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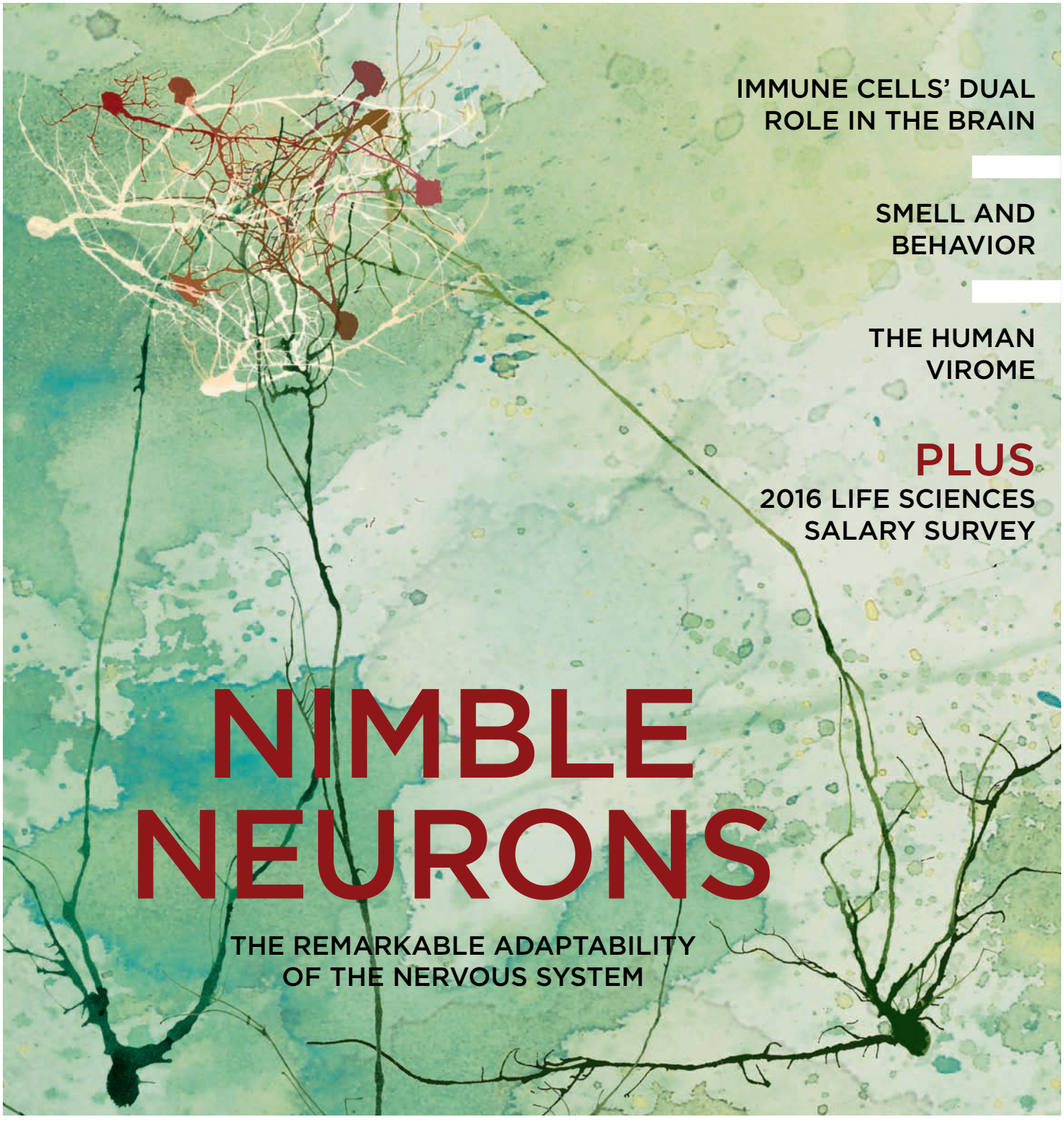

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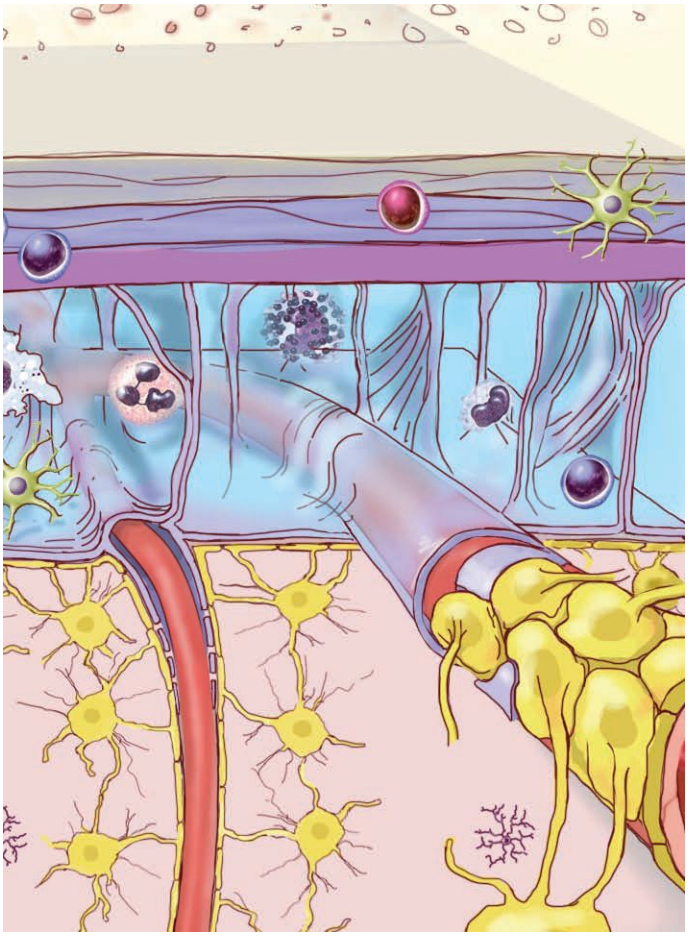

Features

ON THE COVER: GREG DUNN, *OLFACTORY BULB*, INK ON PAPER WITH DIGITAL MANIPULATION, 2010
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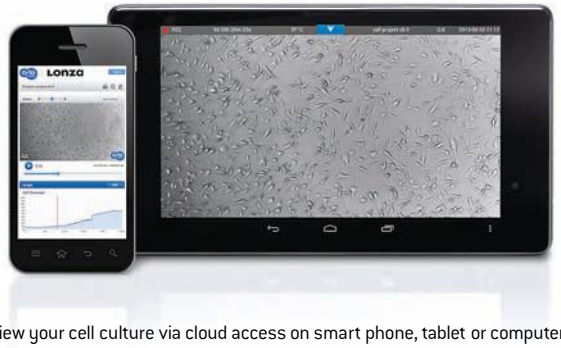

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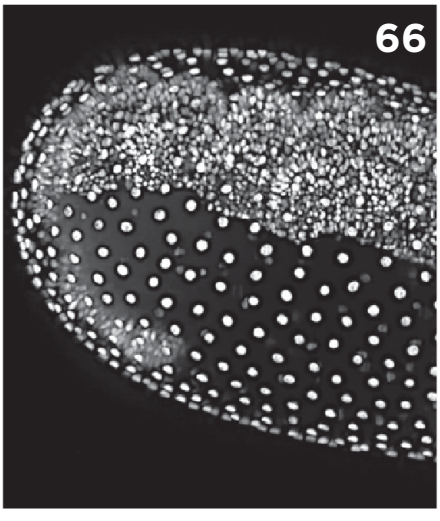
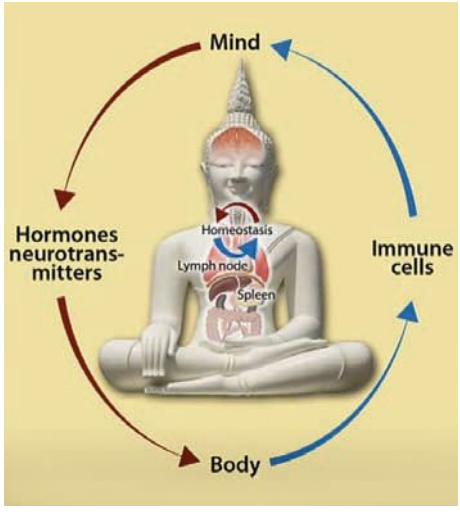


CORRECTION:
In "Pharma for Furry Friends" (*The Scientist*, October 2016), the Zoetis kinase inhibitor Apoquel for treating canine atopic dermatitis was not derived from the same JAK inhibitor used to treat human patients for rheumatoid arthritis.

The Scientist regrets the error.

Online Contents



THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Resurrecting Smells
Visit the Monell Chemical Senses Center in Philadelphia, where researchers are striving to find a cure for anosmia, the loss of a sense of smell.

VIDEO
Sealing the Deal
Irish researchers convert seals into remote oceanographic sensors by attaching tags containing temperature probes and other technologies to their heads.

VIDEO
From Body to Brain
Researcher Michal Schwartz discusses the promise of neuroimmunology to treat neurological disease.

AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

Coming in December

HERE'S WHAT YOU'LL FIND IN NEXT MONTH'S ISSUE:

- The nuclear pore: Structure and function
- The rise of the pangenome
- Annual Top 10 Innovations Awards
- Cell-fate mapping techniques
- RNA therapeutics

AND MUCH MORE

TheScientist TOP 10 INNOVATIONS

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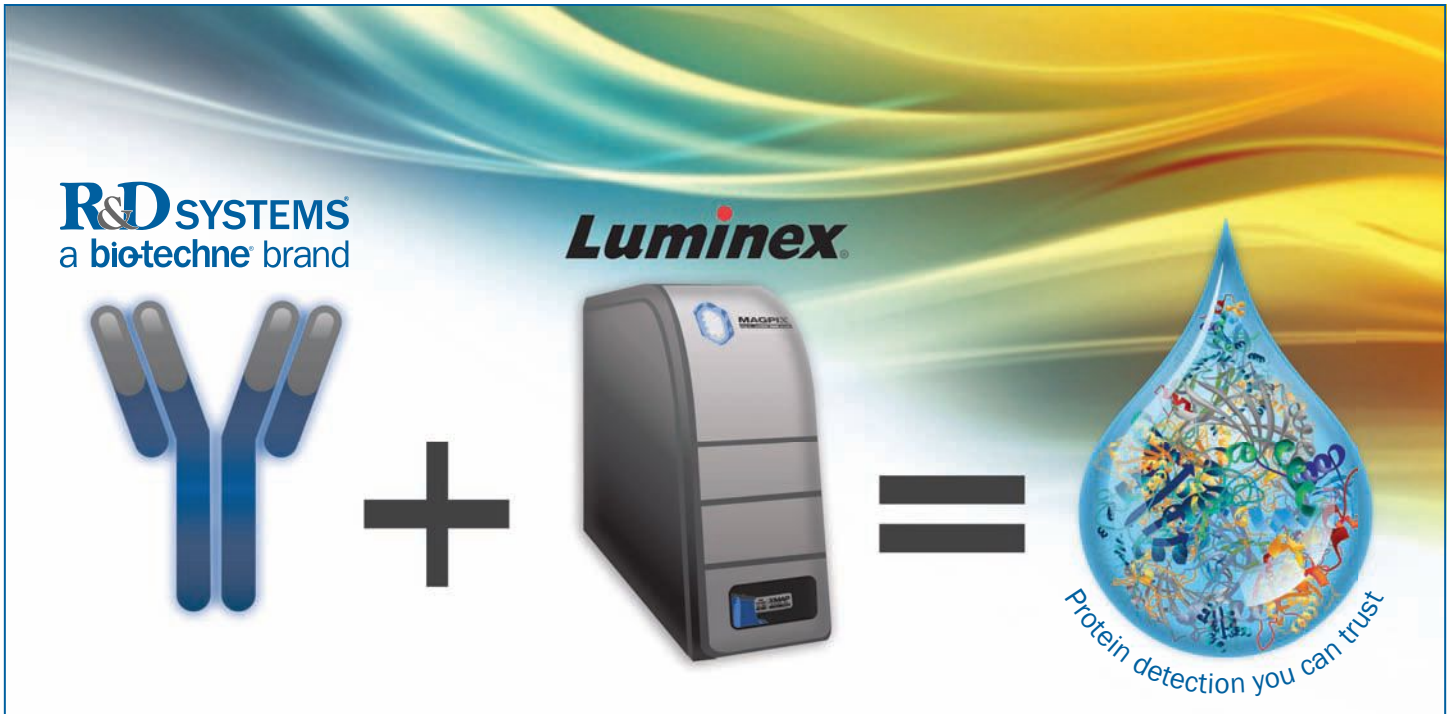

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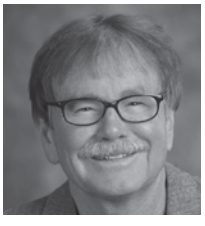
Contributors

Eric Delwart moved from Switzerland in 1982 to work at a biotech firm in San Francisco, an early epicenter of the AIDS epidemic. When he began a PhD in 1984 at the University of Wisconsin–Madison, HIV had just been identified as the retrovirus responsible for AIDS, and that drew him into the field of retrovirology. "It was very exciting," he says, to work on a subject so urgent, and "there was then a lot of optimism about an HIV vaccine." Delwart continued studying HIV during a postdoc at Stanford University and while a PI at the Aaron Diamond AIDS Research Center through the 1990s.

Delwart turned his attention to the field of emerging and unknown viruses—which far outnumber identified viruses—as a PI at the Blood Systems Research Institute, an affiliate of the University of California, San Francisco. Before modern genomic methods, "we didn't have the technology to see these viruses," Delwart says. "This whole universe is being revealed to us in the form of sequence information. What's lagging behind is our knowledge of what these viruses actually do."

Delwart delves into that universe in a feature article "The Good Viruses" (page 40).



In the early 1970s, **Paul Nunez**, armed with a master's and PhD in engineering and physics, was working as a theoretical physicist when he made a dramatic career change. He met Reginald Bickford, a pioneering neuroscientist at the University of California, San Diego, who at the time was looking for technically skilled scientists to help with electroencephalography (EEG) experiments. "I had always been interested in the brain," Nunez says, "but I didn't even know what a neuron was." Nevertheless, he dove into the field and spent the next 10 years as a postdoc under Bickford. The unexplained phenomenon of alpha rhythms, a type of brain wave now associated with vision and attentiveness, particularly interested Nunez, and his theories on the topic began to garner some recognition.

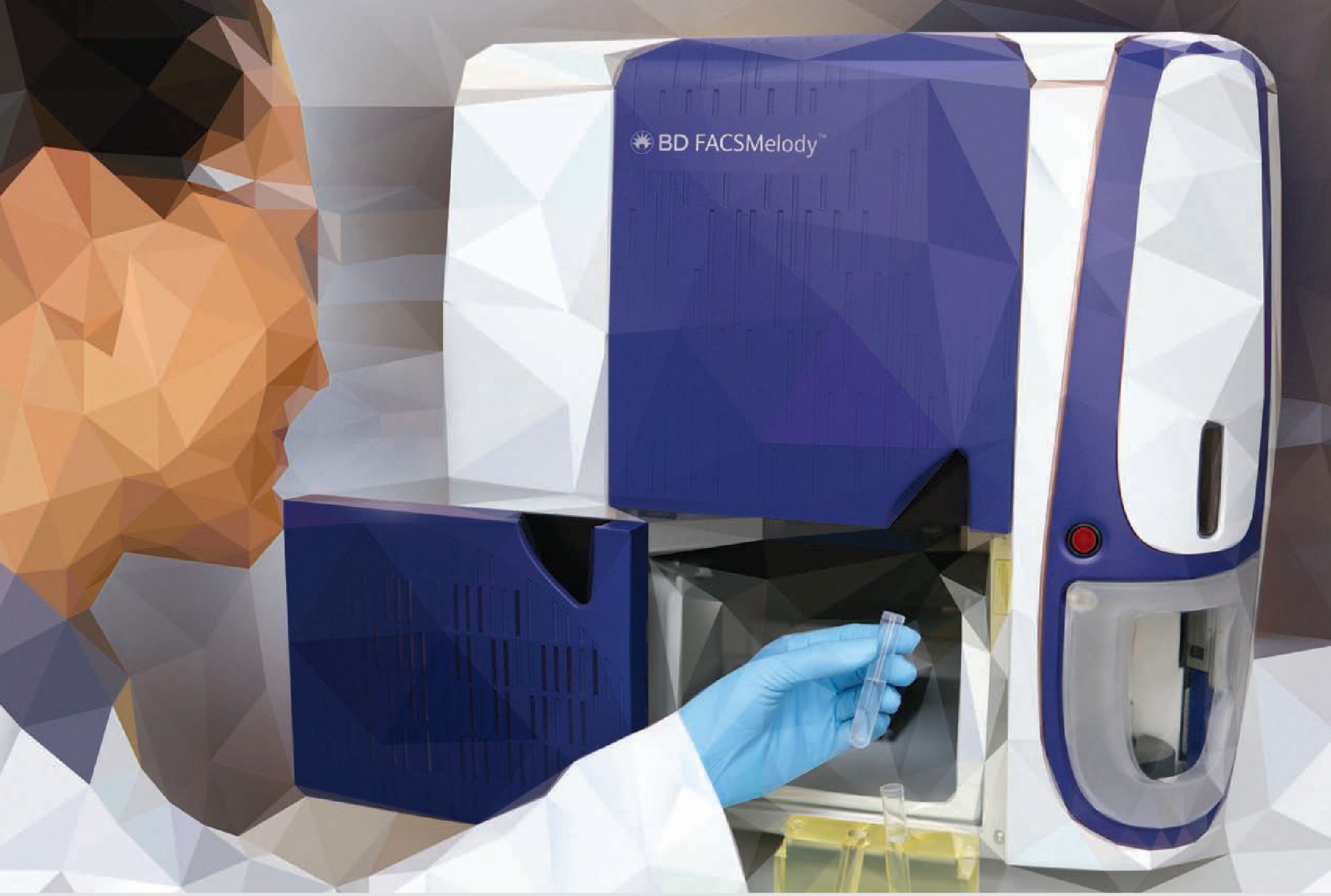
Nunez has authored more than 100 scientific papers and 5 books on the brain and complexity science, touching on medical applications such as epilepsy, coma, and pharmaceutical research. He is an emeritus professor at Tulane University and the owner of a small consulting firm, Cognitive Dissonance, LLC. His career has centered not just on neuroscience but also on the more elusive problem of how the brain gives rise to consciousness.

Nunez's most recent book, *The New Science of Consciousness: Exploring the Complexity of Brain, Mind, and Self*, examines this problem, which he discusses in an essay on page 73.

A self-proclaimed "super-nerd," **Ben Andrew Henry** always really liked science, but he also loved to read and write. Both in high school in Goffstown, New Hampshire, and at Middlebury College in Vermont, Henry split his time between science, English lit, and writing classes. In his junior year of college, he participated in a research program at Woods Hole Marine Biological Laboratory in Massachusetts, where he studied whether restoration projects to remove dykes and other barriers from Cape Cod salt marshes affected nutrient cycles. The Woods Hole scientists "basically just said, 'Alright, design a project. You have all these facilities, have at it. Let us know what you need.'" He also spent some time mapping plant communities in Vermont forests, and followed that up with a summer at the University of Maryland, where he worked with computer models that predicted the nitrogen cycle in Chesapeake Bay sediments.

While Henry found research fascinating, "I wanted to be writing more," he says. So back at Middlebury for his senior year, he designed an independent study in science journalism, for which he interviewed researchers visiting campus and wrote stories about their work. Then, during January break, Henry interned at *Popular Mechanics*, where he looked over the shoulders of the editors and writers in the newsroom. After graduating in May 2016, Henry traveled the country writing poetry before accepting the intern position at *The Scientist*. You can read his work on pages 23, 55, and 61 in this issue. As for what's next? "It's still up in the air," he says. "A part of me wants to be super-negligent and go for an MFA in creative writing, but I also might more realistically go for journalism. . . . That makes the most sense when I'm telling my story on a cover letter."

COURTESY OF ERIC DELWART; COURTESY OF PAUL NUNEZ; COURTESY OF BEN ANDREW HENRY



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Nosing Around

Covering neuroscience research means choosing from an embarrassment of riches.

BY MARY BETH ABERLIN

ANDRZEJ KRAUZE

Bananas. That's what I thought I was when I realized that I couldn't smell the fruit's unique odor. Nor could I smell the obviously soured milk in my refrigerator or the exhalations of smokers on the city sidewalks. Gourmet meals were now wasted on me, where once I had loved teasing apart their flavors. This affliction went on for months, long enough for me to seek medical help, which ruled out anything awful but offered no cure. Only much later, after a course of antibiotics taken for something else, did my sense of smell return.

So why am I telling you this? Every November, *The Scientist* focuses on neuroscience, and we pick out some new and exciting developments in the field to zero in on. In choosing this year's cover, we turned again to the artwork of Greg Dunn, a former neuroscientist whose stunning painting of pyramidal neurons graced our November 2013 cover. When looking through Dunn's portfolio, his print of cell connections in an olfactory bulb glomerulus hit a nerve, so to speak. It reminded me of my bout of anosmia (not to mention our October 2013 issue devoted to olfaction research).

For this month's issue, Notebook section editor Bob Grant had assigned two articles related to altered senses of smell. The first (page 17), by Senior Editor Kerry Grens, took me right back to my own period of odor disturbance. She describes a serendipitously discovered treatment for smell restoration that involves sniffing a collection of aromatic oils. After weeks of this therapy, the sense of smell reappears for some 30 percent of people. How it works remains unclear, but the potential for recovery likely relates to the constant regeneration of sensory neurons in the nose.

In another twist on olfactory neuronal plasticity, Senior Editor Jef Akst reports (page 20) on research showing that male mice continually exposed to the scent of female urine change their behavior toward females, seemingly as a result of losing a male-specific subset of neurons from their odor-processing organ. Coincidentally, those researchers monitored the changes in the neuron population using light-sheet microscopy, a technology that is the subject of one of this month's Lab Tools (page 66).

As always, neuroscience is a gold mine of interesting research. One intriguing component of neural health is the role of diverse immune cells. Contrary to the long-held belief that the central nervous system is sheltered from the body's immune system except in response to injury, researchers now recognize that T cells, macrophages, and other defenders reside in the brain and spinal cord, affecting not only pathogen defense, but also behavior, learning, and memory. Amanda Keener ("Unlikely Allies," page 32) reports on the good-cop role played by these immune cells and what happens when they go rogue.

Other neuro research covered in this issue includes a technique called MAP-seq, which uses mRNA barcoding to rapidly track single neuron pathways over long distances in the brain (page 31); a newly discovered neural network involved in the control of breathing (page 54); and findings about synaptic vesicles' ability to release their contents even in the absence of a structural network at the axon's end (page 55). There is also a profile of University of Pennsylvania neuroscientist Amita Sehgal, who studies the genes that regulate circadian and sleep rhythms (page 56); a Scientist to Watch article (page 61) about olfactory researcher Jason Castro of Bates College; and a Foundations description of the invention of two-photon microscopy (page 80).

I look forward to nosing around the annual Society for Neuroscience meeting later this month. With a cast of tens of thousands and mountains of new research being unveiled, the conference is always an exciting way to discover what's grabbing neuroscientists' attention (and a sneak peek at what you'll be reading in future issues of *The Scientist*). ■

Editor-in-Chief
eic@the-scientist.com

Speaking of Science

Eventually, it becomes hard to take the selections seriously, because we have no idea what factors are taken into consideration, except that somehow, it ends with only white and Asian men receiving the prize.

—**Chanda Prescod-Weinstein,** a theoretical astrophysicist at the University of Washington in Seattle, on the lack of diversity among science Nobel laureates (*LiveScience*, October 5)

As wonderful as it is to advance physics, chemistry, physiology or medicine, it's at least as wonderful to tackle environmental problems, predict natural disasters, demystify how species interact, and educate a population to grapple with complex and important scientific topics. The Nobel organization should take a bold leap into the present and shine its bright light more widely—and unshackle itself from a 19th-century vision of what makes good science.

—**Gabriel Popkin,** science and environmental writer, in a *New York Times* op-ed on the need to expand the categories for which the Nobel Prize is awarded (October 3)

The support (from my cash prize) would last only five years at most. It is important to put in place a permanent system that assists young researchers.

—Cell biologist **Yoshinori Ohsumi** of the Tokyo Institute of Technology, on his decision to use his $930,000 Nobel Prize winnings to set up a fund for scholarships and research grants for young researchers (*The Asahi Shimbun*, October 4)

I am very disturbed by the talk coming out of the U.K. at the moment. Anything that stops the free movement of people is a big negative for science.

