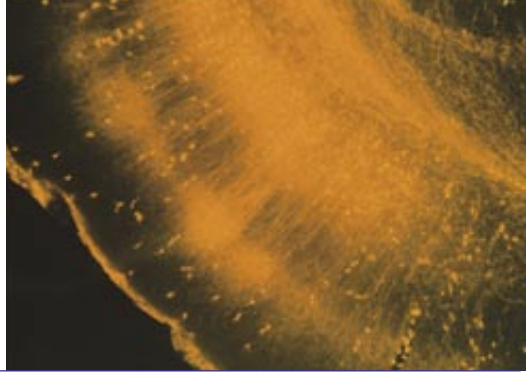
The ability to predict a rat's behavior demonstrates that a pattern-recognition algorithm can decipher sensory information encoded in the animal's neural activity. When fed recordings from the brains of rats participating in the experiment shown at the right, an artificial neural network (ANN) could determine whether an animal would correctly discern the width of an opening. As might be expected, the ANN performed (*graph*) at the level of chance before the rats broke a light beam at the entrance to the experimental chamber (zero seconds). After the animals began exploring the opening with their whiskers (0.1 to 0.25 second), the algorithm's prediction accuracy rose rapidly.



IN THE EXPERIMENT, a rat used its whiskers to feel an aperture formed by two movable bars flanking a nose poke. The animal then reported its judgment about the size of the opening by seeking a reward in an outer chamber at one of two stations it was trained to associate with "narrow" or "wide."

MELISSA THOMAS (*graph*); SOURCE: DAVID J. KRUPA ET AL. IN *SCIENCE*, VOL. 304, PAGES 1989–1992; JUNE 25, 2004 (*graph* and *photographs*)

How can our brains endow each of us with such a unique and irreproducible existence?



accuracy whether rats were going to correctly identify a wide versus a narrow aperture on any given try.

Dynamic Network

OUR ABILITY TO PREDICT the animal's behavior from neural firing patterns alone suggested that we were on the right track toward learning to interpret the language of the nervous system. It was already abundantly clear that instead of relying solely on the activity of specialized individual neurons or even linear columns of barrel-shaped modules, the mammalian brain more likely depends on highly distributed neural ensembles, dynamically formed by broadly tuned cells, to endow animals with their exquisite perceptual capabilities.

A single neuron's membership in those ensembles is probably fluid and might change from moment to moment, and one neuron can participate in many of these assemblies simultaneously. An individual cell's firing properties can also change continuously as a result of the state of the sensory periphery, the animal's past perceptual experiences, its internal brain dynamics, whether it is actively or passively sampling its environment, and the animal's expectations for the future.

We humans share with rats the same basic features of brain architecture, physiology and cell biology. And like them, we navigate our sensory environment aided by complex neural networks producing multiple representations of the surrounding world, shaping perception from moment to moment on a minute scale according to variations in attention, motivation and mood and taking into account our previous sensory experiences.

But how do all these by-products emerge from the tiny electrical discharges of billions of neurons? How can our brains make us all behave so similarly at times and yet endow each of us with such a unique and irreproducible existence? Most neuroscientists would agree that the intricate details of that puzzle will remain a profound mystery for some time.

Yet our research group's work toward deciphering the neural code has already allowed us to put our cursory understanding of this language to practical use by reading neural firing patterns from the motor cortex of a monkey and using computer algorithms to translate that information, in real time, into instructions for moving a robot arm [see "Controlling Robots with the Mind," by Miguel A. L. Nicolelis and John K. Chapin; SCIENTIFIC AMERICAN, October 2002]. Our hope is that one day soon we will also master sufficient syntax to talk back to the brain, which would allow us, for example, to build a human prosthetic arm laden with sensors to send tactile feedback into the somatosensory cortex of its user.

Although the neural code is far from cracked, we are able to catch, and to speak, a few syllables now, and that was not true just 10 years ago. One important reason that we can already use this idiom is its inherent adaptability, which in turn stems from the network properties of communication through neural ensembles. Even if a few words are dropped, the message still comes across, much the way a robust technological network can rapidly compensate for the loss of a few nodes.

Another crucial influence on progress in this field has been the evolution of basic experimental equipment. Decades ago neuroscientists were limited to recording lone neurons, using stiff metal electrodes that damaged brain tissue if moved too violently. As a result, investigators were also forced to study brain activity while an animal was anesthetized or at least sedated and restrained. As our own group's experience demonstrates, once scientists could listen to dozens of neurons in multiple brain structures simultaneously, a new population-based view of neural activity was possible. And new flexible electrode materials made permanent implantation of recording devices in the brain feasible, permitting us today to listen in on the activity of as many as 500 individual neurons, over long periods, in an awake animal engaging in normal behaviors.

It is perhaps no wonder that monitoring neurons one at a time encouraged a linear, neuron-centric view of neural communication. Those early methods could be compared to hearing only one voice during the performance of an opera—no matter how talented the soloist, one would still find it hard to follow the story. When combined into large and widely distributed neural ensembles, however, the collective interactions of these neurons yield exquisitely accurate descriptions of our surrounding environment. Thus, whenever a rat escapes another charging cat, its salvation is most likely thanks to a symphony of electrical pulses playing in its head. SA

MORE TO EXPLORE

Brain-Machine Interfaces to Restore Motor Function and Probe Neural Circuits. Miguel A. L. Nicolelis in *Nature Reviews Neuroscience,* Vol. 4, pages 417–422; May 2003.

Layer-Specific Somatosensory Cortical Activation during Active Tactile Discrimination. David J. Krupa et al. in *Science,* Vol. 304, pages 1989–1992; June 25, 2004.

Global Forebrain Dynamics Predict Rat Behavioral States and Their Transitions. Damien Gervasoni, Shih-Chieh Lin, Sidarta Ribeiro, Ernesto S. Soares, Janaina Pantoja and Miguel A. L. Nicolelis in *Journal of Neuroscience,* Vol. 24, No. 49, pages 11137–11147; December 8, 2004.

International Institute of Neuroscience of Natal: **www.natalneuroscience.com**

MIGUEL A. L. NICOLELIS