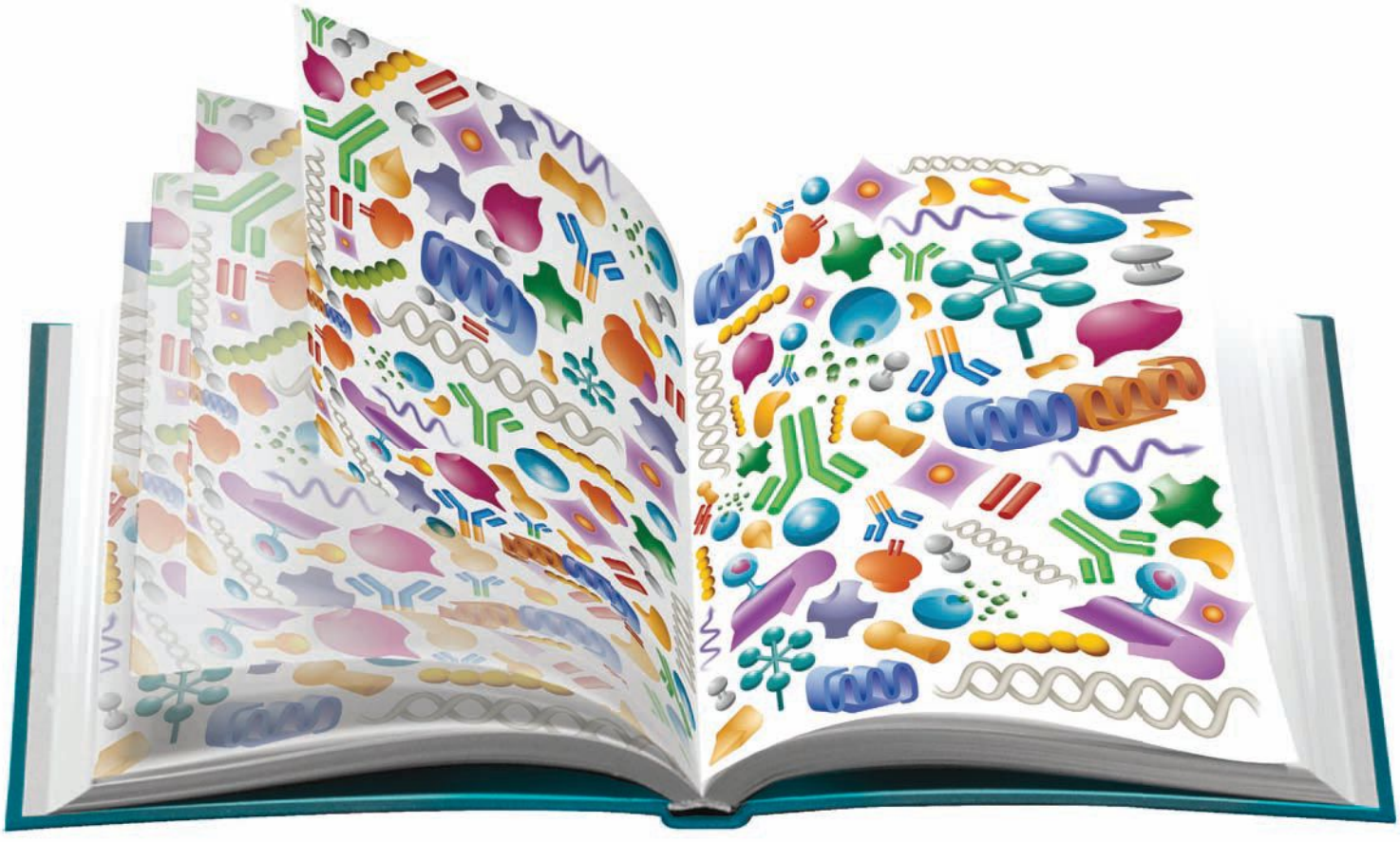
—**Sir Fraser Stoddart,** a Scottish chemist who was awarded a Nobel prize last month for his work on molecular nanomachines, on the potential harm the Brexit may have on research (*The Guardian*, October 6)

A source of common frustration for people at the NIH is to realize that in general the public doesn't understand that the money that goes into the NIH ends up supporting scientists whose work has widespread public benefit and that the advances that occur can frequently be traced back to discoveries that were made 10, 20, 30, 40 years earlier.

—Cancer researcher **Harold Varmus,** former director of the National Cancer Institute, in answer to a question about the pace at which science goes from basic research to clinical relevance (*Undark*, September 20)

We're not trying to assign blame or criticize anyone or call anyone consciously sexist. Rather the point is to use the results of this study to open up meaningful dialogues on implicit gender bias, be it at a departmental level or an institutional level or even a discipline level.

—**Kuheli Dutt,** a social scientist and diversity officer at Columbia University, on her *Nature Geoscience* study of hundreds of postdoc letters of recommendation that found female applicants were only half as likely to get superlative letters as their male counterparts (*Scientific American*, October 6)



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Notebook

NOVEMBER 2016

PHOTO BY CLIVE R. MCMAHON

OMMAGERD, YOU'RE A SCIENTIST!: In Prydz Bay, Antarctica, a male southern elephant seal sports a state-of-the-art miniaturized conductivity-temperature-depth sensor that links to a data-collecting satellite.

Flippered Fieldworkers

In the winter of 1999, Guy Williams took a trip to the Antarctic. A PhD student in oceanography at the University of Tasmania, he had become fascinated with the role polynyas—unfrozen expanses of water surrounded by ice—play in the cooling of large water masses at the Earth's poles. Joining a research expedition, he travelled south from Tasmania by ship through the ice to the Mertz Glacier polynya in East Antarctica. The goal: to take measurements that would help model ocean circulation.

The survey was successful, but the voyage itself was something of an eye-opener. "I was very excited about going to Antarctica," Williams recalls, "but I soon realized that Antarctica in wintertime is another kettle of fish. I got a real insight into how difficult it is to take those sorts of measurements, and how inhospitable those areas are."

With air temperatures dipping below –20 °C, and sea ice more than a meter thick, the Antarctic winter poses a challenge to researchers collecting even basic oceanographic data, such as water conductivity (a correlate of salinity), temperature, and density (CTD). And it's not just scientists who struggle. Technologies such as Argo floats—devices designed to sink and rise in the water column while taking measurements that are then relayed by satellite—are also hampered by ice cover, making them less practical data collectors beyond 60 degrees latitude.

But for Williams and his colleagues, data arrived from an unlikely source. A few years after that bone-chilling expedition, a serendipitous meeting with marine biologists turned up a new data set, one collected by ocean dwellers that are quickly becoming the go-to data loggers in hard-to-access parts of the world's oceans: seals.

"These seals were carrying micro-CTD devices" to take measurements of the water

around them, explains Williams, now a research fellow at the Antarctic Climate and Ecosystems Cooperative Research Center in Tasmania. "It's really the bread and butter for oceanographers. If you have a vertical profile with these parameters, you get everything you need to know about the structure of the ocean."

Designed by scientists at the University of St Andrews's Sea Mammal Research Unit, the specialized telemetry tags are now routinely used around the world to track the movements of seals as they traverse marine environments. Weighing just a few hundred grams, the devices are attached to the seals' heads using an epoxy resin glue, and remain affixed to the animal with negligible impacts until molting, which usually occurs once a year. Onboard sensors take CTD measurements and location data up to every four seconds, and send a portion of those data via satellite back to researchers' desks whenever the animal surfaces.

"When you come into the office, you log in and see where they've been," says biologist Rob Harcourt of Macquarie University, New South Wales, Australia, who collaborates with Williams to study the behavior of elephant seals. "You can see their tracks as they're moving across the Southern Ocean and look at the vertical profiles taken on individual dives—all in beautiful colors, of course."

Seeing an opportunity to "fill in the map" for Antarctic waters, Williams and an international team of researchers examined data collected from 2011 through 2013 by elephant seals as they visited Prydz Bay, another polynya region in East Antarctica. The group knew that the region was involved in forming cold, dense water masses that help drive the circulation of nutrients and dissolved gases around the planet's oceans. But the seal data set revealed something more.

"We found that [glacial] ice shelves are really holding back the system," says Williams. Freshwater input from melting ice, the data showed, hinders the production of these dense water masses—which over a long time scale, Williams says, could lead to the slowing of global ocean currents as the planet continues to warm (*Nat Commun*, 7:12577, 2016).

"It's a valuable piece of the puzzle", notes Greg Johnson, an ocean climate researcher at NOAA. He adds that for now, the seal data set provides more information on current mechanisms than on future evolution. The next step will be to create time series, he says. "If you have data for decades, you can look at variability, and, eventually, changes."

It's taken quite a long time to get the approach really accepted and its value appreciated, but now the ball's rolling I think we've got it up to a useful speed.

—Mike Fedak, University of St Andrews

Such future data sets may also come from seals. Since 2004, several countries, including the U.S., China, and Brazil, have launched national oceanographic programs using marine mammals—particularly seals—to explore hard-to-access regions all across the globe. "It's exactly what I wanted to see," says St Andrews marine biologist Mike Fedak, who helped develop the now internationally used tags and has been a proponent of such collaborations since the early 2000s. "It's taken quite a long time to get the approach really accepted and its value appreciated . . . but now the ball's rolling I think we've got it up to a useful speed."

Of course, seals can't solve everything. They don't swim under ice shelves, for example. To go there, oceanographers would need underwater vehicles, says Williams. And seal-collected data is "a bit of a lottery," he adds. "You watch [the seals], and you're wondering where they're going to go this year. Even on a daily basis, you're watching them, going, 'Please turn left. I want you to go left.'"

But as one of a suite of methods, these animals are filling gaps in data sets that are critical to the accuracy of global ocean modeling. "It fits into a general momentum in oceanography now, which is to be clever about how you collect data," notes Fedak. "It's not all about going out with a bunch of bearded guys in jumpers and ships. I think oceanographers are only too clear now that oceanography needs this mix of data collection technologies."

It's a view that Williams shares. "Seals are providing data to improve the accuracy of our models" by working in complement with other methods, he says. After all, "when you're looking at something the size of Earth," he notes, "especially from extreme environments, you use whatever you can."

—Catherine Offord

Smell School

As a postdoc in the late 1970s, Charles Wysocki visited a lab in New York City, where he helped put together olfactory test kits to be used in determining the genetics of smelling certain odors. One of the compounds in the battery was androstenone, a pig pheromone that for about half the human population evokes either woody muskiness or stale urine. The other half—Wysocki included—can't smell it at all. "Little did I know I'd be getting it all over my clothing," he says, which probably made for a few wrinkled noses on the subway ride home.

His stint putting together the kits lasted just a week, and Wysocki didn't have the need to test androstenone again until 1980, when he launched his own research group at the Monell Chemical Senses Center in Philadelphia (he's now an emeritus member of the faculty). He was testing various strains of mice to find one that couldn't smell androstenone, when after a few months he realized that he was becoming sensitive to the compound—he could pick out which bottles contained androstenone by their musky odor.

At the time, "nobody would believe my story," Wysocki says, so he embarked on years of experimentation that demonstrated that repeated exposure to the

compound can unlock people's ability to perceive the scent. In a study of 20 volunteers who initially couldn't detect the odor, half became sensitive after sniffing the compound three times a day for six weeks (*PNAS*, 86:7976-78, 1989). The results suggested that the inability to smell the substance was not necessarily an immutable trait. But it would be another decade before clinicians would seize upon the olfactory system's plasticity to cure cases of anosmia—total olfactory loss.

For much of history, treating anosmia has been an exercise in futility. Every week for years, Thomas Hummel saw patients at the Smell and Taste Clinic of the Technische Universität Dresden in Germany and sent them home miserable. Some had lost their sense of smell from a head trauma, others following a viral infection. "There's very little that can be done to help them," he says. "That inspired us to find new ways to treat these patients."

Hummel was aware of Wysocki's work and of studies by a German physiologist who had experimented with essential oils decades earlier. So about 10 years ago, he decided to try out an exposure regimen on some patients. He asked 40 anosmic volunteers to sniff four essential oils—rose, eucalyptus, citronella, and clove—twice a day for 12 weeks. Thirty percent of the patients who completed therapy could smell better (*The Laryngoscope*, 119:496-99, 2009). "That was the first study," says Hummel. "Now, it's been replicated over and over."

A meta-analysis of olfactory training studies published this year by Justin Turner, a Vanderbilt University otolaryngologist, and colleagues found that the therapy works for some anosmics (*Int Forum Allergy Rhinol*, 6:299-307, 2016). And he's witnessed its success among his own patients as well. "Since there's really nothing else out there that's effective, I really see no downside to doing it," Turner says.

For Christine Kelly, an American expat living in England, smell training restored a perceptual experience she had lost completely after a viral infection four years earlier. Her anosmia led to depression, and during this low point Kelly met a clinician who introduced her to smell training with essential oils. On her train ride home from that initial visit, Kelly decided that if she were to do this, she would observe herself along the way. "In that respect I became a student of anosmia, rather than a victim," she says.

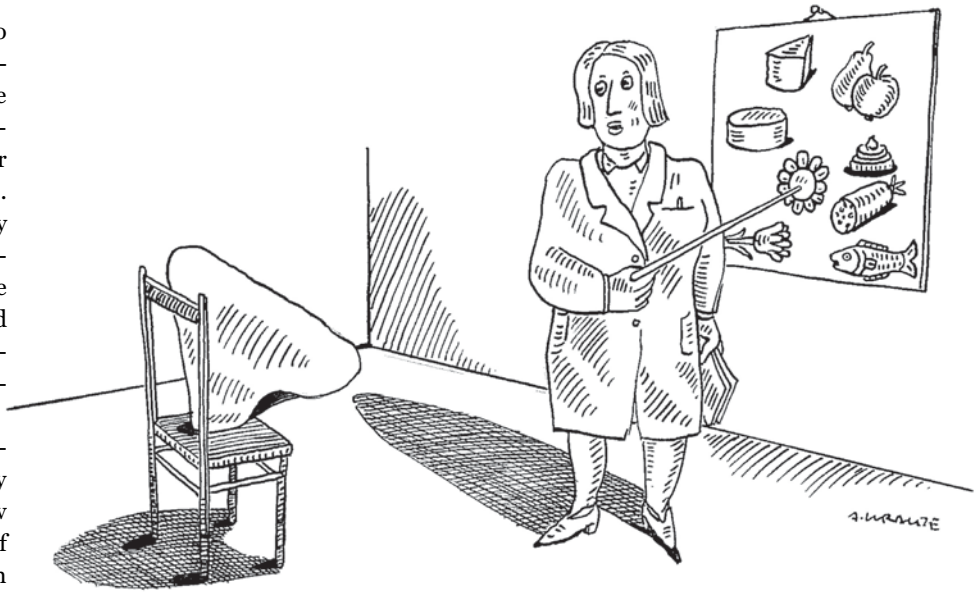
She kept careful track of her training—noting how well she could pick up a scent, distinguish it from others, and smell it the same way she had before the infection (a common problem among those who suffer from a loss of the sense of smell is parosmia—scent distortion).

After a few months of training, odors began to come into focus for Kelly. At first, they were terrible. "Everything had an unearthly, disgusting smell that would vacillate between burning Teflon frying pans [and] spoiling ham sandwiches that had been left inside a camper van in the rain for three months," she recalls. Then, with continued training, Kelly was able to get a fix on lemon. More and more smells followed.

It's not entirely clear how olfactory training works on a neurological level. Part of it, says Hummel, is mere attention: focusing on one's olfactory impressions makes a person better at recognizing them. "Cognitive processing is improved," he says.

The olfactory system is also known for its regenerative abilities; the subventricular zone of the mammalian brain is one of the few regions that births new neurons into adulthood, sending the cells to the olfactory bulb. And the peripheral nerve cells in the nose renew every few weeks. Wysocki says this turnover might be the reason why androstenone anosmics can come to smell the compound—there's some positive feedback, perhaps by a few neurons that present the receptor for androstenone, that triggers the production of more cells bearing the receptor. "What might be going on is that what you're seeing is some selective pressures existing in the olfactory system that are taking place over the course of months, rather than over the course of eons," he says.

While the mechanisms for smell recovery remain mysterious, Veronika Schöpf, a neuroimager at the University of Graz in Austria, has observed some of the brain's changes after olfactory training. She and her colleagues recently subjected 10 anosmics to fMRI before and after a 12-week training session. The participants had far fewer functional connections between odor-processing areas of



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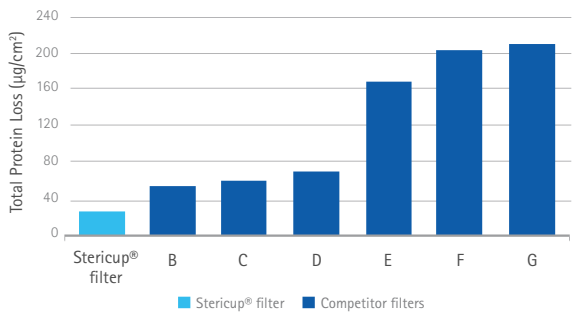

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the brain before the exercise (*Neuroimage: Clinical*, 9:401-10, 2015).

Interestingly, the neural networks that were activated in healthy controls by smelling odors were also activated among anosmics before and after olfactory training. "We were able to visualize in the first instance [that] these areas of the brain are still intact," says Schöpf. The way she describes it, these networks are ready and waiting to process olfactory information in anosmics—and smell training gives the neurons the input they've been waiting for.

Kelly's success with olfactory training has made her an evangelist for the therapy; she gives talks and offers guidance to anyone who asks. But her renewed sense of smell is not the same as before. "I truly have developed my own, new lexicon of smells," she says. She continues to suffer from parosmia; coffee, for instance, is an entirely different scent to her than it was before. And the loss of those old scents took with it the deep emotional upwelling that often accompanies odors—something smell training has not been able to recover. "It is an indescribable bereavement," Kelly says. "A unique bereavement all anosmics have, I think."

—Kerry Grens

Now I Smell You, Now I Don't

A few years ago, Pei Sabrina Xu, then a graduate student in Timothy Holy's lab at Washington University School of Medicine in St. Louis, was trying to design an enclosure for mice that allowed males to smell (but not interact with) females, and vice versa. The animals were usually kept in separate cages, but Xu wanted them to be exposed directly to chemicals released by the other sex.

She brought up the project at a lab meeting, and one of her colleagues suggested that she create a stacked enclosure. She could house the females in the top half and the males in the lower, or vice versa, allowing the urine from the upper deck to drip down into the lower layer. Xu headed to the university's machine shop, where she built the two-layer cage, and then ran her experiment.

Xu's goal in designing the stacked cage was to understand how individuals' neuronal populations differed from one another and how they changed over time. "We know everyone thinks differently, behaves differently. That's largely because of differences in the nervous system. But the brain is so complicated," says Xu, now a postdoc at the University of California, San Francisco. "We even don't know whether different individuals have different types of neurons."

In the mouse vomeronasal organ (VNO), more than 300 different types of sensory neurons respond to a vast array of odor molecules. "The number of different genes for olfactory receptors is huge," says Holy. "About 300 different genes—about 2 percent of the whole mouse genome—are devoted to making these receptors. Each cell picks one of these receptors to express at high levels. The gene that gets picked is sort of the identity of the cell."

Taking advantage of a light-sheet microscope developed by Holy, Xu exam-

NIFTY SNIFFERS: Sensory neurons in the olfactory organs of mice are incredibly plastic.

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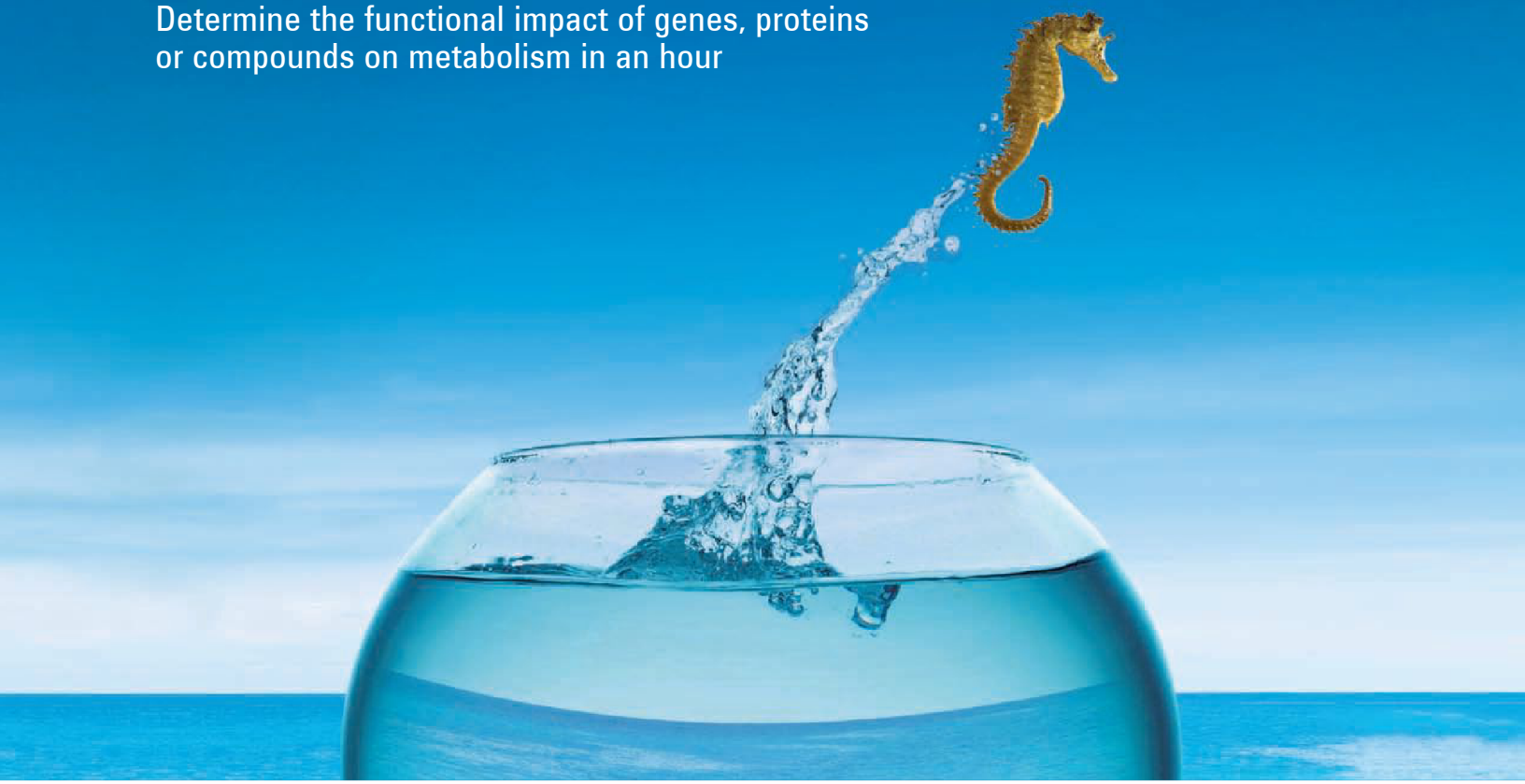

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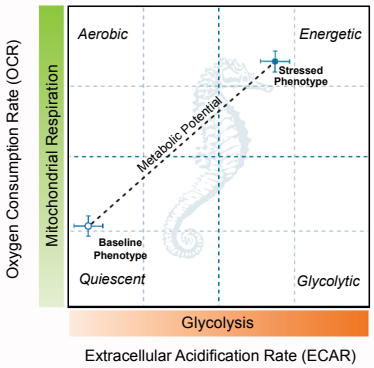

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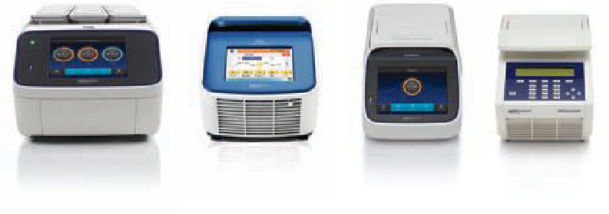

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ined how the stacked living arrangement affected neurons in mice's VNOs. She extracted the organs from the mice and used the microscope to simultaneously monitor the activity of approximately 10,000 neurons in response to various chemicals, revealing their neuronal types. She documented dramatic individual variation in the cellular makeup of each mouse's VNO, and discovered distinct neuron types that existed only in males. And Xu found that, after 9 to 13 weeks of living in the stacked cages, the VNOs of males living beneath females completely lost those male-specific neurons that responded to the scent of female urine. In male mice that lived beneath females and were then housed alone for another two months, those male-specific neurons came back (*Neuron*, 91:878-92, 2016).

About 300 different genes—about 2 percent of the whole mouse genome—are devoted to making these receptors. Each cell picks one of these receptors to express at high levels.

—Timothy Holy, Washington University School of Medicine

"To me, that was a surprising result, in the sense that this is a part of the nervous system involved in pheromones," says Holy. For such a biologically important system, Holy expected differences in VNO cell composition and function to be innate, but "contrary to my own prediction, [the results were] consistent with [the differences] being entirely based on the experience of the animal."

Brandeis University's Leslie Griffith, who studies neuronal plasticity but was not involved in the study, agrees. "It gives you another level of plasticity in sensory systems that I think people have not at all appreciated," she says. "People think about primary sensory systems really at the beginning as being labeled lines: you have a neuron that senses a particular chemical or temperature or whatever . . . and the plasticity has always been thought of as being downstream of those primary sensory neurons. Here you have a really nice example of where it's not necessarily a labeled line system; the system itself is plastic."

Xu confirmed that the scent of female urine was driving the neuronal change by providing male mice in nonstacked cages with bedding material soaked in female urine. Same result, she says: "male-specific neurons disappear." And the same held true when she soaked the bedding material in epitestosterone sulfate, a chemical that activates the same male-specific neurons as female urine. "[Xu] ended up basically eliminating particular subsets of cell types from the nose simply from giving the animal extended experience with those odors," Holy says. Xu even compared the two VNOs of individual animals, one from each nostril, after one nostril had been blocked, and saw changes in the VNO from the unblocked nostril only.

Notably, these neuronal changes affected the animals' behavior: males exposed to female urine or to epitestosterone sulfate became significantly less interested in the smell of female urine. "Normally [males] come and sniff a lot," says Xu, but animals exposed to epitestosterone spent much less time investigating a urine-soaked cotton swab. "We find that this specific neuronal type largely mediates the male interest in the female urine."

"The interesting thing for me from the plasticity standpoint is they're looking at males being interested in females. This is often thought to be very innate," says Max Fletcher, who studies how learning affects neural encoding of sensory information at the University of Tennessee Health Science Center.

As for how the changes are taking place, Holy and Xu suspect that male-specific, urine-detecting neurons simply are not replaced after they die. Because the olfactory system is exposed to the external environment, the cells are constantly turning over. The average lifetime of a VNO neuron is only a couple of months—almost exactly the length of time it took for Xu to see the changes to the neuronal population. "It really does jibe with the time course," says Griffith.

Holy says his lab is currently investigating this mechanism further, and he's got the tools to do it. His microscopy setup enabled Xu to image some 500,000 neurons. "That's a ton," says Fletcher. "That's way more than most people get close to."

Griffith agrees: "From a purely technical point of view, [this study] really is a beautiful example of how you can record from many different neurons and actually get something coherent out of it. . . . This is likely the tip of the iceberg."—Jef Akst

Depression and the Metabolome

In 2002, psychiatrist Lisa Pan, a depression and suicide prevention researcher at the University of Pittsburgh Medical Center (UPMC), met Kyle, a 19-year-old suffering from depression (name altered to preserve confidentiality). He was among the estimated 15 percent of depression patients in the U.S. for whom treatments such as antidepressants or therapy do not help. He "had been through every available treatment" including electroconvulsive therapy, but nothing worked, Pan recalls. "At one time, he was on 17 medications simultaneously." The teenager had attempted suicide, and doctors determined that he was at risk for similar episodes. The next step for him would be state hospitalization.

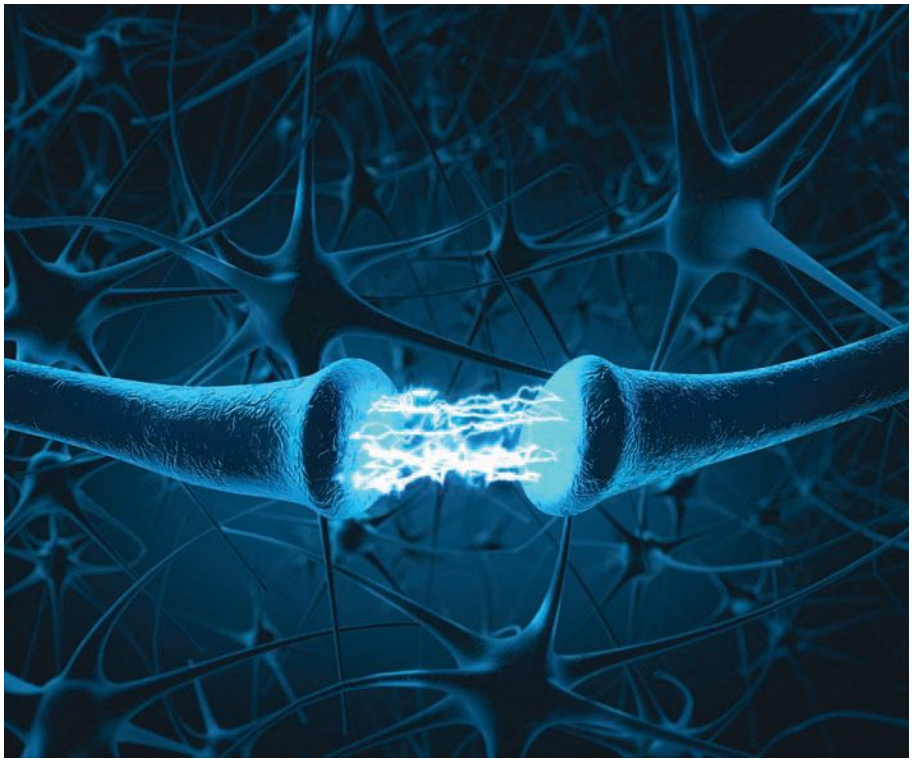
Having exhausted conventional treatment options, Pan went off script. She enlisted a colleague at UPMC, geneticist David Finegold, to run tests on Kyle's neurometabolic system, which supplies nutrients necessary to maintain neurons with a healthy supply of neurotransmitters. The tests revealed that Kyle underproduced tetrahydrobiopterin (BH4), a necessary cofactor

for enzymes involved in making serotonin, dopamine, and other compounds that modulate emotions and mood. Pan gave him sapropterin, a synthetic form of BH4, to bring his system back into balance. "It took some time, but he got better," she says. Kyle left the hospital and went on to graduate from college.

"We felt like we might be onto something," Pan says. She began exploring the possibility that metabolic imbalances affected others for whom standard depression treatment had failed. She and her colleagues conducted an array of blood, urine, and cerebrospinal fluid tests in 33 such individuals, each of whom had shown negligible response to at least three different maximum-dose depression medications administered for six weeks or more. Ranging from teenagers to middle-age adults, the group included some who also suffered posttraumatic stress disorder (PTSD), anxiety, or attention deficit hyperactivity disorder (ADHD).

When the tests came back, about a third of the patients showed a deficiency in the levels of folate in their spinal fluid, another key compound for producing a variety of neurotransmitters. Taking a folate supplement to correct this imbalance improved patients' depression symptoms, lowering their scores on a questionnaire for suicidal thinking and another for mental and physical signs of depression. And Pan says that as each patient continued to take the supplement, their symptoms continued to improve (*Am J Psych*, appiajp201615111500, 2016).

Since the publication of these results, "we have gotten hundreds of phone calls from people just asking for help," she says. As Pan returns these calls, she explains that her findings are preliminary. Her study documents relatively few individual treatment outcomes and is not a formal clinical trial. As the study continues and expands to include more patients, Pan and her colleagues will work to identify the cause of folate deficiency and to investigate whether folate supplementation could become a standard treatment. But these results suggest that treating an underlying neurometabolic imbalance can alleviate depression at least in some cases, which should open a door to new research. Receiving the influx of phone calls "makes me want to work more," Pan says.



STAVING OFF DEPRESSION: Deficiencies in key compounds that help the body make neurotransmitters may contribute to the intractability of depression in some people.

Characterizing depression in broad strokes is almost impossible, cautions David Brent, a former advisor to Pan and coauthor on the study. "There are a lot of people who look the same that probably have very different causes" of depression. Other psychological conditions, including PTSD and anxiety, frequently coincide with and complicate the disorder. "We're really just at the beginning of extricating" these interacting factors, Pan says, and at this stage, developing solutions that can be standardized for treating many people is difficult.

Even so, Finegold notes that preliminary studies like this one have a way of offering hope to people who have not found a successful depression treatment. He, too, receives phone calls from people living with depression, and some express their gratitude simply to know that new aspects of the problem are being explored.

One avenue of Pan's further exploration is to search for genetic markers associated with neurometabolic imbalances. Ideally, genetic testing could be used alongside traditional clinical evaluations to provide physicians with as much patient information as possible, says coauthor David Peters, a geneticist at UPMC. Testing spinal fluid is invasive and time-consuming; a quick DNA swab is much more practical, and Peters says incorporating genetic testing is a "very exciting prospect."

Every six months or so, Pan checks in with Kyle. Having graduated college, he now works in environmental science, and the only medication he takes is sapropterin. In a statement he authorized her to share, Kyle says, "It's safe to say that I owe my life to the successful diagnosis."

—Ben Andrew Henry

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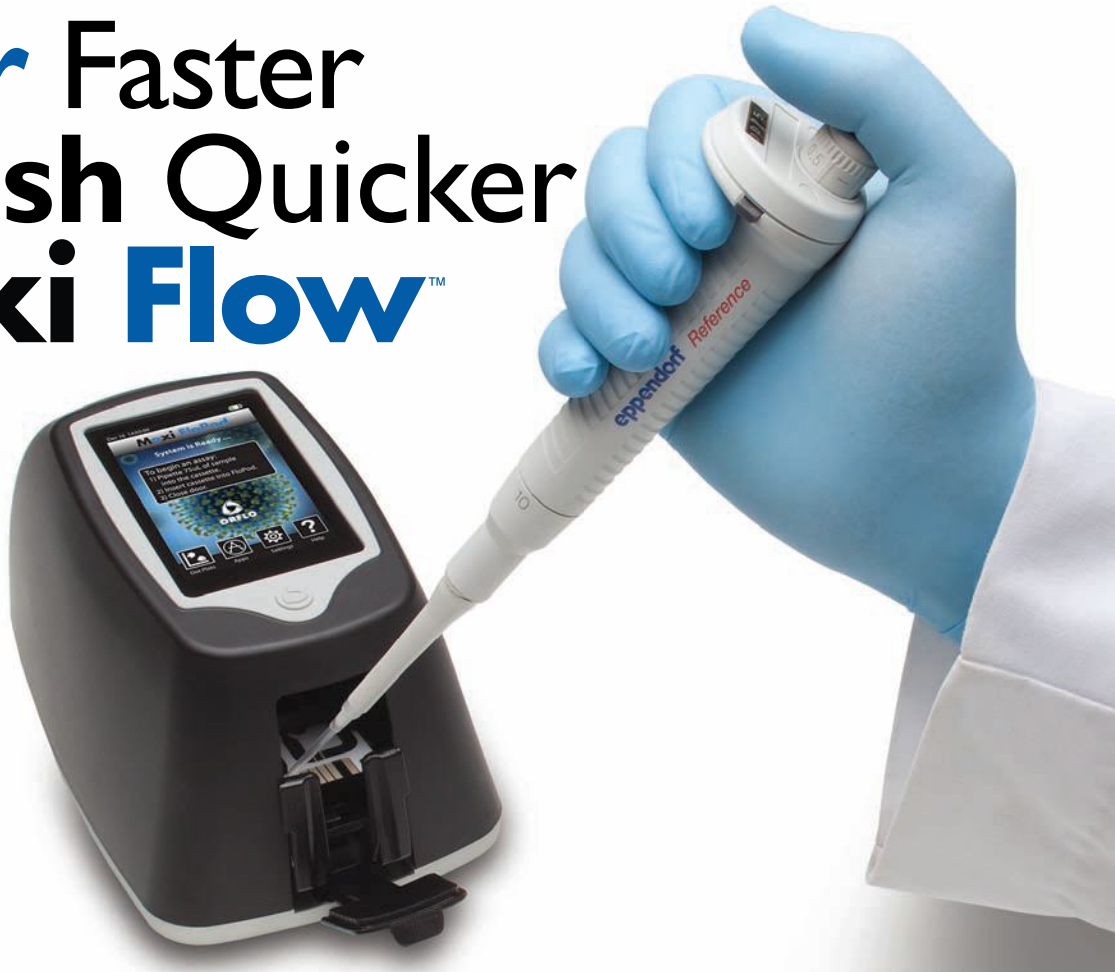

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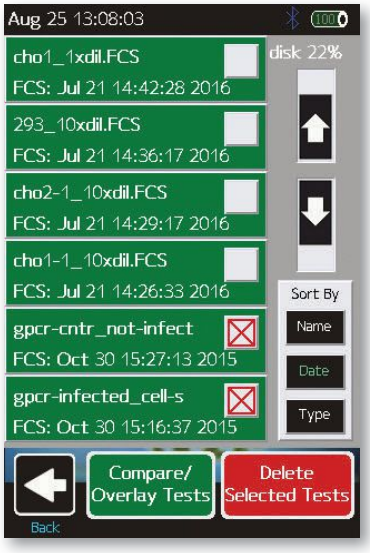

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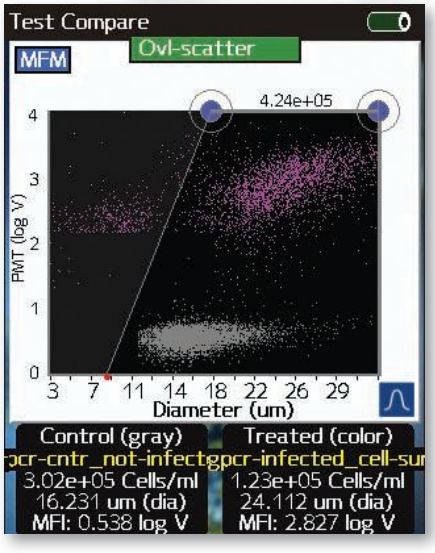

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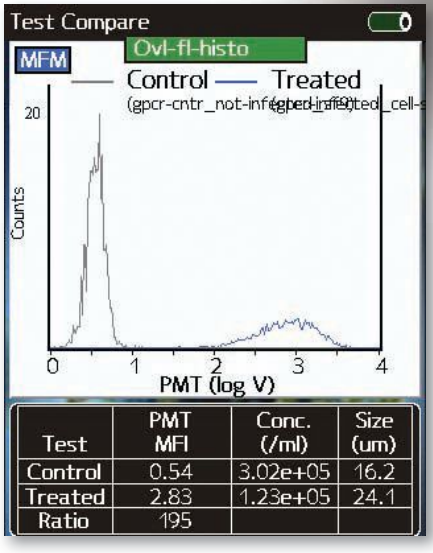

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Toot Your Horn

Why (and how) scientists should advocate for their research with journalists and policymakers

BY JORDAN GAINES LEWIS

Long gone are the days of the lone investigator who discovered a new scientific truth, published the finding in a journal, and continued doing bench research. Nowadays, scientists have to wear any number of different hats: experimenter, data analyst, teacher, mentor, negotiator, financial planner, writer, boss, philosopher, and speaker. We have to be team players, but also self-motivated. We have to pay meticulous attention to detail while also understanding how our research fits into the bigger picture. A good scientist performs well in many of these roles, but one person can't be good at everything.

I am a postdoctoral researcher whose favorite hat is "writer." It's exciting to craft my message, put years' worth of work down on paper, and add my own results to the literature of a decades-old research field. Scientific publications give us the potential to change the status quo in how other researchers approach their own work—and that's a big deal.

But when we pour all our energy into communicating only with other scientists, we miss the mark on targeting two other crucial audiences who can help us make an even bigger impact: journalists and policymakers.

Speak with journalists

The mass media play a huge role in policymaking and agenda setting. For example, a 2012 *Chicago Tribune* investigative series on the dangers of flame retardants in the home prompted US senators to call the Environmental Protection Agency to action. In turn, both the EPA and the Consumer Product Safety Commission began systematically reevaluating a group of chemically similar flame retardants and investigating alternative options for furniture and household products.

Unfortunately, most journalists do not have a strong background in science. In August, *The Washington Post* lamented that the media is ruining science, citing the incentive for journalists to distort scientific findings in order to garner more page views. Even at the institutional level, press releases are beginning to eschew accuracy to attract attention. (By the way, smelling farts won't help you live longer, and squeezing boobs won't prevent cancer.) This shift whittles away the credibility of science and the public's trust in it, and is not without its political undertones and consequences. According to a recent study, there's been a 25 percent drop in the proportion of conservatives who express their trust in the scientific community from 1974 to 2010.

With all the hats that scientists must wear, however, there's hardly time (or readily available training) for most researchers to engage effectively with the media. So don't ignore emails or phone calls from journalists. This is a chance to have your voice heard. Many outlets may want to put an interesting spin or slap a cutesy headline onto the article, but it's your job to accurately convey your scientific discovery and its context to the writer.

Engaging with policymakers

There are other ways scientists can ensure that our voices are heard, and many options for us to interact directly with our policymakers to advance our research.

Meet with your legislators. You can schedule a meeting in their Washington offices or in your home district; going with a small group of colleagues is always best. Many large scientific societies host their own advocacy days for scientists to converge on the capital and meet with their representatives in small, region-specific groups. These societies often

provide resources and a specific message, or ask scientists to share their research during the meetings. If you are having difficulty scheduling a meeting, local town halls are another good place to meet your legislator face-to-face.

Write, call, tweet, Facebook. Legislative representatives have easily accessible contact information on their websites. When certain science or research funding–related items are up for a vote, many scientific societies often craft form letters that you can sign and send. Follow your representatives on social media, track how they're voting, and understand their stances on issues that are important to you.

Invite them for a lab tour. During recesses, when legislators spend time in their home districts, invite them to tour your laboratory. Sometimes the fear of the unknown turns people off, and labs can be weird and scary to people who don't work in them. Engage your government representatives by showing them your equipment, explaining what you study in lay terms, and describing the importance of your work. Let them practice pipetting or check out some cells under a microscope. Most importantly, tell them how their funding and support has helped you, and how their continued support will impact the health and well-being of the citizens and the economy.

Get training on how to reach out to policymakers and communicate with the public. Familiarize yourself with the outreach and advocacy divisions of your favorite scientific societies, as well as your own institution's government relations and public communications departments. If you're having difficulty reaching out to a policymaker, or need resources on how to communicate with a journalist or speak at a public event, these folks can facilitate meetings and often have a slew of resources available.

Vote. Sure, this goes without saying. But one in three US citizens don't vote in presidential elections, and fewer than half tend to vote in midterm elections.

When scientists pour all their energy into communicating only with other scientists, they miss the mark on targeting two other crucial audiences who can help them make an even bigger impact: journalists and policymakers.

What's the point of advocacy efforts if your new representative doesn't support your cause to begin with?

Consider doing a science policy fellowship. For those who want to take their advocacy a step further, a sabbatical year is a great time to participate in a science and technology policy fellowship. Several large scientific societies such as the American Association for the Advancement of Science and the Robert Wood Johnson Foundation have science-specific policy fellowships, as do many smaller organizations.

So whether you only have time for a quick tweet or want to transition into a yearlong policy fellowship, there are plenty of ways for you as a scientist to advocate for your own work. You might not choose to dive into journalism or fly to the capital for a meeting, but policymakers might just read your 140-character tweets—whereas I guarantee they won't read your jargon-filled scientific publications (or your angry Facebook posts). ■

Jordan Gaines Lewis is a postdoctoral sleep researcher at Penn State College of Medicine in Hershey, Pennsylvania. She is also a freelance science writer with a strong interest in science policy.

The Semantics of Aging

Is growing old a disease? Medical science may have pathologized aging without ever realizing it.

BY MUTAZ MUSA

The concept of aging is undergoing a rapid transformation in medicine. The question has long been asked: Is aging a natural process that should be accepted as inevitable, or is it pathologic, a disease that should be prevented and treated? For the vast majority of medicine's history, the former position was considered a self-evident truth. So futile was any attempt to resist the ravages of aging that the matter was relegated to works of fantasy and fiction. But today, the biomedical community is rethinking its answer to this question.

The controversy has been fanned, to a great extent, by one Aubrey de Grey, a Cambridge University–trained computer scientist and a self-taught biologist and gerontologist. Over the past decade, de Grey has undertaken an energetic campaign to reframe aging as a pathologic process, one that merits the same level of attention as, say, cancer or diabetes. Although many of de Grey's claims remain controversial—notably, that the first person who will live to 1,000 years old is already among us—I agree that we can and should pathologize aging. In fact, it seems we already have.

"Aging" is a term we use to describe the changes our bodies undergo over time. Colloquially, we tend to refer to early changes, say from infancy to early adulthood, as maturation or development and reserve "aging" for changes that occur thereafter. The early changes are generally considered good: stronger muscles, wiser minds, and so on. The later changes are far less popular: thinning skin and hair, weakening bones, and other forms of decline.

To complicate matters, the human body comprises a number of different systems that each develop at its own pace. The nervous system seems to reach full maturity in our 20s, for instance, while the skeletal system may peak a decade later. Of course, this physiologic natural history is subject to environmental influence. For example, a diet rich in calcium and vitamin D, along with weight training, can increase bone density and strength. Nevertheless, these environmental factors ultimately act on a foundation that, beyond a certain age, is inexorably deteriorating. There is a finite limit beyond which environmental factors cannot save us.

The changes of aging vary in their specifics from one system to another, but common mechanisms are at work. For instance, wear-and-tear of joints results from depletion of articular cartilage, just as the thinning of skin is due to a loss of elastic connective tissue. Other age-related changes arise from errors in cellular activity or the accumulation of metabolic by-products, the probabilities of which rise over time.

As these natural changes proceed, they lead to readily recognizable disease. The accumulation of fat in blood vessel walls provides a particularly good demonstration of this. Lipids are an essential part of our diet, but as processed lipids continue to accumulate in vessel walls, these vessels harden and narrow, eventually failing to supply the heart with enough blood. If the narrowing blocks vessels entirely, the heart is starved of blood, causing heart muscle death, or heart attack.

This simplified example illustrates that perfectly normal processes that are critical to survival will quite naturally lead to disease. In a biological sense, the mere passage of time is pathological. Importantly, most of the early changes in this progression, such as high cholesterol, are symptomless. Yet they are precursors to life-threatening illness and are therefore considered pathologic entities in their own right, to be prevented and treated. The same can be argued of the more subtle and gradual damages of aging.

There are countless other conditions subject to this dynamic. They include some of the most common and debilitating ailments, such as osteoporosis, arthritis, stroke, diabetes, dementia, and even many forms of cancer. Given enough time, myriad diseases will afflict us as a direct result of the natural aging process.

We can and should view these diseases, whose prevention and treatment are standard medical practice, as the clinical manifestations of natural age-related changes. Doctors have long targeted such changes to prevent disease. For instance, by recommending their patients limit the fat and carbohydrate content of their diets or take statin medications, doctors have strived to stave off heart disease. In so doing they unknowingly have been battling aging itself.

In a biological sense, the mere passage of time is pathological.

Yet there are those who find this view of aging contentious, a reaction that likely stems from the misperception that the terms "natural" and "pathologic" are conflicting. There's a common yet unwarranted sense that these two terms are mutually exclusive; that what is natural can only be right, and what is pathologic cannot be natural. This is untrue. Because "natural" typically describes what conforms to the usual course of events, and "pathologic" describes what is harmful, the question posed in the opening paragraph presents a false dichotomy. Both "natural" and "pathologic" describe aging fairly.

Thus, the controversy is largely semantic. If I were to replace the call for a "fight against aging" with an invitation to "combat age-related changes," I would expect a far more positive response. A call to "prevent the early stages of disease" would surely receive virtually unanimous support. I contend that the three phrasings are synonymous. ■

Mutaz Musa is a physician in the Department of Emergency Medicine at Albert Einstein College of Medicine/ Montefiore Medical Center, a health-care consultant, and an entrepreneur in New York City.

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Mapping Single Neurons Using RNA

Researchers swap microscopy for RNA sequencing to track neural paths in the mouse brain.

BY RUTH WILLIAMS

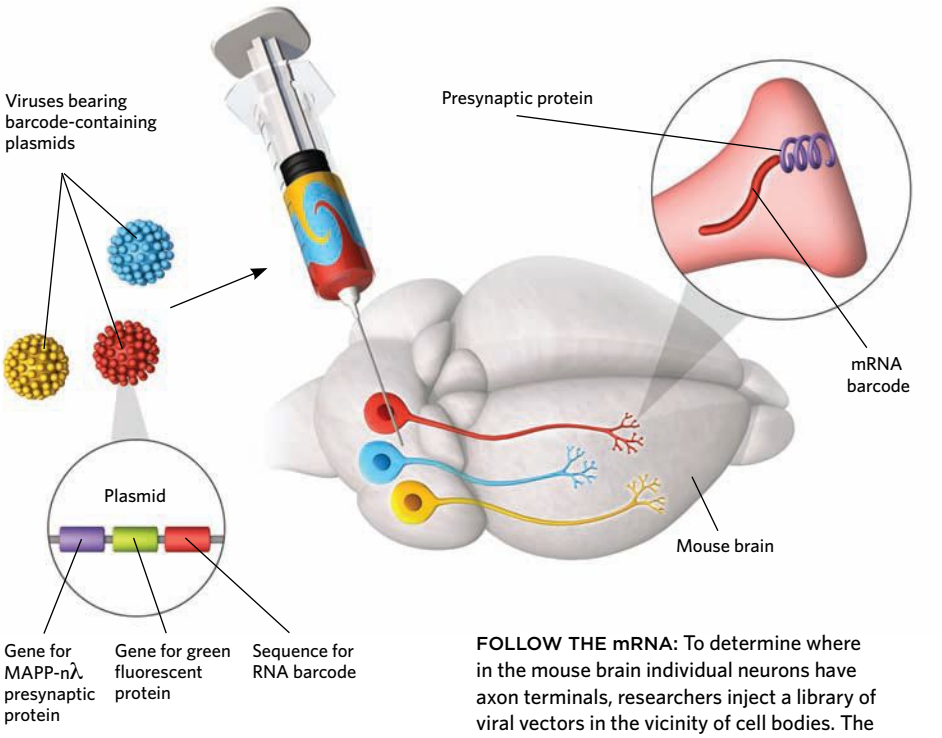
Several ambitious brain connectome projects are underway to diagram the wiring of animals' most complicated organ. For the most part, neural cartographers rely on microscopy, but tracing numerous projections across the large volumes of the brain is "extremely painstaking and slow," says Thomas Mrsic-Flogel of the University of Basel.

Inspired by recent advances in sequencing technology, Tony Zador of Cold Spring Harbor Laboratory had an idea: "If there was a way to convert the mapping problem into one of sequencing, it would be a [big] win in terms of throughput and cost." Now, he and his colleagues have done just that.

To map neurons by sequencing, Zador's team injects a mixture of approximately 10^6 viruses—each bearing a genetic sequence encoding a unique mRNA barcode—into mouse brain regions. By and large, each neuron becomes infected with one virus, and therefore receives just one barcode identifier. Two days later, once the mRNAs have travelled the lengths of the axons (guided by engineered presynaptic proteins), injection sites and target brain regions are dissected, and the RNA is extracted and sequenced. RNAs at the target sites matching those from the injection site thus represent individual neuron projections.

Zador and colleagues used the technique, called MAP-seq, to examine neuron projections into the cortex from the locus coeruleus—a site in the brainstem that is the sole source of the neurotransmitter noradrenaline for the neocortex. The team found that while some neurons branched out to multiple regions of the cortex, others connected with just one or two sites.

The experiment served as "a proof of principle," says Zador, who now plans to perform hundreds of injections in a single mouse cortex to examine connectivity from multiple regions at once. This has the potential to "yield a hugely rich anatomical data set," Mrsic-Flogel says. (*Neuron*, 91:975–87, 2016) ■



FOLLOW THE mRNA: To determine where in the mouse brain individual neurons have axon terminals, researchers inject a library of viral vectors in the vicinity of cell bodies. The viruses contain plasmids each encoding a unique RNA barcode and a presynaptic protein called MAPP-nλ that will shuttle the barcoded mRNA to the ends of the axon. Typically, each cell takes up only one virus, giving that cell an RNA identifier. Dissecting brain regions and sequencing the RNAs reveals which cells project where.

AT A GLANCE

MAPPING TECHNIQUE	CELL LABELING	TARGET SITE ANALYSIS	SPATIAL RESOLUTION	THROUGHPUT
Anterograde tracing	One or a few cells per brain are injected with the same fluorescent label (using additional colors allows tracking of greater numbers of cells).	Illuminated axon branches are observed with a microscope.	High. The precise locations of projections can be determined down to the resolution limit of the microscope.	Very low. It can take more than a week of intensive microscopy to track a single neuron's projections.
MAP-seq	Unique mRNA barcodes label individual neurons.	Tissue is dissected into tiny sections. RNA is extracted and sequenced.	Limited by the dissection method. Cryostat sections were 300 μm thick in this study, but laser microdissection could provide thinner pieces of tissue.	High. Many thousands of individual neurons can be mapped in one week. Multiple injection sites would increase this number further.

Unlikely Allies

Once thought only to attack brain tissue, immune cells turn out to be vital for central nervous system function.

BY AMANDA B. KEENER

In a dark room in Charlottesville, Virginia, a mouse swims in a small pool, searching for a place to rest. In 12 previous swims, with the help of visual cues and training from an experimenter, the mouse eventually tracked down a platform near the center of the pool. But just a day after its last swim, the animal is spending nearly as much time searching for the platform as it did on its first swim. The discombobulated mouse's problem? It has no T cells.

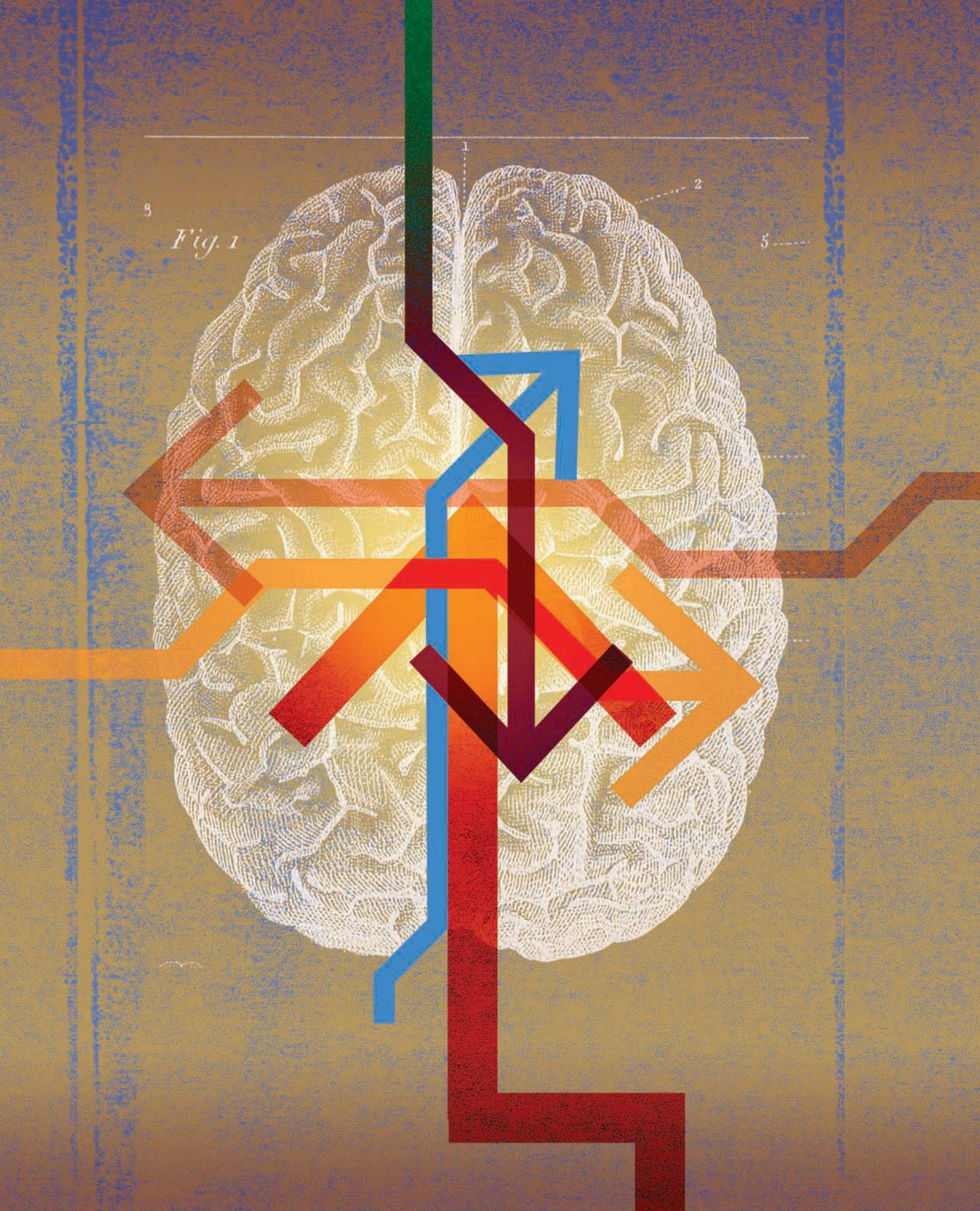
"Mice without functional T cells do not perform cognitive tasks as well as wild-type mice do," says the University of Virginia's Jonathan Kipnis, who first demonstrated a link between the immune system and cognitive function in 2004 as a member of Michal Schwartz's lab at the Weizmann Institute in Rehovot, Israel.[1] He later discovered that T cells' pro-cognitive effect is mediated by the cytokine interleukin-4 (IL-4), which prevents macrophages from generating proinflammatory signals that inhibit a protein important for learning and memory.[2]

Kipnis's work is part of a wave of research changing the way scientists view the relationship between the immune system and the central nervous system (CNS). Until recently, the brain and the spinal cord were considered immune-privileged sites, strictly cordoned off from immune cells unless something went terribly wrong. Researchers knew, for example, that multiple sclerosis (MS) was caused by T cells that breach the selective border called the blood-brain barrier (BBB), enter the CNS, and attack the myelin sheath covering neurons. Even microglia, specialized macrophage-like immune cells that scientists had recognized as normal CNS residents since the 1960s, were mainly studied in the context of disease.

Saying the immune system is always good for the brain, it's wrong; saying it's always bad for the brain, it's wrong. It depends on the conditions.

—Jonathan Kipnis, University of Virginia

But over the past two decades, researchers have recognized that the entire immune system is very much a part of a functional CNS, with vital roles in cognition, injury repair, neurodegenerative disease, and sensory systems. Microglia pervade the CNS, including the white and gray matter that constitute the organ's parenchyma. Other immune cells, including T cells, monocytes, and mast cells, reside in the brain and spinal cord's outer membranes, known as the meninges, and circulate in cerebrospinal fluid (CSF).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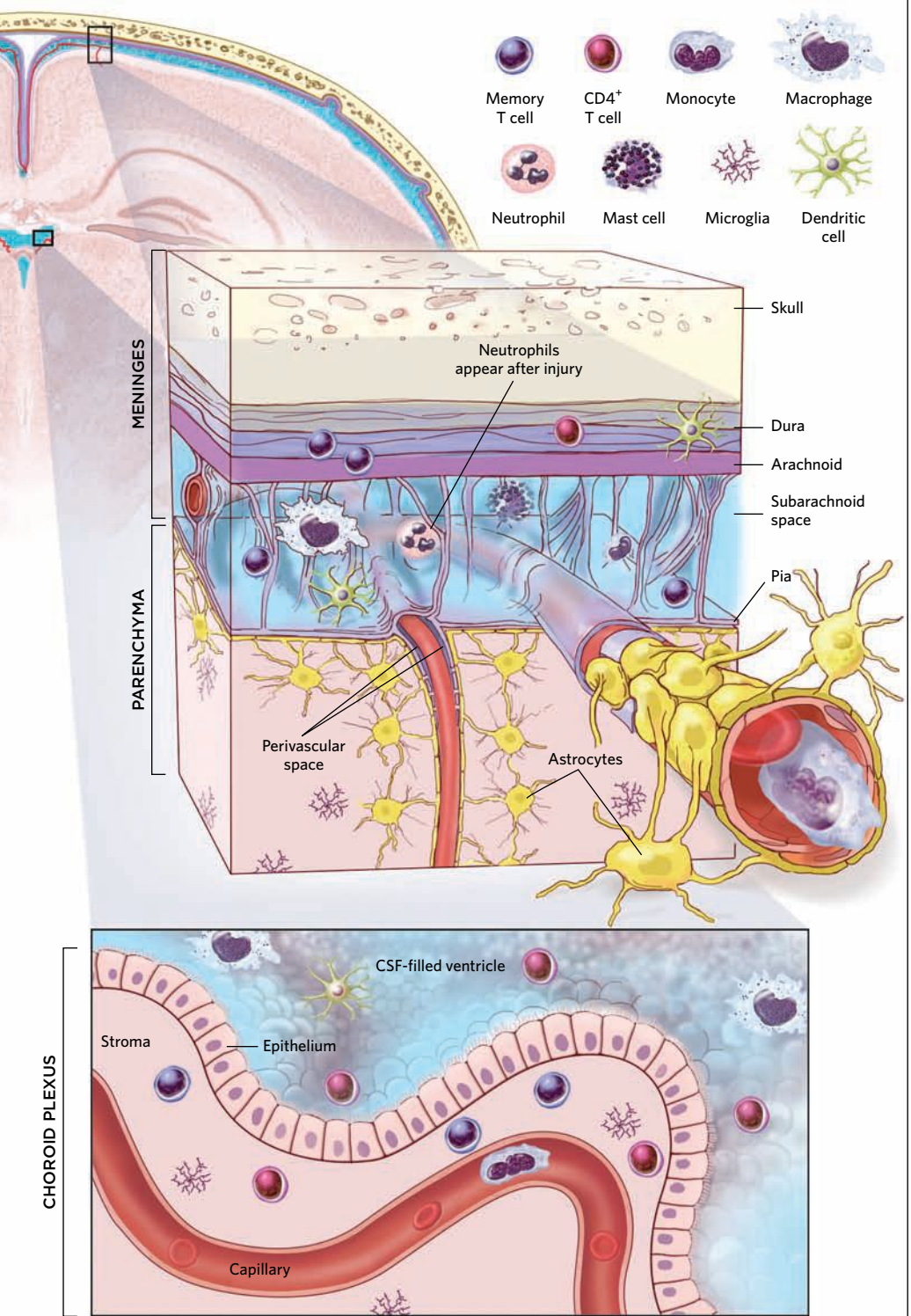
IMMUNITY IN THE BRAIN

Until recently, the central nervous system (CNS) was thought to be cordoned off from the peripheral immune system, reliant only on its resident immune cells called microglia. Peripheral immune-cell breaches anywhere in the CNS were considered signs of disease. But researchers now know that diverse immune cells—possibly by the millions—circulate in the cerebral spinal fluid (CSF) and live in the brain's outer membranes even in healthy individuals.



"Ten or 15 years ago, it was all bad," Serge Rivest, a neuroscientist at Québec's Centre Hospitalier de l'Université Laval (CHUL), says of the relationship between the brain and immune cells. These days, he says, researchers are focused on understanding the good in addition to the bad and the ugly.

Injury patrol

As early as the 1980s, researchers knew that immune cells infiltrated the CNS after injury, but such immune activity was viewed as something to be stymied, not encouraged. In fact, doctors used corticosteroids, which suppress immune-cell activity, to treat brain injuries for many decades. But Schwartz says it didn't make sense to her that tissues as indispensable as the brain and spinal cord wouldn't take advantage of the immune system's ability to protect against pathogens and repair damaged tissues. In the mid-1990s, she began searching for a positive neurological role of the immune system.

After nicking the spinal cords of rats, her team demonstrated that injecting macrophages at the injury site restored the animals' motor function. The macrophages facilitated healing, as they are known to do in other tissues such as liver and muscle.[3] (See "Immune Cell–Stem Cell Cooperation," *The Scientist*, July 2016.) Around the same time, other researchers were finding that eliminating macrophages improved recovery from spinal cord injury in mice and rats.[4] Because of this, Schwartz recalls, her work "was met with a high degree of skepticism."

But over the next decade, Schwartz and others continued to unveil more ways that the immune system promotes CNS repair after trauma. Macrophages, for example, can damage neurons by secreting cytokines, proteases, or reactive oxygen species, but in rat and mouse models of spinal cord injury, they also produce transforming growth factor-beta (TGFβ), which promotes wound healing,[5] and interleukin 10 (IL-10) which helps resolve inflammation.[6] By the late 2000s, researchers recognized that different subtypes of mac-

rophages can benefit neuronal growth in rodents, and that some were critical to recovery.[7] Views also began to change on the clinical side after the 2004 Corticosteroid Randomization After Significant Head Injury (CRASH) study showed that corticosteroids didn't help brain injury patients recover, but increased their risk of disability and death.[8]

More recently, research has revealed that it's not just macrophages and other components of the innate immune system that help maintain CNS health; cellular drivers of adaptive immunity also contribute. In 2013, Schwartz and her colleagues demonstrated in mice that the lining of each of the brain's four ventricles harbors memory T cells whose receptors bind proteins found in the CNS.[9] Although these T cells are specific for CNS proteins, they don't cause autoimmune disease. Schwartz contends that their specificity allows them to respond to local CNS damage.

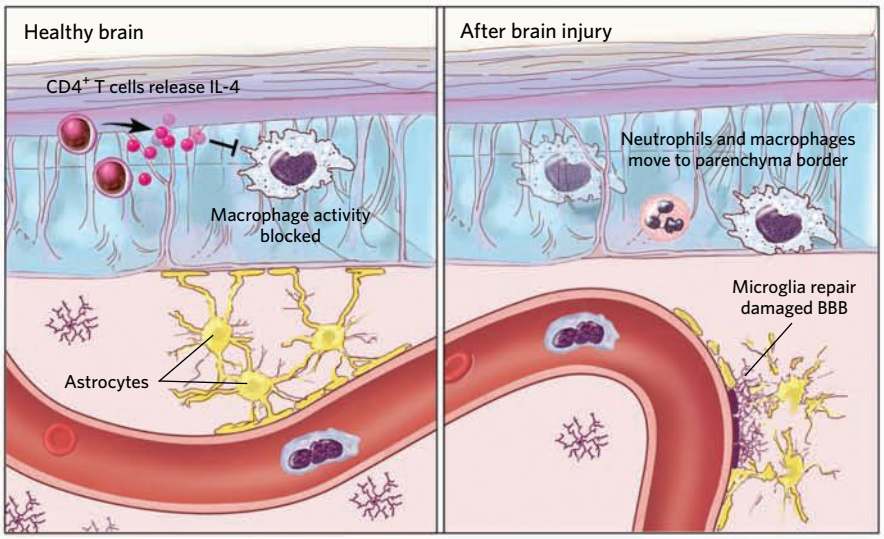
Her team also showed that T cells present in this lining, called the choroid plexus, secrete cytokines such as interferon gamma (IFNγ), which allows selective passage of $CD4^+$ T cells and monocytes from the blood into CSF within the ventricles.[10] In a model of spinal cord bruising, mice deficient for the IFNγ receptor had reduced immune cell trafficking across the choroid plexus and poor recovery of limb movement. And last year, Kipnis's team reported that IL-4 produced by $CD4^+$ T cells in the CNS signals neurons to regrow axons after spinal cord or optic nerve injury.[11]

To better understand how different immune cells contribute to injury repair, Dorian McGavern, an immunologist at the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland, is tracking responses to CNS injury in real time. Using two-photon microscopy to image cells below the surface of brain tissue in living animals, he and his colleagues have found that minutes after injury, microglia clear up debris in the parenchyma. "They'll look around the environment, and basically start street-sweeping and picking up all the dead

IN SICKNESS AND IN HEALTH

The immune system is a critical part of a functioning central nervous system (CNS), even in the absence of injury. But most immune cells are largely relegated to the cerebral spinal fluid (CSF), the brain's meninges, and the epithelium of the choroid plexus. When the CNS experiences a major insult, however, immune cells join microglia in the parenchyma.

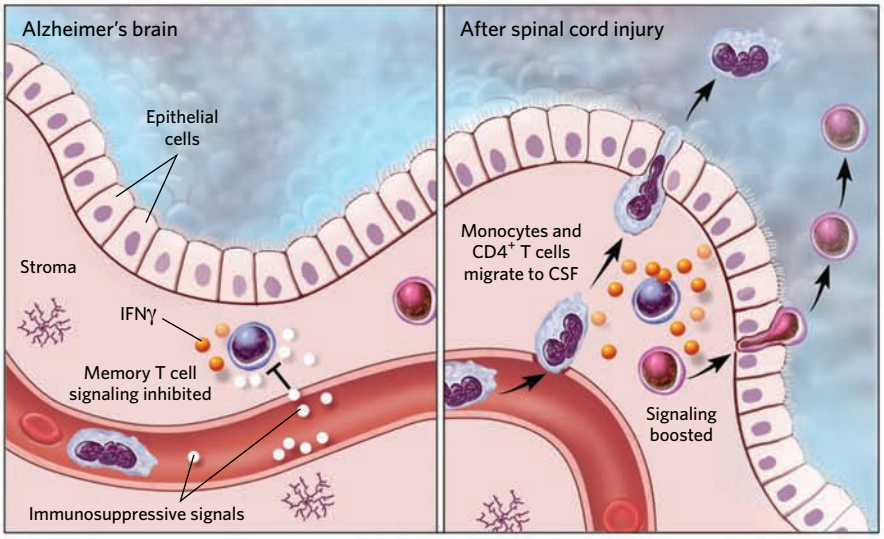
MENINGES



$CD4^+$ T cells in the meningeal lining produce IL-4 cytokine, which prevents nearby macrophages from making proinflammatory molecules. If left unchecked, such proinflammatory signaling blocks a protein that astrocytes in the parenchyma need to support learning and memory.

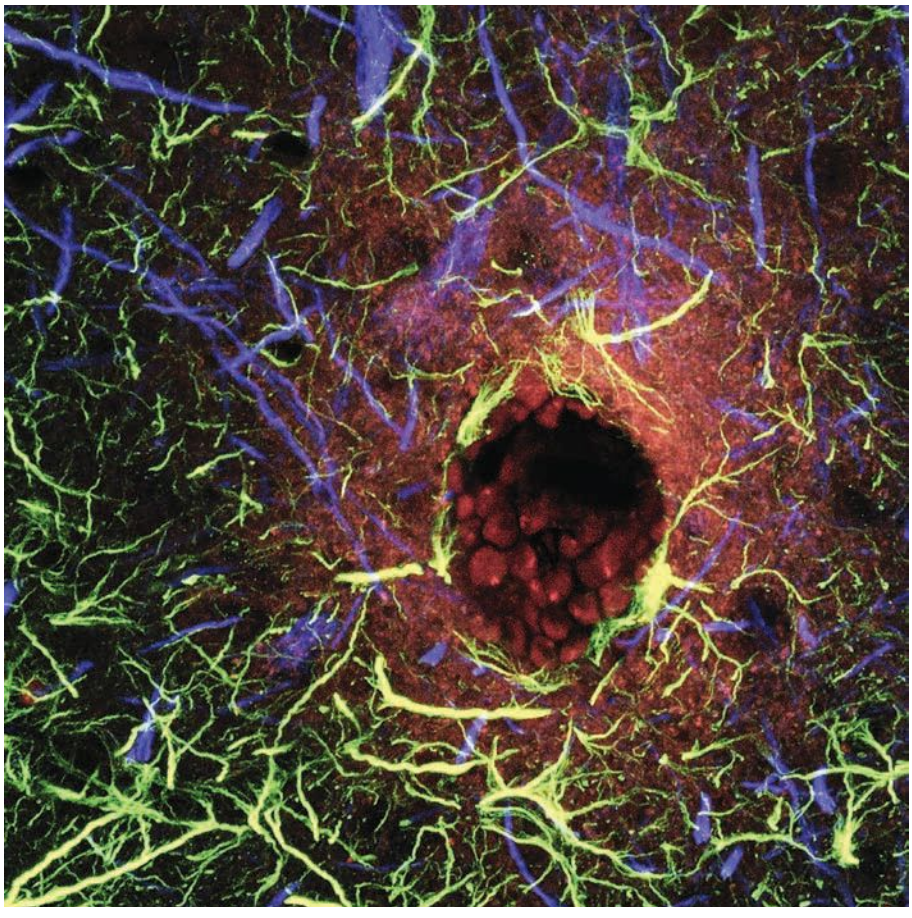
Neutrophils and macrophages migrate to the edge of the meninges, but don't enter the parenchyma. Macrophages clear dead cell debris. Neutrophils are also helpful for resolving injury, though it's not yet clear how. Microglia fill in spaces left by damaged or dead astrocytes to seal a leaky blood-brain barrier.

CHOROID PLEXUS



Immunosuppressive signals from far off regulatory T cells (Tregs) reduce IFNγ signaling. This blocks normal trafficking of monocyte and $CD4^+$ T cells from the blood and stroma to the cerebral-spinal fluid (CSF).

Memory T cells ramp up production of IFNγ, which facilitates the migration of $CD4^+$ helper T cells and monocytes from the blood and stroma into the CSF-filled ventricle, where they can access the site of injury.



BLOOD GUARDS: At the edge of a neural blood vessel (red), tightly packed endothelial cells form a blood-brain barrier (BBB) that mediates which cells enter the central nervous system. When glial cells called astrocytes, whose projections surround the endothelial cells, are damaged, microglia fill in spaces to maintain the integrity of the BBB. (Neurons in blue, glia in green)

material," McGavern says. Meanwhile, other immune cells remain confined to the meninges and to the so-called perivascular spaces between larger cerebral blood vessels and their sheaths of pia mater, the innermost meningeal membrane.

"When we watch the anatomy [after injury], we see that the microglia will stay in the brain parenchyma, and the neutrophils and macrophages will come to the lining of the brain and the perivascular spaces," says McGavern.

When he and his colleagues prevented macrophages from entering the CNS by blocking receptors that respond to nearby cell damage, mice fared worse within the first 24 hours after injury.[12] "In every case, we have created more injury and more [neural] cell death." His team also found that microglia reinforce the BBB, which is composed of endothelial cells, pericytes, and astrocytes. Microglia fill in spaces left by astrocytes killed or damaged during injury. Without a robust barrier, McGavern says, unwanted immune cells may flood the parenchyma and do more harm than good.

Balancing health and disease

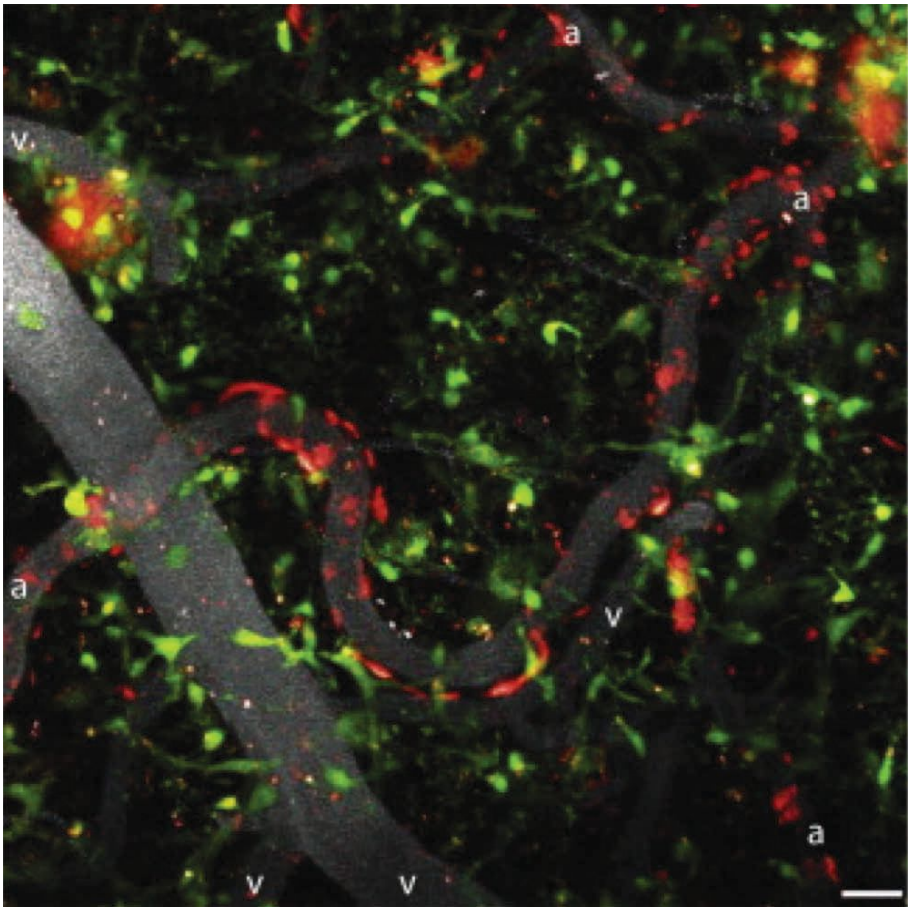
In addition to repairing neural injury, immune cells appear to play a role in fighting neurodegenerative disease. Back in 2006, Rivest and his team made the unexpected finding that microglia derived from blood monocytes are attracted to amyloid-β plaques in the mouse brain, and although the cells don't completely eliminate the deposits, they do restrict their size.[13] At the time, most work on microglia in Alzheimer's disease suggested that the cells' association with plaques caused inflammation and more neuronal damage; Rivest's work suggested the immune cells might help get rid of the neuron-killing amyloid-β protein.

When Rivest first presented his work at a meeting, practically the whole audience lined up behind the microphone to make skeptical comments, he recalls. "I was really not well received there." He adds that he himself was an early skeptic. "At the beginning, we were convinced that very strongly activated [immune] cells were bad for the brain, [but] it turned out that [they] prevent circulation in the brain of proteins that are neurotoxic."

In 2013, Rivest used two-photon microscopy to monitor monocytes in blood vessels of living mouse brains, and he watched as the cells migrated toward and cleared amyloid-β deposits within veins. When the researchers selectively depleted monocytes, the mice developed more amyloid-β plaques in the cortex and hippocampus.[14] And when they knocked out the innate immune signaling protein MyD88, which mediates signals from several monocyte-activating receptors, the mice also experienced more amyloid-β accumulation, accompanied by accelerated cognitive decline.[15]

More recently, Rivest's team found that microglia-forming monocytes are beneficial in a model of MS, where microglia are found within the inflammatory lesions. Last year, the researchers reported that inhibiting monocytes from entering the CNS reduced the clearance of damaged myelin and impeded proper remyelination.[16]

Schwartz has similarly found evidence for the immune system's ability to protect against neurodegeneration. Last year, she and her colleagues reported that the choroid plexus epithelium was less permissive to immune cell trafficking in a mouse model of Alzheimer's disease than in wild-type mice, due to anti-inflammatory signals produced by regulatory T cells (Tregs). They found that depleting Tregs in Alzheimer's mice allowed macrophages and $CD4^+$ T cells into the brain, reduced the number of amyloid-β plaques, and



HUNGRY PHAGOCYTES: Monocytes (green) from the blood are attracted to amyloid deposits (red) in cortical veins of the murine brain. These cells help clear the plaques, improving the mice's cognitive performance.

In addition to repairing neural injury, immune cells appear to play a role in fighting neurodegenerative disease.

improved cognition.[17] Similarly, blocking the T-cell checkpoint protein PD1, which normally supports Treg survival while suppressing the activity of other T cells, reduced amyloid-β plaques in mouse brains and improved the animals' scores in a learning and memory water maze test.[18]

In 1996, Schwartz cofounded Proneuron Biotechnologies, which plans to test antibodies that target PD-1 in Alzheimer's patients. This, she says, would be the first proinflammatory approach to treating a neurodegenerative disease, where immune activation has long been seen as a contributor to neural damage.

But there's a reason that scientists have believed that immune activity contributes to Alzheimer's damage: microglia, perhaps best known for trimming back synapses, have the potential to become overzealous, and excessive synapse pruning can cause neural damage in a variety of CNS diseases. By blocking the cells' proliferation in mice, Diego Gomez-Nicola of the University of Southampton in the U.K. has successfully alleviated symptoms of Alzheimer's disease, amyotrophic lateral sclerosis, and prion disease. And earlier this year, Beth Stevens of the Broad Institute and her colleagues reported that inhibiting a protein that tags synapses for microglial pruning halted over-pruning and loss of synapse signaling strength in two mouse models of Alzheimer's disease.[19]

"You'll probably find just as many papers saying that microglia are good as microglia are bad," says Gomez-Nicola, "and neither one nor the other is true."

Rivest says a fuller appreciation of the benefits of immunity in the CNS could open a lot more doors for potential treatments than simply looking for ways to block inflammation whole hog. "The field is really moving toward that direction," he says.

Behavior modification

Looking beyond immune cells' negative roles in neurological diseases has led researchers to some unexpected immune functions in the CNS, including the role for T cells in learning and memory that Kipnis described. Initially, he and his colleagues observed that mice without T cells are slower to learn in a water maze-based test of memory.[1] The researchers could restore normal cognitive abilities to these mice by injecting them with wild-type T cells.[20]

Kipnis says regulation of stress may be linked to T cells' role in learning. Stress can signal macrophages to secrete proinflammatory cytokines, some of which block a protein called brain-derived neurotrophic factor (BDNF), which astrocytes need to support learning and memory. $CD4^+$ T cells in the meninges make more IL-4 cytokine after mice have been trained in a water maze—a stressful exercise for the animals—suggesting the signaling molecule might let macrophages know when the brain is dealing with the stress of learning something new, not the stress of an infection. "They tell macrophages, 'Don't overshoot,'" says Kipnis. In mice whose meninges are depleted of $CD4^+$ T cells and thus deficient for IL-4, macrophages secrete proinflammatory factors unchecked in times of stress, disrupting their ability to learn and form memories.[2]

Last July, his group also reported that mice lacking B and T cells were less social: while control mice spent more time investigating other mice than inanimate objects, immune-deficient mice had no preference.[21] The researchers observed the same behavior shift in immunocompetent mice when they blocked a protein on T cells that facilitates migration to the CNS, or when they knocked out IFNγ, which Schwartz's work has shown facili-

CELL REPORTS, 5:646-53, 2013

tates immune-cell migration through the choroid plexus.

Kipnis speculates about an evolutionary link between immunity and social behavior; IFNγ both encourages social activity and protects animals from many communicable diseases. He and his colleagues observed that IFNγ levels are highest in the brain tissue of social animals, such as rodents and zebrafish, when the animals are wild or housed in captivity together rather than individually, suggesting that social interaction and T-cell immunity in the CNS reinforce each other.

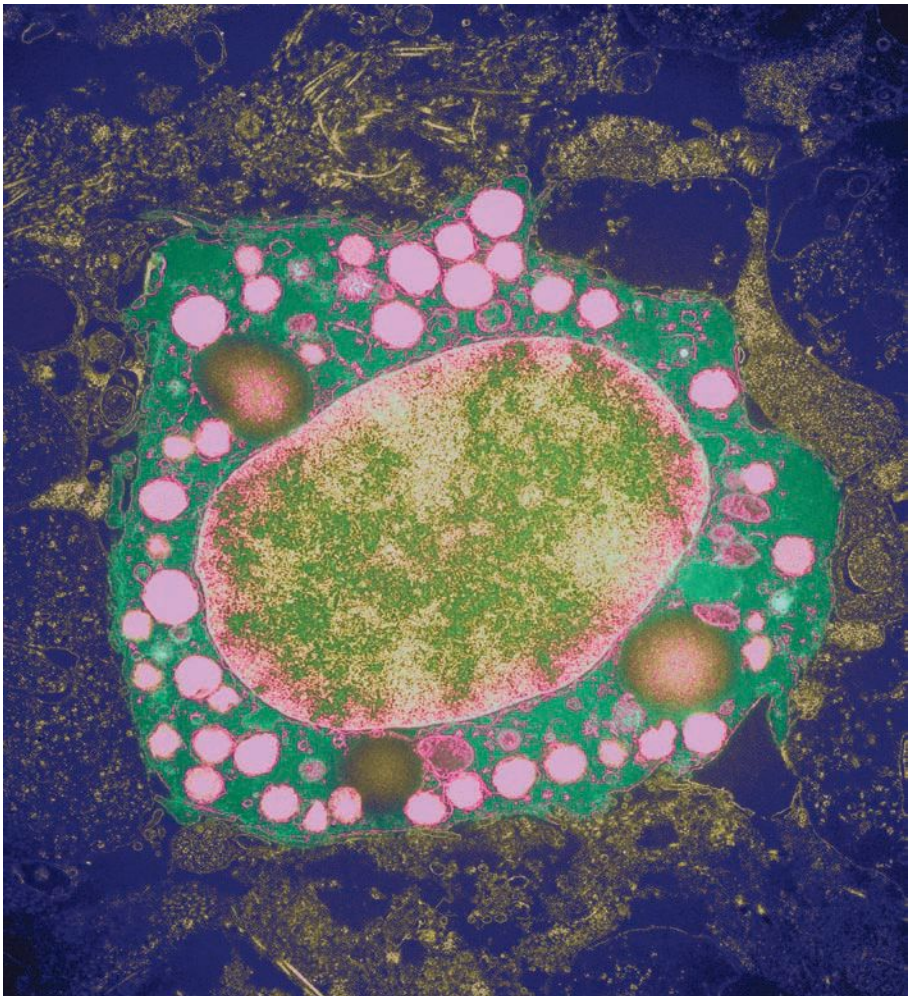
Others have proposed a link between behavior and innate immune cells called mast cells. Best known for their involvement in allergic responses in the upper airway, skin, and gastrointestinal tract, mast cells have been found in the meninges as well as in perivascular spaces of the thalamus, hypothalamus, and amygdala. They are known to quickly recruit large numbers of other immune cell types to sites of inflammation, and to play a role in MS. But mast cells also release serotonin into the hippocampus, where the molecule aids neurogenesis, supports learning and memory, and regulates anxiety.

Stress can signal macrophages to secrete proinflammatory cytokines, some of which block a protein called BDNF, which astrocytes need to support learning and memory.

Mice deficient in mast cells display deficits in hippocampal neurogenesis as well as in spatial learning.[22] The animals also appeared more anxious, taking more time to enter an open space, for example.[23] And Tufts University pharmacologist Theoharis Theoharides has found that human mast cells in culture respond to stress signals by releasing a growth factor that increases blood vessel permeability.[24]

Thus, like microglia, mast cells are a double-edged sword when it comes to neural health. It's a reflection of the entire immune system's love-hate relationship with the CNS, Kipnis says. "Saying the immune system is always good for the brain, it's wrong; saying it's always bad for the brain, it's wrong. It depends on the conditions." ■

Amanda B. Keener is a freelance science writer living in Winston-Salem, North Carolina.



HELP OR HARM: Mast cells (shown here) and other immune cells can support the health of the central nervous system and aid in injury repair and pathogen defense, but overactivation can lead to neural damage.

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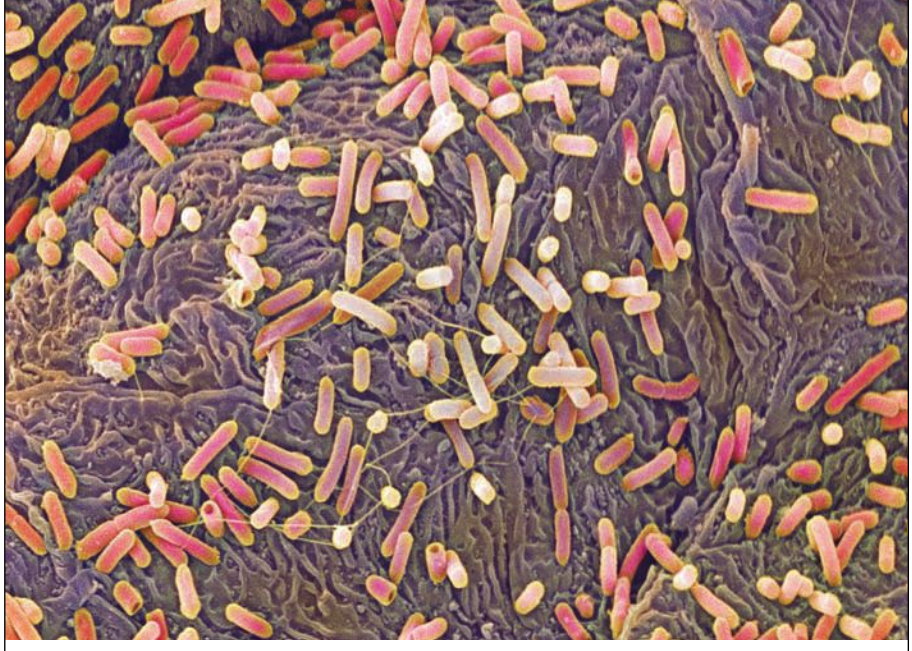


MICROBIOME MEDIATION

Seven years ago, Dartmouth Geisel School of Medicine immunologist Lloyd Kasper and his colleagues found that mice given a cocktail of gut bacteria–eliminating antibiotics were resistant to multiple sclerosis (MS). The mouse model used to study MS, called experimental autoimmune encephalomyelitis (EAE), involves immunizing the animals with peptides found in myelin to generate an immune attack. But when Kasper and his colleagues gave the mice antibiotics prior to immunization with two different myelin peptides, the animals did not develop EAE (*J Immunol*, 183:6041-50, 2009). Antibiotic treatment was also associated with more regulatory T cells (Tregs) and anti-inflammatory cytokines in the lymph nodes of EAE-resistant mice.

The work suggested a role for gut microbes in neuroinflammation. But like so many components of the immune system itself, the microbiome's relationship with CNS health goes both ways. Since his early studies, Kasper has found that resident gut microbes can program immune cells to protect the nervous system against immune overactivation. In 2010, his group showed that a gut bacteria–made sugar called polysaccharide A (PSA) activates a particular subset of Tregs that are induced by most MS treatments (*J Immunol*, 185:4101-08, 2010). Several studies have reported alterations in MS patient gut microbiota, though it's not clear whether those changes are a result or cause of disease. "You have this balance going on in the gut," Kasper says. "As long as everything is in balance, it's homeostatic and everybody's happy. But something happens in the [MS] disease process that that balance is lost."

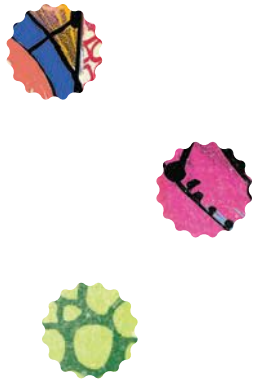
The gut microbiome's influence on the immune system has also been documented in mouse models of stroke, and appears to have similarly conflicting consequences. A study published last year showed that disrupting the gut microbial community with antibiotics reduced T-cell trafficking to the brains of mice after induced strokes, reducing neuronal damage (*Nat Med*, 22:516-23, 2016), for example, while another study indicated that a healthy gut microbiome is an asset for stroke recovery (*J Neurosci*, 36:7428-40, 2016). The authors of the latter study suggested that whether Tregs or proinflammatory T cells migrate from the gut to the brain after stroke may determine if the response promotes neuronal healing or damage.

The field is still young, but Kasper wonders if it might be possible to use gut microbes' immune-modulating capacities to enhance regulatory responses rather than suppress total immunity. This is especially needed for MS treatment, where most current drugs block immune activity wholesale. "We should be using agents that drive a positive response," he says. "If we can drive the immune system from the gut upward and do it in a positive way, I think that will give rise to greater benefit and far less side effects than [current drugs]."

The Good Viruses

Researchers now recognize that not all viruses in and on us are bad; some may even be beneficial.

BY ERIC DELWART



Since the dawn of microbiology, researchers have focused on pathogens that make us and our domesticated animals and plants sick. Because the onset of symptoms was the only way to know if specific viruses were present years ago, the most well-studied viruses are those that cause disease. But many viruses chronically infect humans without inducing disease, except perhaps in the very young, the very old, or the immunosuppressed.

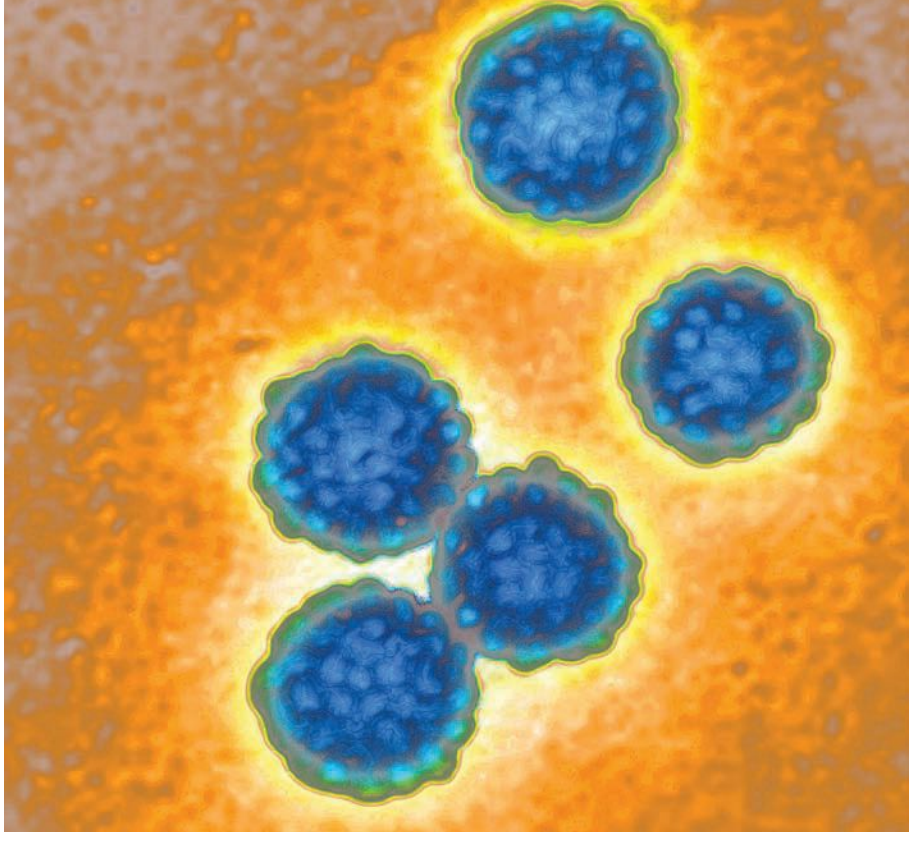
In recent years, great leaps in genomic sciences have allowed researchers to detect viruses living in and on the human body—collectively called the human virome. Recent genomic explorations of human samples have revealed dozens of previously unrecognized viruses resident in our gut, lung, skin, and blood. Some of these newly identified viruses may underlie mysterious, unexplained diseases, but it is also possible that some of these viruses are harmless in most people, most of the time. Knowing how these newly discovered viruses affect humans will allow us to determine whether they are to be prevented, treated, ignored, or even encouraged.

A spectrum of viruses

Researchers can now identify viruses present using metagenomic analyses. This is achieved by comparing the genetic information from next-generation sequencing of clinical samples to the genomes of all known viruses. These include viruses that infect all branches of life, from humans to plants and bacteria. When a sample contains a previously identified virus, its genetic sequences can show upward of 80 percent similarity to viral sequences in public databases such as the National Center for Biotechnology Information and the European Nucleotide Archive. Such similarities are easily identified computationally.

More challenging are novel viruses whose DNA or RNA genome does not show a significant match to that of any known viruses. In these cases, researchers can translate viral genes into proteins in silico and computationally search for related viral protein sequences. Due to the redundancy of the genetic code and the need to maintain basic protein structures and active sites, protein sequences evolve at a slower rate than their genes, and are therefore recognizable over longer evolutionary time.

Great leaps in genomic sciences have allowed researchers to detect viruses living in and on the human body—collectively called the human virome.



OCCASIONAL PATHOGEN: Human papillomaviruses can induce skin infections and cervical cancer, but many strains are commonly found on the skin of healthy people. Viral diameter approximately 55 nm.

The perception that every human virus causes disease is therefore yielding to a much more complex biological reality.

With this new ability to rapidly characterize viral genomes, data acquisition is outpacing our understanding of the viruses' role in health and disease. A few years ago, only two polyomaviruses were known to infect humans. Using metagenomics approaches, researchers have identified 13 known human polyomavirus strains, and have linked some of these with diseases ranging from neurological or kidney damage in immunosuppressed transplant and AIDS patients to skin cancers.[1] Most of these polyomaviruses infect a majority of people during childhood and are then silently carried until a weakened immune system unleashes them to wreak havoc.

Such occasional pathogenicity is typical of viral families found in humans. For example, some human papillomaviruses are found on the skin of most healthy adults and go unnoticed,[2] while a few specific papillomaviruses can induce cervical or anal cancers (now preventable by early vaccination). Similarly, herpesviruses are nearly universal infections in adults, where they set up lifelong, symptom-free residence in neurons or cells of the immune system. Later in life or following immunosuppression, latent herpesviruses can reactivate and induce diseases ranging from cold sores to meningitis, lymphomas, or Kaposi's sarcoma.

A rarely studied group of viruses called anelloviruses may claim the prize as the most common human viral infection; they can be detected in the blood of almost 100 percent of adults.[3] Anelloviruses are transmitted very soon after birth and multiple strains can establish persistent viremia in the same person. Because of their level of genetic diversity—the highest of any viral family—anelloviruses may infect different tissues with different consequences. And as with papillomaviruses, it is conceivable that only a subset of anelloviruses may turn pathogenic.

Whether such common and persistent viruses affect health is still being sorted out. A frequent consequence of chronic and acute viral infection is immune overstimulation. The increasing concentration of anelloviruses seen in immune-suppressed individuals indicates that anelloviruses remain under immunological control and may therefore result in low-level chronic inflammation, known to result in myriad health problems. (See "Is It a Pathogen?" on opposite page.)

Despite this potential for affecting health, there is as yet no direct evidence that anellovirus infections are harmful. Their ubiquity and lack of acute pathogenicity does point to a long and successful coevolution with humans. Because anelloviruses infect nearly everyone, however, their potential impact on heath is particularly difficult to determine. Fortunately, scientists have recently discovered anelloviruses in monkeys and rodents, providing means to study these viruses' pathogenicity in these animal models both in isolation and together with other common infections.

Beside the nearly universal blood-borne viruses described above, a cornucopia of other recently discovered viruses can be detected in respiratory and fecal samples of healthy persons, particularly children. These viruses include a growing number of astroviruses, parvoviruses, picornaviruses, picobirnaviruses, and others whose roles in health and disease also remain largely unknown. (See illustration on pages 44–45.)

IS IT A PATHOGEN?

Research funding has generally followed the actual or anticipated disease burden caused by clearly pathogenic viruses such as HIV, HCV, or, recently, Zika virus. Given the large number of viruses detected in healthy hosts, it is likely that some of the viruses initially found in sick hosts are simply harmless coincidental infections. Thus, before newly characterized viruses are deemed pathogenic, and therefore worthy of public or commercial investments, their disease-causing abilities must be stringently vetted.

To assess pathogenicity, researchers still rely on the four postulates for pathogenicity established by German physician and microbiologist Robert Koch in the late 1800s: 1) the agent is found in only those people with the disease, 2) the agent can be isolated from diseased individuals, 3) inoculation with the agent causes disease, and 4) the virus can be reisolated from the inoculated individuals.

But satisfying these postulates for human viruses is a tall order. Firstly, many viruses cannot be purified and grown in culture. Moreover, because human inoculations are unethical, researchers need to use animal models, such as rhesus macaques and mice—and many human viruses only infect humans.

Alternatively, researchers can try to demonstrate that the virus is found replicating at the site of pathology: the liver for hepatitis, for example, or the brain for encephalitis. Detecting only a single virus in diseased tissues—a feat made possible by deep sequencing—can also provide supporting evidence for its culpability. But this approach also has its limitations, as human necropsies are costly and thus rarely performed, often leaving blood as the only available tissue type for study. In such cases, measuring the emergence of antibody response to a new virus to show that the timing of the viral infection corresponds to the onset of the immune response can help identify a likely culprit.

Case-control studies that compare virus detection rates in patients or animals with similar symptoms versus healthy controls can provide powerful evidence of virus-disease association. Such studies control for age, geographic origin, gender, socioeconomic status, and even time of year of sample collection, leaving only the disease state to differentiate the two groups. Most viruses are neither consistently pathogenic nor always harmless, but rather can result in different outcomes depending on the health and immunological status of their hosts. The less pathogenic a virus is—the lower the percentage of infected people who become sick—the larger such case-control studies need to be to detect a difference between the groups.

This flood of new information regarding our virome indicates that, even when in perfect health, we are chronically infected by several types of viruses and often transiently infected by yet others. The perception that every human virus causes disease is therefore yielding to a much more complex biological reality.

Benefiting from our viruses

Viral infections at a young age may help our immune system develop properly, providing protection against later infections and preventing immune overreactions that lead to allergies. Viral infections of the respiratory and gastrointestinal tracts of healthy infants are now known to be common and often asymptomatic, likely thanks to protection by maternal antibodies delivered across the placenta and via breast milk. Such attenuated infections might provide a form of natural vaccination against later infections with related, more-pathogenic viruses. Just as the proper development of the human gut and immune system in infants is dependent on the presence of a bacterial gut microbiome, a recent study found that early enteric viral infection could have a similar beneficial effect in mice.[4] Specifically, mouse norovirus, a commensal relative of a common human pathogen, restored intestinal morphology and immunological function that was perturbed in germ-free or antibiotic-treated newborn mice.[5]

Commensal viruses may also provide protection against pathogenic infections with other viruses. Unexpectedly, a virus in the same family as hepatitis C virus (HCV), Zika, and dengue has been reported to mitigate the consequences of HIV infection. This virus, known as pegivirus C or GBV-C, was originally discovered in an unexplained case of acute hepatitis,[6] but researchers subsequently showed it to be a common infection unrelated to the disease. It's estimated that three-quarters of a billion people are persistently infected with pegivirus C, while even more possess antibodies from earlier, cleared infections.[7] Multiple studies have shown that HIV patients infected with pegivirus C tend to live longer than HIV-

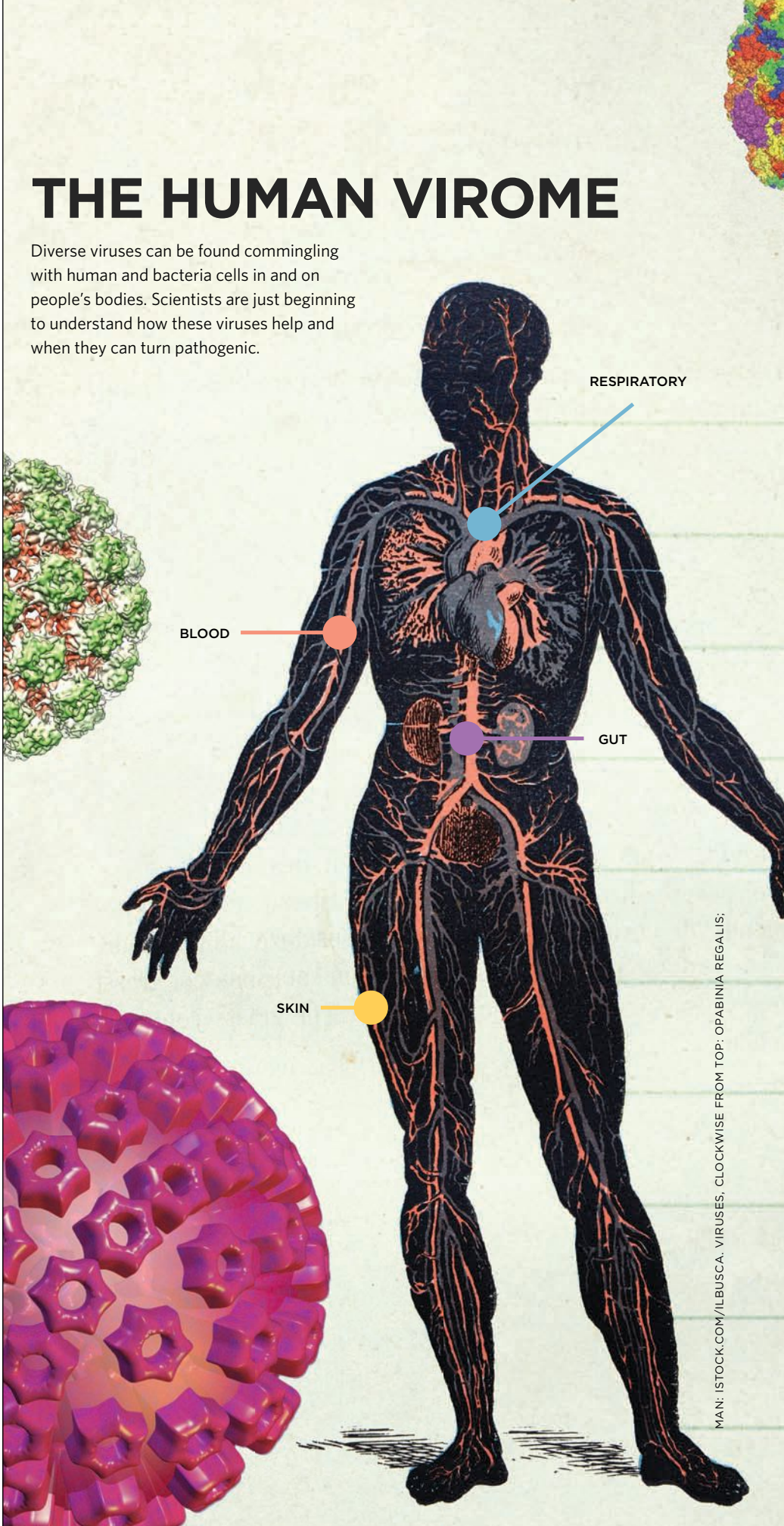
infected subjects without the coinfection.[8] The mechanism behind the phenomenon is unknown, but may involve blocking interactions with cell-surface receptors or intracellular components required for HIV replication.

Another potential benefit of resident viruses is related to their preference for rapidly dividing cells. Anecdotal observations of spontaneous cancer regressions coincidental with viral infections have indicated that viruses may preferentially infect cancer cells, and several promising oncolytic viral therapies are being developed to fight human tumors.[9,10] Whether viral infections and lysis of cancer cells is a common natural phenomenon remains an intriguing question.[11]

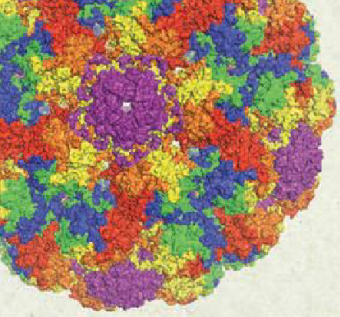
Viruses in our DNA

In addition to the viruses that can infect us, humans (and all other vertebrates) have traces of past viral infections integrated into our very own genomes. About 8 percent of the human genome consists of retroviral DNA sequences that have inserted themselves into the human germline, where some of their functions have been adopted to serve essential functions for their host's survival and development.[12]

Expressed proteins from such endogenous retroviruses can bind to and block cellular receptors that might otherwise be used by exogeneous, pathogenic retroviruses.[13] The membrane fusion activity of some endogenous retroviruses has also become essential for certain cellular functions of the host. For example, endogeneous retroviral envelope proteins are responsible for fusion of trophoblast cells into the structures of the mammalian placenta that mediate nutrient and gas exchange between maternal and fetal systems.[14] Recently, researchers found that one of these viral proteins essential to placental development, called syncytin, also increased fusion of myoblast cells during muscle-fiber formation: male mice, but not females, lacking this retroviral gene for syncytin showed a 20 percent reduction in muscle mass.[15] The same virus-descended gene involved in the formation of the placenta is also involved in a sexual dimor-



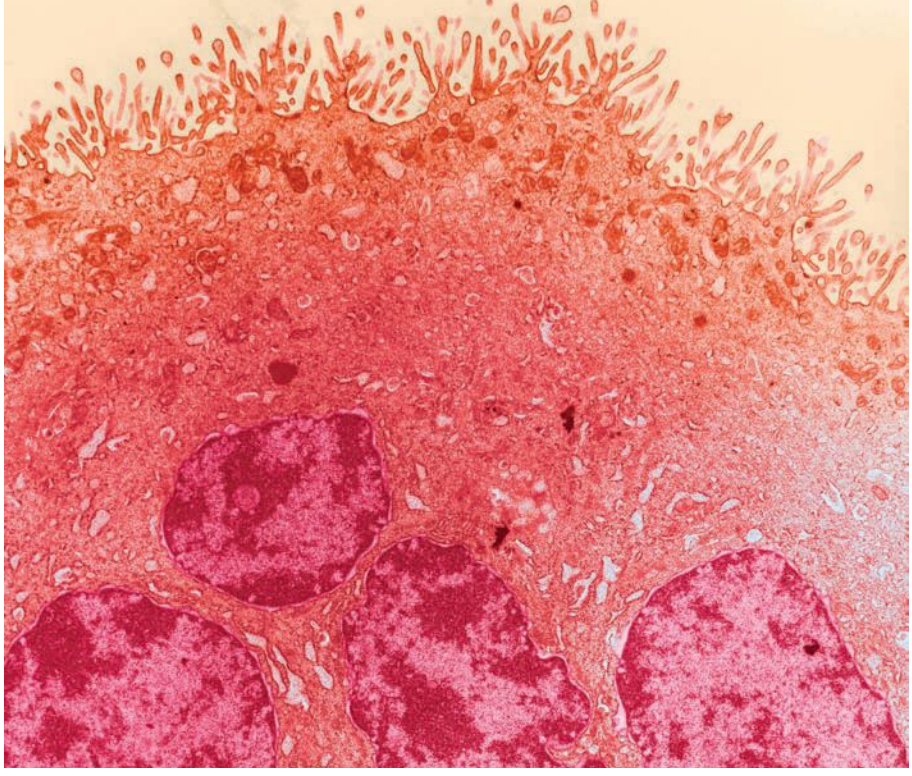
MAN: ISTOCK.COM/ILBUSCA. VIRUSES, CLOCKWISE FROM TOP: OPABINIA REGALIS;



VIRAL FAMILY	GENETIC DIVERSITY	WHERE IN BODY				PATHOGENIC AND/OR COMMENSAL?	
		BLOOD	GUT	RESPIRATORY	SKIN	PATHOGENIC	COMMENSAL
Adenoviridae			●	●		✓	✓
Anelloviridae		●				?	✓
Astroviridae		●	●			✓	✓
Flaviviridae		●				✓	✓
Herpesviridae		●				✓	✓
Papillomaviridae					●	✓	✓
Parvoviridae		●	●			✓	✓
Picobirnaviridae			●			?	✓
Picornaviridae		●	●	●		✓	✓
Polyomaviridae		●		●	●	✓	✓

LOW HIGH

CREDIT: NEUMANN, G.S., ROSSMANN, M.G.; MANUEL ALMAGRO RIVAS; BRUCEBLAUS; VOSSMAN; ALL WIKIMEDIA COMMONS



VIRUSES IN THE GENOME: Endogenous retroviral proteins are essential to cell fusion events that occur during the development of the mammalian placenta. A recent study also found that this same protein, called syncytin, increased myoblast cell fusion in male mice, helping them acquire muscle mass. (Shown here is a color-enhanced electron micrograph of a multinucleate syncytiotrophoblast layer of placenta.)

phism (greater muscle mass in males) typical of placental animals.

Vertebrates have also coopted a number of integrated retroviral promoters to provide a means for tight, coordinated control of the expression of multiple genes during early embryonic development.[16] Clearly, our very long evolutionary history in a bacteria- and virus-rich environment has driven human adaptation to many such infections, from the cellular level—domestication of retroviral genes and hyperreactive immune systems—to the cultural: adaptations intended to reduce the burden of infectious diseases.

Future of human viromics

The detection of viral infections will become faster and more sensitive as sequencing and computational platforms continue to improve, and as researchers generate a more complete catalog of human-infecting viral genomes. The ability to analyze a blood drop, respiratory swab, or fecal sample and report its complete viral content within hours or even minutes could enable public-health workers to rapidly understand and better control infectious disease outbreaks, and may one day become standard practice in diagnostic labs or even used directly by consumers. The rapid identification of known viral pathogens could reduce needless antibiotic use and the corresponding spread of antibiotic resistance in bacteria.

Genomic approaches will also allow large molecular epidemiological studies to measure exactly which viruses are associated with what diseases in different geographic regions. This information will determine which viruses are responsible for the greatest disease burden and help determine those vaccines and transmission-reduction steps that will be most effective. Ambitious plans are also afoot to sequence all viruses in all mammal species and to predict which are most likely to spill over into humans. It's also possible for human viruses to become more pathogenic through mutation or by recombination with animal viruses. A better understanding of what makes some viruses pathogenic, alongside constant monitoring of the human virome in health and in disease, particularly in hot spots of human-animal interaction, may provide early warning signs of the next great viral pandemic. ■

Eric Delwart is a senior investigator at the Blood Systems Research Institute and an adjunct professor in the Department of Laboratory Medicine at the University of California, San Francisco, where he investigates human and animal viromes.

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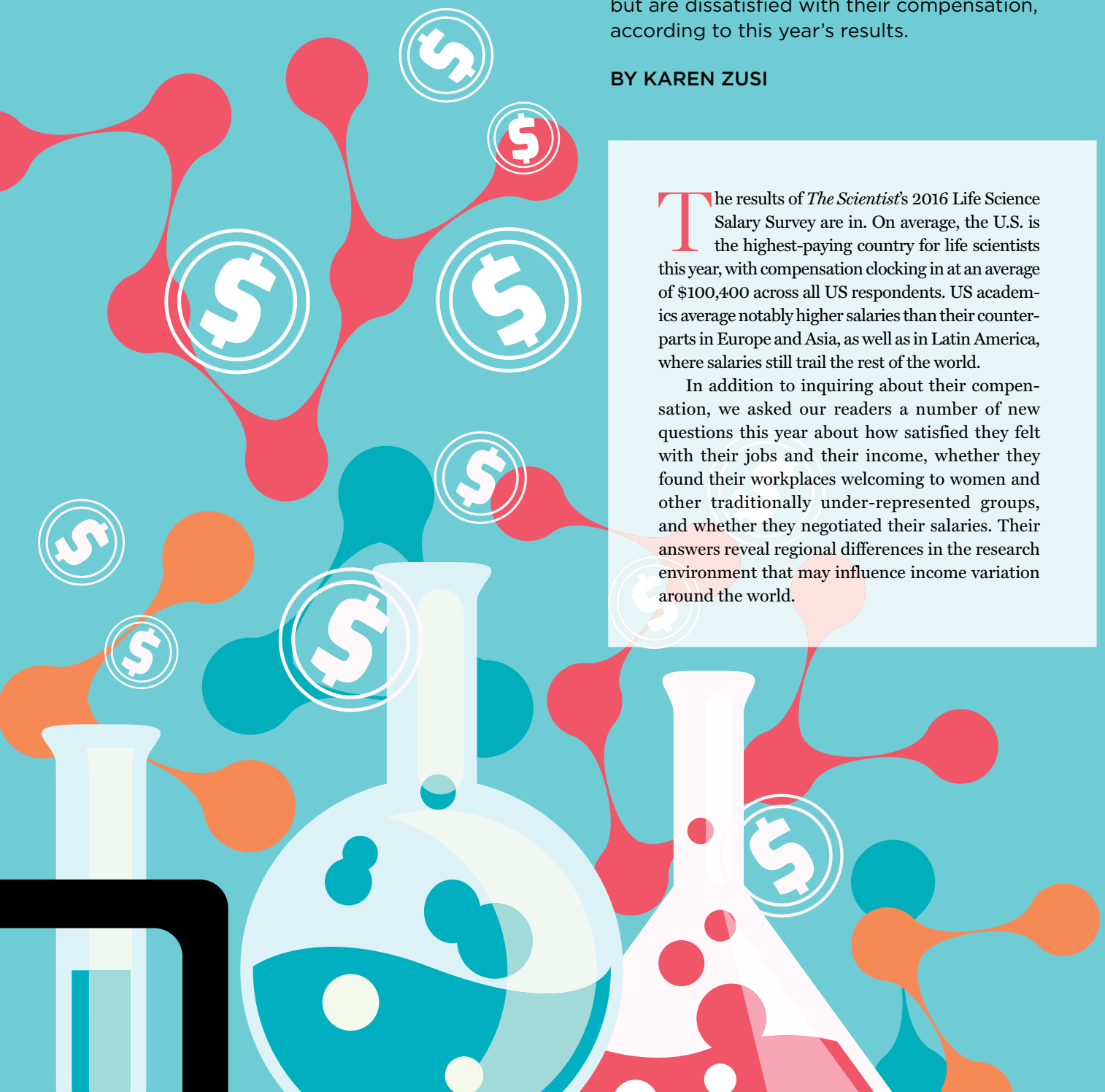
2016 LIFE SCIENCES SALARY SURVEY

Most researchers feel stimulated by their work but are dissatisfied with their compensation, according to this year's results.

BY KAREN ZUSI



The results of *The Scientist*'s 2016 Life Science Salary Survey are in. On average, the U.S. is the highest-paying country for life scientists this year, with compensation clocking in at an average of $100,400 across all US respondents. US academics average notably higher salaries than their counterparts in Europe and Asia, as well as in Latin America, where salaries still trail the rest of the world.

In addition to inquiring about their compensation, we asked our readers a number of new questions this year about how satisfied they felt with their jobs and their income, whether they found their workplaces welcoming to women and other traditionally under-represented groups, and whether they negotiated their salaries. Their answers reveal regional differences in the research environment that may influence income variation around the world.

LIFE IN ACADEMIA

Among academic scientists in the U.S., average salaries for different positions vary by a few thousand dollars from those in the 2015 salary survey, with a few exceptions. College or university department heads, for example, report the highest salaries at an average of $186,727—about $27,000 more than last year's reported average salary of just $159,022.

Comparing academic salaries in the U.S. and Canada with those in Europe—the two regions with the most responses—reveals that the disparity between regions increases as researchers move higher up the academic ladder. While graduate students have comparable salaries of around $29,000 on both continents, and postdocs in Europe actually earn an average of a few thousand more than postdocs in the U.S. and Canada, full professors in the U.S. and Canada make nearly twice as much as European professors, according to *The Scientist*'s data.

Academic salaries across Europe are set at the national level, creating an environment where institutions have less individual leeway in determining compensation. "The ways in which salaries are done in Europe are just so much different," says Stephan. "The local campus has virtually no ability to bargain with you or anything."

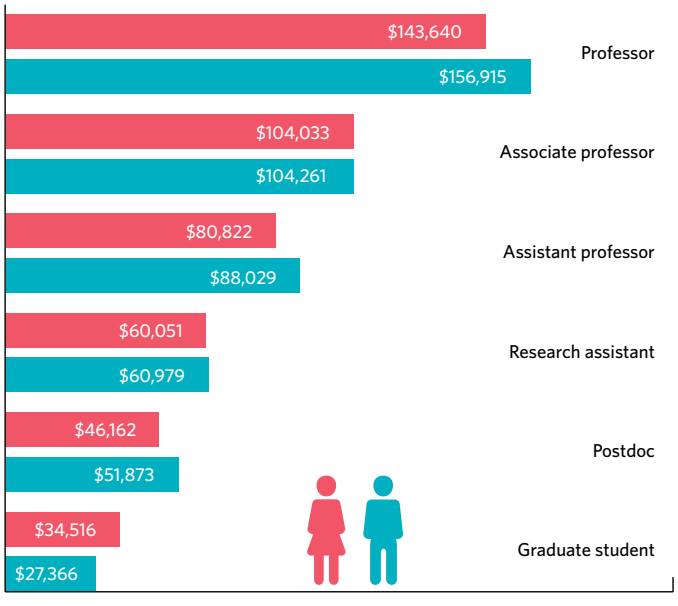
Gender gap in US academia

While female graduate students who responded to *The Scientist*'s 2016 survey make several thousand dollars more on average than their male counterparts, the tables turn among researchers further along in their careers. The largest gap is among full professors; females earn an average of $13,000 less than their male counterparts.

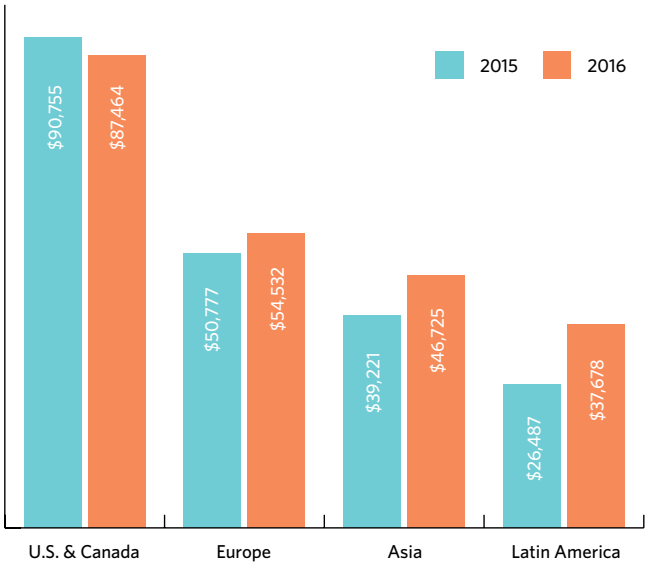
Economists have proposed a number of contributing factors to explain the gender gap in academia, including area of specialization and the nature of salary and start-up package negotiations. A study published earlier this year also suggests that marital status and the presence of children contribute significantly to the divide between men and women in both academia and industry (*American Econ Rev*, 106:333-38, 2016). Women in STEM careers surveyed for the study make 31 percent less than males a year after receiving their PhDs, and while much of the gap is attributable to their chosen research specialty, 11 percent is explained by marriage and kids.

It could be that women tend to work fewer hours or take time off when juggling family commitments, which can severely impact early-career wages. "You could say, 'Well, women are choosing to spend more time with their families and children, and that's a voluntary decision to scale back their hours,' but you could also say to yourself, 'Why is it that women make those decisions whereas men don't?'" asks Bruce Weinberg, an economist at Ohio State University and a coauthor on the study. "There's some indication that these jobs, these career tracks, are not fully family-friendly."

AVERAGE US SALARIES BY GENDER



AVERAGE ACADEMIC SALARIES



Postdocs in the U.S.

US postdocs responding to the 2016 salary survey earn an average of $48,907—notably more than the national average for federally funded postdoc salaries, which typically falls in the low $40,000 range, says Stephan. For example, in fiscal year 2016, the National Institutes of Health (NIH) National Research Service Award stipend for new postdocs was set at $43,692. "It makes me think either very senior postdocs have replied to [*The Scientist*'s] survey or there's something a little odd about the postdoc salary," Stephan adds. "It definitely does not reflect first- or second-year postdocs."

The national salary average is set to increase, however. Earlier this year, the NIH issued new guidelines regarding postdoc pay, requiring institutions to either raise the base salary for postdoctoral researchers to $47,476 across the board or start tracking hours to pay postdocs for overtime work. The new guidelines go into effect on December 1. "If you don't adopt it, you're going to have to be monitoring overtime work all the time, and that's a very costly thing to do," says Stephan. As a result, she suggests, universities may end up with fewer postdocs overall to avoid raising their budgets. Stay tuned for next year's salary survey to gauge the impact of the NIH's new program.

SPECIALIZATION OF US RESPONDENTS BY GENDER

SALARY BY SPECIALIZATION

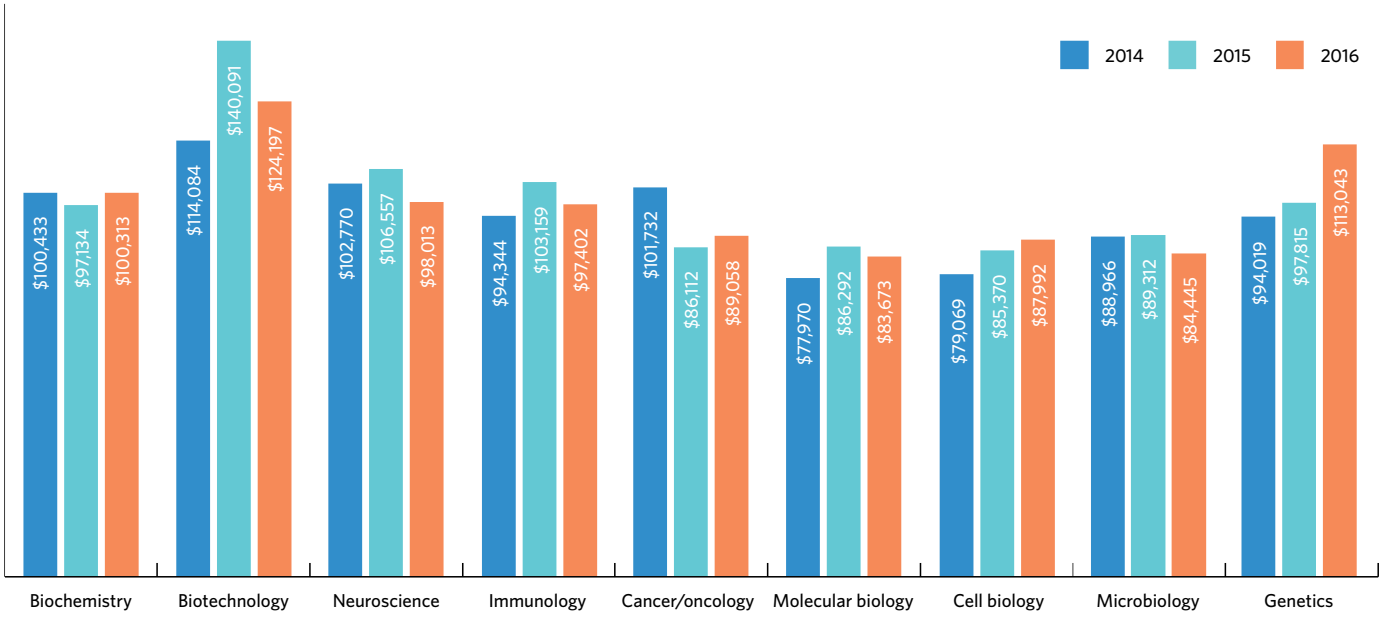
As in previous years, *The Scientist*'s 2016 salary survey documents notable differences in wages across fields of specialization. And the income variation by field may be skewed by a subject area's popularity among industry scientists.

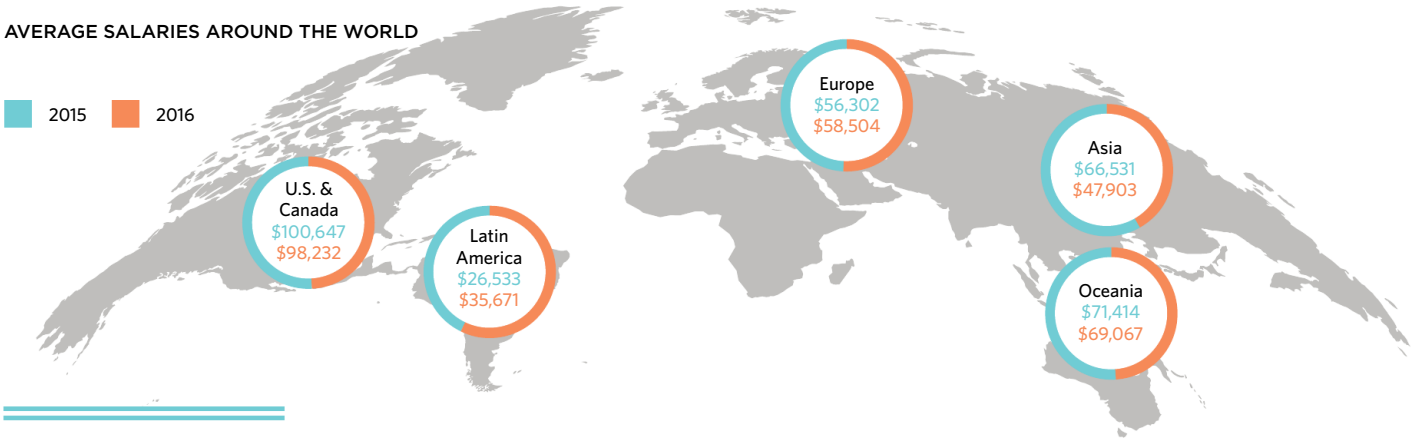
Of the fields with the most responses this year, biotechnology continues to lead the pack in the U.S., though by a smaller margin than in 2015. In 2014, the average biotech salary was $114,084, but in 2015's salary survey, that number skyrocketed to $140,091. This year, it's a bit closer to 2014 levels, at $124,197. Other fields, such as genetics, continue to grow; the average geneticist salary increased by more than $15,000 this year over 2015.

The 2016 survey responses also highlight variation in the gender ratios among scientists in different specialties. Only 23 percent of respondents studying bioinformatics are female, for example, and in the fields of biochemistry, chemistry, drug discovery and development, and environmental science, women account for only about a third of respondents. Cell biology, genetics, microbiology, and virology trend in the opposite direction, with females making up more than 55 percent of respondents.

In Weinberg's study of US women in STEM careers, his team found that two-thirds of the wage gap was explained by field of study. "You could say that's a choice," Weinberg says. "But you can also ask, 'Why is it that women choose one set of fields and men choose another set of fields that are generally better compensated?' I think there are a variety of explanations, some more benign and some less benign."

AVERAGE US SALARIES BY FIELD





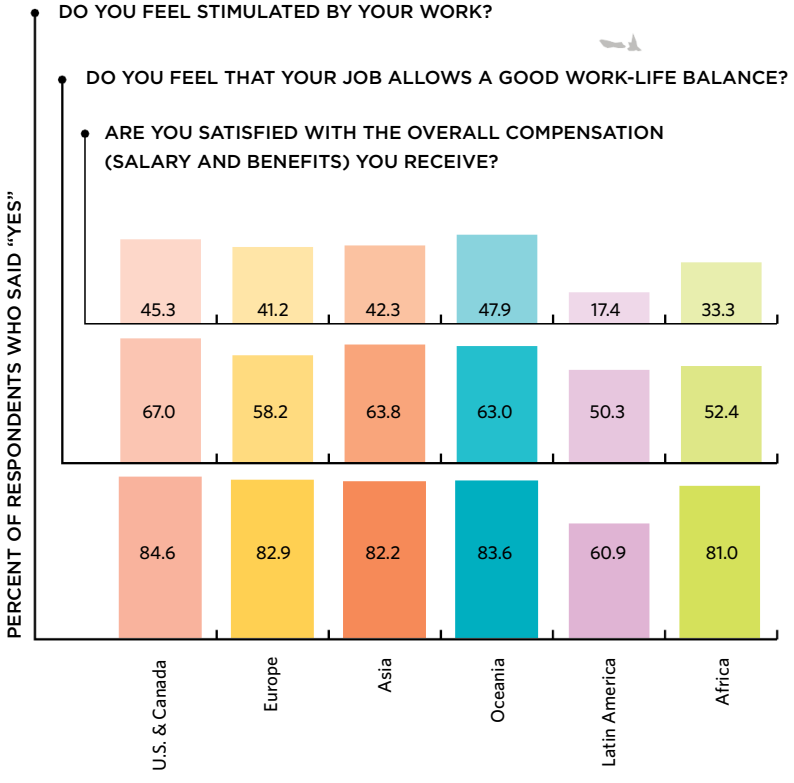
JOB SATISFACTION

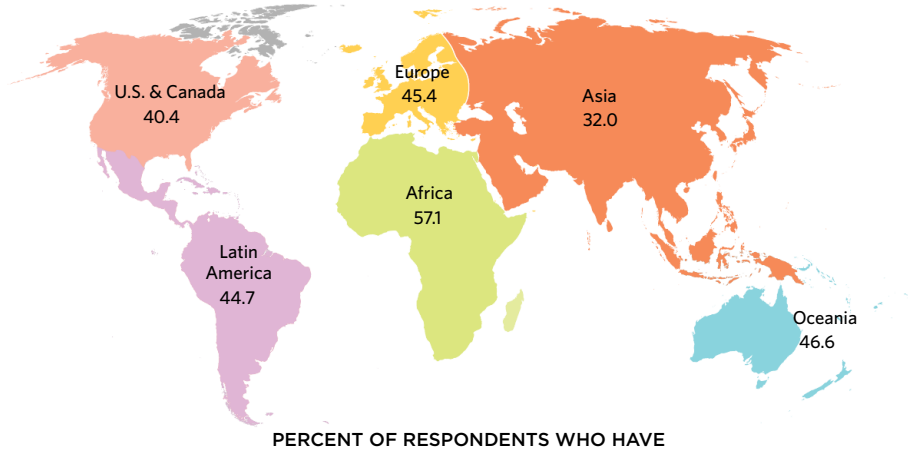
The world over, more than 80 percent of scientists say they feel stimulated by their work. However, fewer respondents feel that their job allows for a good work/life balance, and fewer than half of surveyed scientists in any given region are happy with their salary and benefits. Correspondingly, nearly half of respondents from most regions report having looked for new jobs within the past year.

Researchers in Latin America appear to be the least happy, with respondents reporting the lowest rates of feeling stimulated, having adequate work/life balance, and being satisfied with how much they make. This may stem in part from the region's low average salaries, which rank significantly below those in other parts of the world. In addition, "working conditions are far from ideal in the entire region," Natalia Muñoz Barreda, national coordinator for the Chilean scientist advocacy group Ciencia Con Contrato, wrote in an email to *The Scientist*.

In Chile, for example, the vast majority of salaries are funded by short-term grants, which have severely impacted the availability and shelf life of staff scientist positions at universities. "Research assistants and lab managers have been especially hit by our grant programs, not only with low salaries and nonexistent job security, but also with the lack of basic labor rights, such as maternity leave, the ability to unionize, [and] access to health insurance and pension plans," Muñoz Barreda wrote.

At the other end of the spectrum, respondents from the U.S. and Canada have the highest or second-highest scores in each of these job-satisfaction categories. Nearly 85 percent of US and Canadian researchers report being stimulated by their work. But Stephan is skeptical that the responses truly reflect the state of North American academia, especially in the overworked realm of post-graduation research positions. "I was astonished that two-thirds of people in the U.S. and Canada said 'yes' [to having a good work/life balance] given that one of the big concerns seems to be that postdocs, at least, are spending 2,500 or 2,600 hours a year working in these jobs," she says.





PERCENT OF RESPONDENTS WHO HAVE LOOKED FOR ANOTHER JOB IN THE PAST YEAR

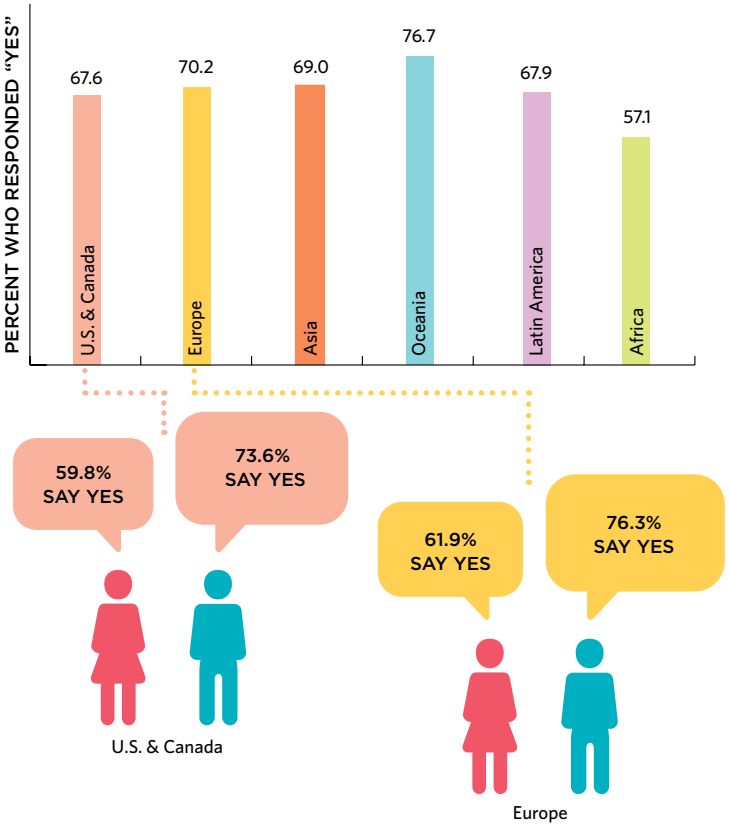
TOWARD AN INCLUSIVE ENVIRONMENT

Despite the wage gaps, representation of women and other traditionally under-represented groups in the life sciences is reaching higher levels in many areas of the world. When asked if women and minorities were adequately represented at their organization, most respondents answered yes. Oceania had the highest score, with 77 percent of scientists believing that these groups were represented adequately, and Africa had the lowest at 57 percent. Women in Africa face unique challenges to entering the sciences, says Unoma Okorafor, founder of the nonprofit Working to Advance STEM Education for African Women. "In many African communities still, almost 100 percent of the burden of caring for the home falls on women," she explains. "That's a big barrier for a lot of women to pursue professional careers." Moreover, across much of the continent "the sciences are seen as professions mostly reserved for men," Okorafor adds. "There is a huge lack of role models for other women to look up to."

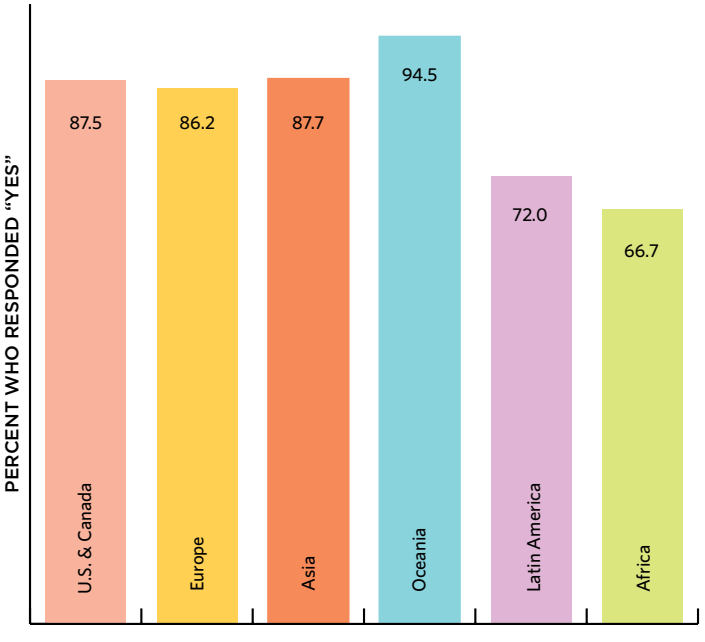
Among respondents from the U.S. and Canada and from Europe, breaking down these data by gender reveals that men were more likely to answer positively; approximately 75 percent of male respondents feel that women and minorities are adequately represented at their workplaces, while only 60 percent of women agree. But, even if women and minorities are not well represented, that might not mean they aren't welcome. When asked if their organizations were a safe and welcoming place for women and minorities, a greater percentage of respondents responded positively. A whopping 95 percent of respondents from Oceania feel their workplaces are welcoming to women and minorities, for example, and more than 86 percent of respondents from the U.S. and Canada, from Europe, and from Asia agree. Africa again had the lowest scores, with 67 percent of scientists agreeing. "We're making slow progress when it comes to helping communities understand the benefit of educating girls, and that is also filtering into the scientific realm," says Okorafor.

The Scientist's 2016 results suggest that scientists in Europe and Asia are the most mobile, with nearly 30 percent of researchers holding citizenship in a country other than where they work. In contrast, only 17 percent of respondents from the U.S. say that they are noncitizens. Stephan notes that this value seems quite low, however; for example, in a 2011 survey of biology, chemistry, materials, and earth and environmental sciences researchers, she and her colleagues found that 38 percent of researchers in the U.S. had a different country of origin (*Nat Biotechnol*, 30:1250-53, 2012). The U.S. is a global leader in educating foreign-born researchers, says Stephan, and when it comes to attracting scientists to the nation for training, the "research opportunities and the prestige of what the country has to offer are terribly important."

DO YOU FEEL THERE IS ADEQUATE REPRESENTATION OF WOMEN AND/OR MINORITIES AT YOUR ORGANIZATION?



DO YOU FEEL YOUR ORGANIZATION IS A SAFE AND WELCOMING PLACE FOR WOMEN AND/OR MINORITIES?



A SUCCESSFUL INDUSTRY

Of all the life scientists surveyed this year, directors in US industry have the highest average earnings at $204,016, a leap from last year's reported $178,457. Averaging across industry positions reveals that salaries in the sector maintain their dominant lead over academic salaries, with life scientists working in US industry earning an average of $132,121, compared with just $89,284 for those in academia.

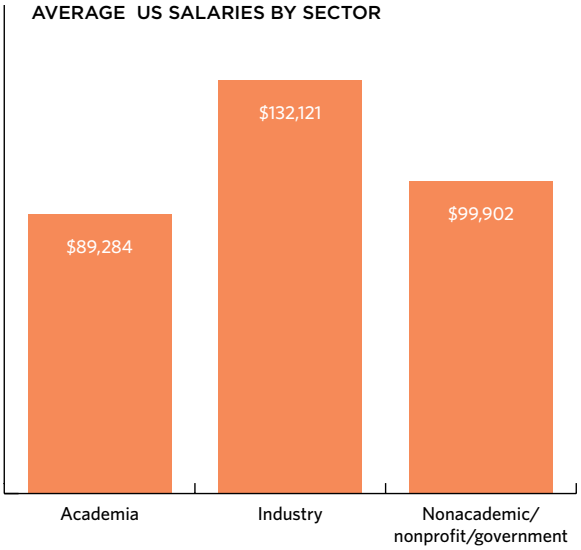
These sector-based income differences may contribute heavily to the salary gender gap when life scientists are grouped together across the board, says Shulamit Kahn, an economist at Boston University currently working on a study of the pay gap for women in STEM fields. "The single biggest contributing factor when you put everyone together is that way more men work in industry, with the industry pay scale," she says. Comparable numbers of men (890) and women (804) in US academia answered this year's survey, but 60 percent more male respondents (334) than female (213) work at for-profit companies in the U.S.

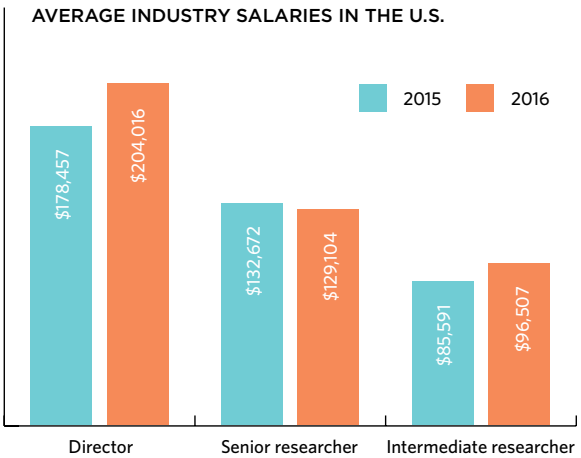
The higher wages could be explained by the fact that "industry simply has more resources because they're selling products," says Henry Sauermann, a business professor at Georgia Tech who studies the career paths of scientists. But there are other factors at play, he adds. Life-science companies vary widely in how much research freedom they offer their scientists, for example, and employees may have to seek permission to publish findings or collaborate with outside parties. "If it's true that industry gives you less freedom, and might require you to study something that you find less interesting, then they may have to pay more to make up for that," he says.

In Europe and in the U.S. and Canada, academics responding to this year's salary survey do have a slight edge over industry scientists when it comes to feeling stimulated by their work. However, a greater percentage of industry researchers report satisfaction with their work/life balance and salaries compared to those in academia.

Of course, salary is only one factor on the table when graduate students and postdocs make career choices. "People go on to a PhD because of their passion for doing research and because of their interest in science, not because of the expectation of making a high salary," says Michael Roach, an economist at Cornell University and a frequent collaborator of Sauermann. If income were a primary motivator, he adds, "I think there'd be more selection early on in undergrad [toward] careers that would lead into banking or consulting." ■

Karen Zusi is a freelance science writer living in southern Connecticut.





See more data and discussion at the-scientist.com, including information on salary negotiation trends in the U.S.

SURVEY METHODOLOGY

The Scientist collected data via a Web-based survey, which was open from March 18 to July 19, 2016. Participation in the survey was promoted by email and advertising to readers of *The Scientist* and visitors to the-scientist.com. The responses were carefully filtered to eliminate duplicate or misleading answers, as well as to eliminate reported salaries greater than $1 million or less than $10,000. We received usable responses from 4,036 individuals from around the world.

The survey asked respondents to provide demographic data about themselves in 18 categories, and to report their base annual salary and other monetary compensation. All international salaries were converted to US dollars on July 30, 2016, and analyses were done using the US-equivalent amount. For year-over-year comparisons, data from previous surveys were first converted into USD using the conversion rates from July 30, 2016, to control for inflation. The data reported are averages of the total compensation reported for a given category. For average salaries, all categories reported received a minimum of 50 responses; for other questions, all categories reported received a minimum of 20 responses.

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- An improved tool-kit for interpreting and qualifying ELISpot assays

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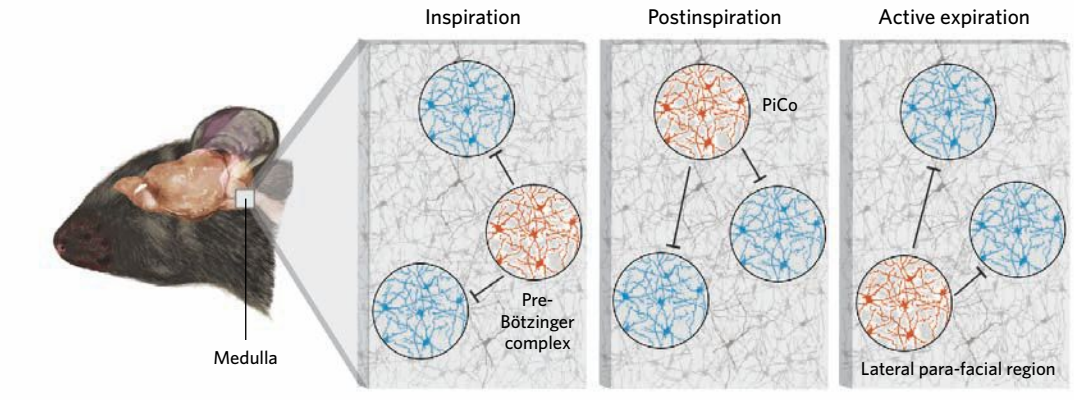
The Literature

EDITOR'S CHOICE IN NEUROSCIENCE

Waiting to Exhale



THREE-PART RHYTHM: There are three stages to mammalian breathing: inspiration, passive expiration (postinspiration), and active expiration—a conditional phase used during labored breathing. Inspiration and active expiration have been linked to rhythm-generating excitatory neural circuits in the medulla: the pre-Bötzinger complex and the lateral parafacial region, respectively. A recent study has revealed a third excitatory network—the postinspiratory complex (PiCo)—that drives postinspiration, suggesting that the coordination of breathing may rely on alternating inhibitory interactions between three networks.

THE PAPER

T.M. Anderson et al., "A novel excitatory circuit for the control of breathing," *Nature,* 536:76-80, 2016.

A lot can happen after we take a breath—from swallowing a sip of coffee to singing in the shower—and the nervous system has to coordinate all these behaviors without sending fluids into the lungs or disrupting airflow. But studying the neural control of breathing has been a challenge, not least because researchers haven't found all the circuitry involved.

Two breathing phases, inspiration and active expiration (the forced expulsion of air during labored breathing), have each been linked to rhythm-generating excitatory networks in the medulla, the lowest portion of the brainstem. But scientists have been stumped as to the source of excitation generating the third: the passive release of air from the lungs after breathing in, or postinspiration. From this incomplete picture, most models of breathing have assumed that just two rhythm-generating circuits—inspiratory and expiratory—set the timing of all three breathing phases, with coordination coming about as each active phase inhibits the other two.

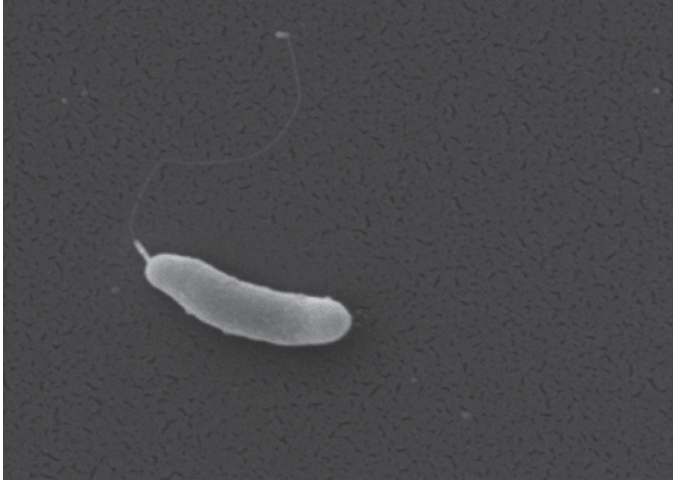
Jan-Marino Ramirez, a neuroscientist at the University of Washington, has spent much of his career working to refine this model. Over the last decade, he and his colleagues have developed a preparation of horizontal brain slices from baby mice that provides a broad in vitro view of neural activity in the medulla. Using this preparation, the team has finally discovered the excitatory network that generates postinspiration, which the group has named the postinspiratory complex (PiCo).

After so many years of unsuccessful searching, "I thought, 'Oh, maybe it's something else,'" Ramirez remarks about initially finding the PiCo. "But then we started the research—isolating this area and showing it's an independent neural network."

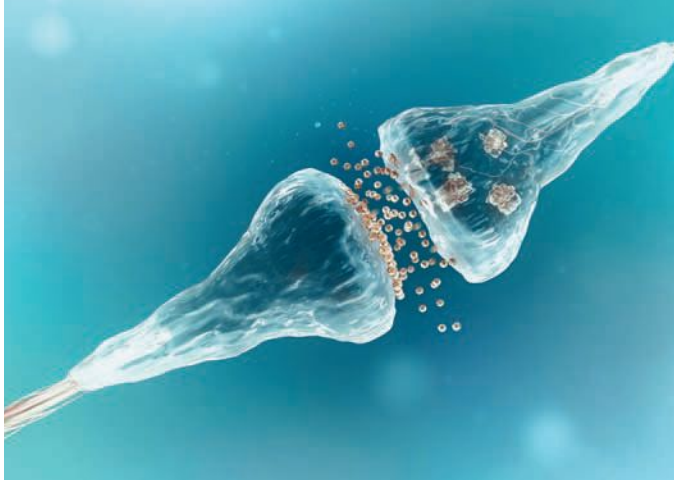
Through pharmacological and optogenetic experiments, the researchers demonstrated that the PiCo is necessary and sufficient to generate postinspiration in vitro and in adult transgenic mice. What's more, like the networks driving inspiration and active expiration, the PiCo appears to generate its own rhythm. "That was astonishing to us," Ramirez says. The team is now exploring a model of breathing coordinated by the interactions of three, not two, rhythm-generating excitatory networks.

"There are a number of seminal results presented here," says neurobiologist Jeffrey Smith of the National Institute of Neurological Disorders and Stroke who was not involved in the work. He adds that the experiments themselves were "technically sophisticated and involved a variety of elegant approaches," but that the network's architecture and activity in vivo will require further investigation.

Ramirez plans to examine the PiCo's influence on actions occurring during postinspiration. "Can we interrupt vocalization, for example, or swallowing?" Such experiments might explain what happens when the coordination of breathing goes wrong—a common problem that leads to aspiration pneumonia in people with certain neurodegenerative diseases. It could also open a window on how and why we coordinate certain other behaviors, such as the holding of breath during concentration, Ramirez notes. "I think these circuits will allow us to probe higher brain functions," he says, "which I find very, very exciting." —Catherine Offord



HOOKED: *Marinomonas primoryensis* (about 1.5µm in length without the flagellum) latches onto ice floes in the Antarctic Ocean.



DOCKED: Vesicles continue to release neurotransmitters into the synapse even after researchers disable docking scaffolds.

MICROBIOLOGY

Ice Fishing

THE PAPER

M. Bar Dolev et al., "Putting life on ice: Bacteria that bind to frozen water," *J R Soc Interface*, 13:20160210, 2016.

ANTARCTIC ANOMALY

In 1999, Jack Gilbert set off for Antarctica looking for a natural antifreeze. Out among the sea ice, the microbial ecologist, now based at Argonne National Laboratory, found a bacterial antifreeze protein (AFP) called *Mp*IBP that was hundreds of times larger than other known AFPs. It was an enigma, he says. "For years, I've been telling people we don't really know what this protein does."

FISHING FOR ICE

Scientists now have a handle on its function. Ido Braslavsky of the Hebrew University of Jerusalem and colleagues placed *Marinomonas primoryensis*, which produces *Mp*IBP, into a microfluidic flow chamber with a copper wire kept at sub-zero temps and embedded in the middle. Micrographs of bacteria streaming by the ice crystals around the wire showed the cells latching on. When the team introduced antibodies that disabled *Mp*IBP, the bacteria slid off the ice, suggesting that the protein—which is shaped like a fishing line with a hook on the end—enables bacteria to cling to ice floes in their ocean habitat, says Braslavsky. It's the first bacterial adhesion molecule discovered that sticks to ice.

CRYSTAL-CLEAR CUSTOMIZATION

The researchers built their device to have temperature control with millikelvin precision, crucial for studying ice because "it can just disappear" mid-experiment, says Braslavsky. Plus, the chamber's thin design allowed for high-resolution images of individual bacteria bound to the ice.

BEYOND ANTARCTICA

Manipulating the adhesion protein with antibodies allowed Braslavsky's group to disable specific structural regions one at a time, showing that only one domain in the "hook" at the very tip of *Mp*IBP grabs onto ice. Adhesion-blocking antibodies could help prevent biofilm formation, says Braslavsky.

—Ben Andrew Henry

NEUROSCIENCE

Resilient Neurons

THE PAPER

S.S.H. Wang et al., "Fusion competent synaptic vesicles persist upon active zone disruption and loss of vesicle docking," *Neuron*, 91:777-91, 2016.

HAIR TRIGGER

Neurons send each other signals by firing neurotransmitters across synapses. A stash of neurotransmitter-packed vesicles hunkers close to the presynaptic membrane so the vesicles can fuse with the membrane and release their cargo as soon as an electrical impulse pulls the trigger. An elaborate scaffold of proteins coordinates this vesicle docking, and scientists long assumed these proteins were essential to neuron signaling. For years, Harvard Medical School neuroscientist Pascal Kaeser has wanted "to test that very fundamental hypothesis" by "entirely [removing] that structure."

STILL TALKING

Kaeser and his colleagues recently got their chance. By genetically altering mice to disable key scaffolding proteins, they prevented vesicles from docking at the presynaptic membrane in cultured neurons. The results "blew us away," Kaeser says. He expected a total shutdown of neuron signaling, yet vesicles continued to fuse with the presynaptic membrane, though much more slowly.

A SIMPLER TIME

By stripping out scaffold proteins, the Harvard team might just have turned back the evolutionary clock, says Reinhard Jahn of the Max Planck Institute for Biophysical Chemistry in Germany. Neurons evolved those proteins as improvements to accelerate regular pathways for vesicle secretion, says Jahn. "Maybe we reduced a synapse to one of these more simple pathways of secretion, and that pathway still works," Kaeser observes.

REASSEMBLY

Defective docking sites are implicated in a range of neurological disorders, from autism to schizophrenia, Kaeser says. "If we could disrupt the entire thing, maybe we could start understanding . . . how it is put together."

—Ben Andrew Henry

Time, Flies

By studying the sleep-wake cycle of fruit flies, Amita Sehgal is revealing how the body's circadian and sleep rhythms are regulated.

BY ANNA AZVOLINSKY

In January 1983, 22-year-old Amita Sehgal arrived in New York City from India to visit her oldest sister, who was due to have a baby. Sehgal had just been rejected from the molecular biology PhD programs at Rockefeller University and Columbia University. "I felt that I had no prospects," says the University of Pennsylvania professor of neuroscience. She had heard about a Cornell University in NYC, so she and her other sister walked the streets of Manhattan asking its whereabouts. "Someone told us Cornell was hundreds of miles away in Ithaca, and that I must have been asking about the medical school. I had no idea, but I said 'Yes' and was directed to the Upper East Side." Sehgal walked into the medical school, inquired about their PhD program, and was told that the application deadline for the program was that very day. "I sat in the office and filled out the application, wrote my essay, and handed it in!" she says. A few months later, Sehgal was admitted into the genetics program.

"If someone during my postdoc had asked me if flies sleep, I would have said, 'Sure, why not.' But no one asked."

Sehgal's parents had also joined the visit and were returning to India in July, shortly before she started the PhD program. "It was fortuitous the way things worked out. My parents were comfortable leaving me in New York because my oldest sister was living there." One month later, however, her sister and family moved to Florida, and Sehgal was alone, living in Cornell housing. "The first six months were really, really rough," she says. Cornell had dissolved the genetics program to which Sehgal had been admitted and offered her tuition support with no stipend—and that only for the first semester. "My parents and sister were in no position to help me financially," she says. Sehgal found a professor at the adjacent Memorial Sloan Kettering Cancer Center (MSKCC), Raju Chaganti, who gave her part-time work with no expectation that she join his lab. She had little money and survived on ramen noodles. "Everyone jokes about living on ramen noodles in college, but I literally did. You could buy four to six packets for $1 depending on how good the sale was." But by the end of her first semester, Sehgal's situation took a turn for the better. The chair of the cell biology program picked up Sehgal's tuition and stipend support. By the end of the year, she had joined Moses Chao's lab and was trying to clone the gene for the human nerve growth factor (NGF) receptor.

After focusing on neuronal development during her PhD studies, Sehgal switched to studying circadian rhythms in fruit flies as a postdoc. As a professor, she continued to unpack the molecular mechanisms of biological clocks—and since discovering that fruit flies sleep, Sehgal's laboratory has continued to use *Drosophila melanogaster* to probe the molecular circuits that govern sleep. Here, Sehgal talks about how, as a graduate student, she set off the emergency shower in the lab; her rash decision to study circadian rhythms; and why she has never generated beautiful data.

SEHGAL SEEKS

On the move. Sehgal was born in New Delhi. Because her father worked in the Indian government's tourism department, the family moved around while she was growing up, first to Frankfurt, Germany, where Sehgal attended kindergarten and first grade, then to Calcutta, back to Delhi, and on to Kashmir. When she was in ninth grade, Sehgal's parents sent her to live with relatives in Meerut, a city near Delhi, so she could attend a high school they thought was better than the ones in Kashmir. When she had to select an education track toward the end of high school, she chose a science track because that's what her friends were doing, but coupled it with English literature, which she most enjoyed.

Uninspired science. Sehgal attended an all-girls' college at Delhi University, graduating in two years with a degree in biology. The two-year option was available for specific majors because the college system had not yet been synced with India's new 12-grade high school program. The curriculum was old-fashioned chemistry, botany, and zoology with an emphasis on rote memorization, according to Sehgal. "Part of the reason I was not interested in science was the way it was taught. There was no effort to make it interesting." After graduating in 1980, Sehgal was accepted to law school. She also applied to a prestigious molecular biology master's program at Jawaharlal Nehru University in Delhi. To her surprise, she got in and, following the advice of friends and family, chose the master's program.

At a crossroads. While Sehgal was completing her master's, her family moved to Perth, Australia. She joined them after completing the program in 1982 and worked in a laboratory at the Royal Perth Hospital, studying DNA repair in muscular dystrophy patients. But she hated the monotonous cell irradiation and counting. "I was worried that I needed to make a career decision and didn't know what to do," says Sehgal. With four years invested in science, she decided to apply to graduate school in the U.S.

JEFF FUSCO PHOTOGRAPHY

AMITA SEHGAL

Professor of Neuroscience, Perelman School of Medicine, University of Pennsylvania
Howard Hughes Medical Institute Investigator

Greatest Hits

- In *Drosophila*, identified *timeless*, the second circadian rhythm clock gene found
- Described how light sets the timing of the *Drosophila* clock
- Discovered that the fruit fly's rest phase is actually a form of sleep and established *Drosophila* as a genetic model of sleep
- Isolated sleep-regulating genes and a site in the *Drosophila* brain that controls sleep
- Discovered molecules that regulate sleep, such as sleepless; identified the cAMP pathway as a regulator of sleep and identified the mushroom body in the fly brain where the pathway acts

What bands? "The first semester was miserable for many reasons," says Sehgal of her initial time at Cornell. "I didn't know if I would be there after the semester, and the classes were really hard. I had never read a research paper before. We would discuss bands on gels and I had no idea what anyone was talking about! 'What is a gel and what is a band?' I thought." Sehgal met an older Indian graduate student from Canada. She confided in him how worried she was about failing out. "'One thing you need to learn about Americans is that they always act like they know everything,' he said to me. He turned out to be mostly right. After our first exam in biochemistry, I got the second highest score, which was a big shock to me. I thought everyone knew more than me. But it was also a shock to the other students. Five people had failed the exam, and someone asked me if I was one of them."

SEHGAL STEADIES

Last place. During her rotation in Chao's lab, she tried to clone the gene for the human NGF receptor (NFGR) by transfecting human DNA into mouse fibroblasts. By the time she moved on to her other rotations, Chao had verified that the human DNA left after her transfections was indeed the *NGFR* gene. When the gene cloning was published in *Science*, Chao made Sehgal last author. "I can only guess, but I think he had to be first author because cloning this gene was something he had always wanted to do; it was his project. At the same time, he wanted to give me the next-best authorship," Sehgal says.

Fly clock. For her postdoc, Sehgal wanted to continue to study neuronal development but switch to *Drosophila*, a system that was malleable to genetic manipulation. Rather than doing a postdoc in California as she had planned, Sehgal applied to join Michael Young's lab at Rockefeller University in NYC, as her fiancé and now husband, Jeffrey Field—presently a professor of pharmacology at Penn—had recently been promoted to staff member at the Cold Spring Harbor Laboratory.

During her interview, Young had handed her an accepted manuscript on circadian rhythm, and Sehgal switched to working on biological clocks. Following discussions with Young, she began a genetic screen to identify novel circadian rhythm mutants. "I was very naive. Had I stopped to think whether I should do this screen, the answer would certainly have been 'no,' because it had been 20 years since the first clock gene was identified." Sehgal screened mutations for deviation from the timing of eclosure—the emergence of adult flies from their pupae—which occurs at dawn

in wild-type flies. She also had to set up equipment to monitor flies' rest-activity cycle (used as a readout for circadian rhythm mutants), which required tracking down discontinued Apple 2E computers that could support the analysis software. After about a year of setup, another postdoc, Jeff Price, joined the project, and together they identified *timeless*, a novel circadian rhythm mutant with alterations in both rest-activity behavior and eclosure timing. The *timeless* gene—not linked to the mobilized P-elements used to mutagenize the flies—took yet another year to clone, which Sehgal and Young accomplished after she became a Penn faculty member in 1993. In an accompanying paper, Sehgal described how the periodicity of *timeless* transcription controls the accumulation and nuclear localization of the protein of the other known circadian rhythm gene, *period*. In 1996, Sehgal's graduate student Melissa Hunter-Ensor found that the Timeless protein is degraded by light, "providing a mechanism of how the clock is reset daily," says Sehgal.

Fly sleep. "It was known that flies had a rest-activity cycle, which we used to determine what the internal clock was doing, but sleep was not studied as a behavior in and of itself. If someone during my postdoc had asked me if flies sleep, I would have said, 'Sure, why not.' But no one asked," says Sehgal. It was only after she came to Penn that she began to interact with researchers at the university's Sleep Center and started to wonder if flies actually did sleep. Joan Hendricks, a Penn School of Veterinary Medicine sleep researcher, came to Sehgal's lab to learn molecular biology. On the heels of using genetics to identify circadian-rhythm genes, Hendricks and Sehgal wanted to see if they could establish the fruit fly as a sleep model. "Joan basically locked herself in the dark room with a safe light and watched the flies, because we needed to see what the behavior looked like. We decided to forget about EEG tracking in flies and looked for other sleep characteristics: control by a circadian clock, reversibility, an increased arousal threshold to sensory stimulation, and most importantly, the need to make up for loss of the rest state when deprived of it," says Sehgal.

In 2000, they showed that the flies' rest-like state is indeed sleep and that human sleep-perturbing drugs can also alter sleep patterns in flies, suggesting similar neurochemistry is at work. "The definition for fly sleep is five minutes or more of inactivity. Less than five minutes and they are still responsive to gentle stimulation," explains Sehgal. To monitor the flies' sleep and activity states, her lab continues to use an activity monitoring system similar to the one she set up during her postdoc: researchers place flies individually in small tubes, and assay their movement through deflection of an infrared beam projected into the tube. For sleep deprivation, they attach vials of flies to a shaker that rotates randomly, giving the insects jolts that wake them up.

Sleep and memory. In 2006, postdoc William Joiner homed in on the cAMP/protein kinase A (PKA) pathway as a promoter of fly wakefulness and identified the mushroom body, the part of the fly brain associated with memory, as the region of the brain that regulates sleep. "This supported the idea that sleep may be required for consolidation of memory," says Sehgal.

New sleep gene. Kyunghee Koh, another postdoc in the lab, collaborated with Joiner and postdoc Mark Wu on the lab's first unbiased genetic screen for short-sleeping fly mutants in 2005. They found that inactivation of the *sleepless* gene in flies results in a greater than 80 percent reduction in sleep and cuts their life span in half. The gene codes for a small molecule enriched in the fly brain that modulates ion channel activity.

SUPER SEHGAL

Blooper reel. "I was really green when I started graduate school. I didn't know simple things like what ethidium bromide was, and when I did my first library screen, I threw away the nitrocellulose with the bacterial colonies and tried to plate the spacer on the petri dishes. My graduate story is filled with disasters. I started a fire in the lab with the flame that was kept in the hood and the firemen came. Another time, I flooded the lab. I was talking to someone and playing with the emergency shower string that was hanging above me. I gave it too strong a tug and the showers went off. I was jumping to catch the string trying to turn it off. Meanwhile, the guy I was speaking to walked away and came back with a bar of soap and a towel for me as a joke."

"Sleep also affects organs other than the brain, although the sleep field has been very brain-centric. I am very interested now in whether peripheral organs contribute to sleep."

Convincing results? "I have really bad lab hands. I have never generated beautiful data. Every experiment I did had to be done a billion times, to convince myself and everyone else that I had a result."

Beyond the brain. "We don't know if there are dedicated sleep molecules. All of the genes we find in our screens also regulate neural activity and plasticity and some roles in learning and memory. [The genes are] not like the dedicated biological clock genes. Sleep also affects organs other than the brain, although the sleep field has been very brain-centric. I am very interested now in whether peripheral organs contribute to sleep."

Getting your ZZZZs. "Since I was a kid, sleep has always been super important to me. My parents never had to make me go to bed. Yet my daughters don't like to sleep, and my older daughter in particular had problems with sleep when she was little. I tease her that I want to sequence her clock genes because I think she has delayed sleep phase syndrome."

Homebody. "We still live in the same house that we moved into when we first started at Penn in 1993. After being such a nomad, I never thought I would end up living in the same house for more than 20 years." ■

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Jason Castro: Passing the Smell Test

Assistant Professor, Bates College. Age: 37

BY BEN ANDREW HENRY

As a graduate student in the field of olfactory neuroscience, conducting what his former mentor describes as ambitiously clever research, Jason Castro felt something was missing. "I wanted to use science to make a connection with people," he says, not just to churn out results.

In 2012, the 34-year-old Castro accepted a faculty position at Bates College, a small liberal arts school in Maine, in order to "do the science equivalent of running a mom-and-pop—a small operation, working closely with students, and staying close to the data and the experiments myself," he says. Students who passed through his lab or his seminars recall Castro as a dedicated mentor. "He spent hours with me just teaching me how to code," recalled Torben Noto, a former student who went on to earn a PhD in neuroscience.

After he arrived at Bates, Castro, along with two computational scientists, enlisted big-data methodologies to search for the olfactory equivalent of primary colors: essential building blocks of the odors we perceive. Their results, based on a classic set of data in which thousands of participants described various odors, identify 10 basic odor categories.[1]

Castro launched another project a few months later, when a paper published in *Science* reported that humans could discriminate between at least a trillion different odors. A friend from grad school, Rick Gerkin, smelled something fishy about the findings and gave Castro a call. "We became obsessed with the topic," says Gerkin, now at Arizona State University. The researchers spent almost two years pulling apart the statistical methods of the study, finding that little tweaks to parameters such as the number of test subjects created large swings in the final estimate—a sign that the results were not robust.[2] This August, the original study's authors published a correction in *Science*.

Nathan Urban, Castro's graduate advisor at the University of Pittsburgh, says Castro is driven by fundamental questions. As Gerkin put it, "If you sit down and have a beer with him, you're going to be talking about higher-level ideas in human cognition." Castro has more formally pursued the philosophical nuances of olfaction, as well. He coauthored a 2014 paper with a colleague in the philosophy department, weaving detailed neuroscience into phenomenological argumentation, arriving at a fundamental, if speculative, point: odors are perceived as "objects," much like visual or tactile objects, and are organized in the brain according to their ecological significance.[3]

In setting up shop at a small college, Castro did not sacrifice scientific ambition. Last spring, he received a National Science Foundation Faculty Early Career Development Grant, a recognition usually awarded to researchers at larger universities. Because his work has a "computational front end," plumbing data sets available online, Castro says he doesn't need a university's deep pockets to fund data-gathering of his own. "I'm certainly able to do the science I want to do."

The influx of federal money will fund a project to mine data from the Allen Brain Atlas, a map of gene expression in the mouse brain, to address more fundamental questions regarding olfaction. He is exploring, for example, the possibility that our sense of smell is actually an amalgam of many sensory channels tailored to specific purposes. With so much data at his fingertips, Castro says, "I'm only limited by how creative an analysis I might want to try." ■

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A Day in the Life

Improved methods for tracking lab animals' behaviors yield better data—and tons of it.

BY AMBER DANCE

Biomedical engineer David Meaney uses mouse models to study the pathophysiology and behavior deficits caused by traumatic brain injury. At lab meetings, his students love to joke about their multitasking prowess—they can crunch data while chatting with him or even when sleeping.

Their trick is software the University of Pennsylvania team wrote to analyze videos of mouse behavior 24/7. Not only does the program spare them from tedious hours of watching mice snooze or sniff, it also provides more-reliable results by eliminating variation in how different scientists score behaviors.

"Controlling behavior, ultimately, is the function of the brain," says David Wolfer of the Swiss Federal Institute of Technology (ETH) and the University of Zurich, where he studies the neural basis of cognitive function in mouse models of disease. "[Observing behavior is] probably the most sensitive way to know if something is wrong, or not, with the brain."

Yet classic experiments to assess behavior have problems. Scientists would typically lift a mouse by its tail to transfer the animal from its home cage and place it in a maze or other apparatus. Then, they'd hover nearby as the mouse attempted some task, be it remembering the place where it got an unpleasant foot shock or finding a safe harbor in a pool. Researchers have realized for some time now that mice don't behave normally under such stressful conditions. (See "Mouse Traps," *The Scientist,* November 2014.) Plus, different researchers often get different data, due to the way they act around the animals, their particular smells, or how they score the results. These problems led Wolfer, Meaney, and others to hand the task off to computer software and automated cages that manage the experiments with minimal human interference, often in the same spaces where the animals live.

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Automated systems have been used to analyze mouse models of conditions ranging from Alzheimer's to autism. Although they've been available for a decade, the systems have been slow to catch on widely. For publications, reviewers may still ask for evidence from the classic tests they're used to, warns Wolfer, but he thinks that with more time, the automatic tests will gain acceptance.

While mice and rats are common subjects, many systems work for other animals, and at least one company, CleverSys, Inc., makes a primate version as well. With Meaney's software, "You could do almost any animal that has a nose and tail," reasons Tim O'Brien, director of the Neurobehavior Testing Core at the University of Pennsylvania, which collaborates with Meaney on the program. Scientists are also automating assays of fruit fly behavior. (See "Eye on the Fly," *The Scientist,* January 2015.)

Researchers who adopt the technology face a new challenge, though: oodles of data from hours of mice undergoing experiments or just living normally in their cages. It's gotten even worse for Meaney since he attached mini-microscopes to the heads of some of his mice in order to video their brains along with their behaviors. "It's really cool, but I'm eating hard drives," he grouses. "I'm probably storing 5 gigabytes a week."

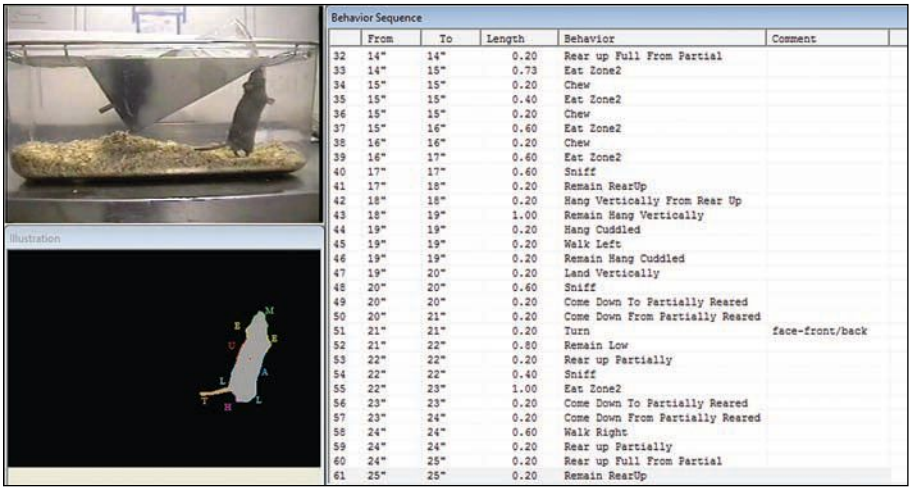
Here, *The Scientist* profiles four options that run or analyze rodent behavior experiments for you.

HOMECAGESCAN

cleversysinc.com/csi_products/homecagescan

What do mice and rats do all day? HomeCageScan, from Virginia-based

CANDID CAMERA: HomeCageScan analyzes a side-view of a mouse in its home cage to identify detailed behaviors.



	From	To	Length	Behavior	Comment
32	14"	14"	0.20	Rear up Full From Partial	
33	14"	15"	0.73	Eat Zone2	
34	15"	15"	0.20	Chew	
35	15"	15"	0.40	Eat Zone2	
36	15"	15"	0.20	Chew	
37	15"	16"	0.60	Eat Zone2	
38	16"	16"	0.20	Chew	
39	16"	17"	0.60	Eat Zone2	
40	17"	17"	0.60	Sniff	
41	17"	18"	0.20	Remain RearUp	
42	18"	18"	0.20	Hang Vertically From Rear Up	
43	18"	19"	1.00	Remain Hang Vertically	
44	19"	19"	0.20	Hang Cuddled	
45	19"	19"	0.20	Walk Left	
46	19"	19"	0.20	Remain Hang Cuddled	
47	19"	20"	0.20	Land Vertically	
48	20"	20"	0.60	Sniff	
49	20"	20"	0.20	Come Down To Partially Reared	
50	20"	21"	0.20	Come Down From Partially Reared	
51	21"	21"	0.20	Turn	face-front/back
52	21"	22"	0.80	Remain Low	
53	22"	22"	0.20	Rear up Partially	
54	22"	22"	0.40	Sniff	
55	22"	23"	1.00	Eat Zone2	
56	23"	23"	0.20	Come Down To Partially Reared	
57	23"	24"	0.20	Come Down From Partially Reared	
58	24"	24"	0.60	Walk Right	
59	24"	24"	0.20	Rear up Partially	
60	24"	25"	0.20	Rear up Full From Partial	
61	25"	25"	0.20	Remain RearUp	

CleverSys, can tell you, in great detail. The system uses a simple video feed, aimed at a caged animal from the side, and identifies nearly two dozen different behaviors, such as jumping, chewing, and urinating. Plus, it categorizes three kinds of grooming—whether a mouse is washing its face, head, or body.

Andrew Steele, a neuroscientist at California State Polytechnic University, Pomona, uses HomeCageScan in his studies of circadian rhythms. It offers him a convenient, noninvasive way to screen for changes in behavior. "We have a much richer picture of what's really happening from the HomeCage data. You could never do this, on a practical level, without the computer vision software." Well, he admits, "you could—it would just take you hundreds of years."

The software works with a typical cage set-up, and comes with wall panels that produce white light, to ensure the camera can see the mouse all day. At night—when mice are more active—it uses infrared light and imaging.

COST $50,000 for four cages, software, camera, and lighting equipment

PROS

- Provides a detailed assessment of animal activity
- A version of the cage can hook up to individual ventilation lines required by most animal-care facilities, making it possible to run experiments in the same location where the animals are housed, says company president Yiqing Liang.

CONS

- Images one animal per cage at a time, though it can do four cages at once. "If you've got a ton of mice . . . this is not the way to go," advises Steele. "This is when you want a lot of detail on just a few animals."
- Because the camera records from a side view, you can't tell much about how the animal travels around its cage. Another CleverSys product called GroupHousedScan combines a top view with the side view, and can track two mice at once, plus their social interactions, Liang says.

PHENOTYPER

noldus.com/animal-behavior-research/products/phenotyper

The PhenoTyper cage, from Noldus Information Technology, can house 1–4 mice and record their performances in experiments. The cage has four customizable walls, plus an infrared video camera in the lid to monitor mouse actions in real time. Before or while monitoring behavior, the addition of food, water, and a shelter translucent to infrared light can transform the enclosure into a home cage. The lid also contains lights and a buzzer to use as signals to the mice.

There are several add-on options. For example, the lickometer measures changes in electrical capacitance every time a mouse uses the water bottle. A feeding monitor notes when a mouse interrupts an infrared beam in front of the snacks. Researchers can track how often a mouse uses a running wheel, too.

The CognitionWall contains a treat dispenser, accessible by three doorways. In one type of behavior experiment, scientists can set up the cage so only one of the three doorways results in a reward, then test how long it takes mice to learn the right door.

COST $8,000 for one cage with the basic EthoVision software platform that tracks and analyzes behavior. Additional software modules—for example, to track multiple animals or synchronize with additional hardware—cost $1,900–$4,500.

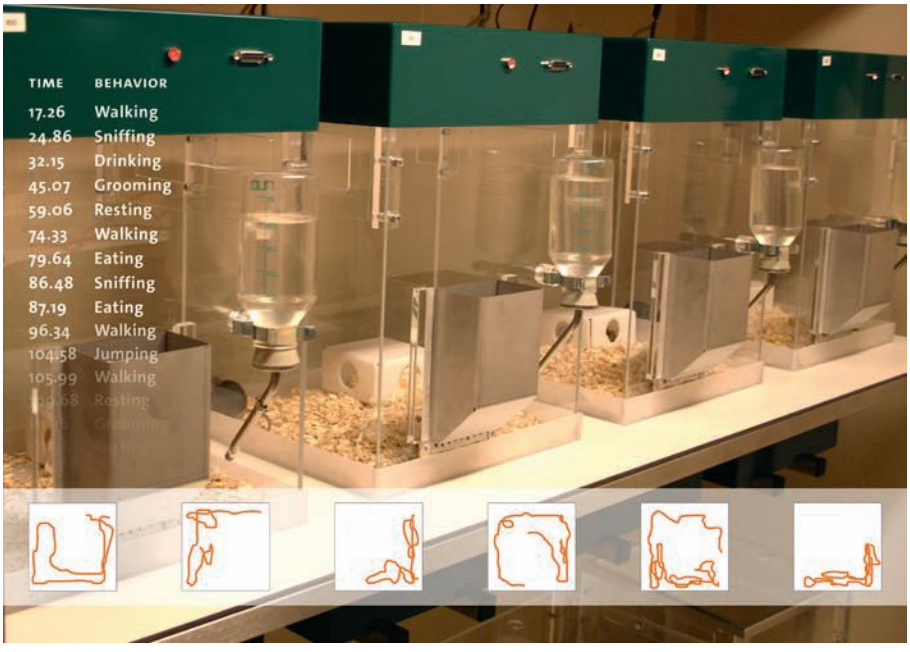
PROS

- The lid can accommodate a cannula to administer drugs, or a fiber optic wire for optogenetic stimulation of the mice during an experiment. For example, the computer can deliver a light pulse every time an animal goes to a certain spot, or approaches another mouse.
- The EthoVision software is easy to learn and use, says Lex Kravitz, a neuroscientist at the National Institutes of Health in Bethesda. He's used it to track mice nonstop for up to 30 days in his studies of obesity.
- It's also straightforward to integrate the PhenoTyper with other equipment, such as an implanted EEG probe, so you can sync the data between the two later on, Kravitz says.

CONS

- The top-view video won't show certain behaviors in as much detail as HomeCageScan, but it is possible to add a side-view camera as well.

COURTESY OF CLEVERSYS, INC.



THE VIEW FROM ABOVE: Using the PhenoTyper's top-view camera, researchers can track a mouse's every move. The EthoVision software can also identify behaviors such as sniffing and grooming.

- In order to use the video feature that tracks locomotion of a specific mouse, you can only have one animal per cage. Alternatively, Noldus Information Technology sells a color camera ($1,200) so researchers can color-tag the different animals, says Abbe Macbeth, regional sales manager for the company in Leesburg, Virginia.
- The video software has trouble dealing with light-haired mice, particularly on gridded floors. Again, a color camera will help.

INTELLICAGE

tse-systems.com/products/behavior/intellicage/index.htm

Mice are social animals, points out Harm Knot, chief technology officer for TSE Systems in Bad Homburg, Germany, so how can scientists expect them to act normally when performing on their own? For example, the main differences in behavior of autistic individuals, be they mouse models or people, occur when around others. TSE's IntelliCage is an environment that monitors experiments on up to 16 mice per cage. A more complex set-up called PhenoWorld can house even more animals, temporarily sequestering certain mice to check their metabolism or behaviors.

One can analyze the spontaneous behavior of mice inhabiting the cage or during experiments. To facilitate those studies, each corner of an IntelliCage contains several features for the mice to interact with. By poking their noses into certain spots, they can gain access to drink, monitored by a lickometer. They might see LEDs in different colors as signals, or get a puff of air in the face as negative reinforcement. Instead of video, the data are output as charts of various actions—the time and number of licks of a bottle or nose pokes in a certain location, for example, or visits to an area of the cage.

The cage can track these data because each mouse has an implanted radio-frequency identification chip (RFID), like the ones used to identify lost pets, and each corner of the cage has a chip reader. That way, different mice can undergo different tests or conditions. For example, some might be able to access a drug-laced water bottle, while control mice get only plain water.

One test scientists can do with the IntelliCage, Knot says, is an index of memory similar to the Morris water maze, in which mice learn and recall the location of a safe platform. Scientists train mice to know that they will only receive liquid refreshment in corner #1, then change the beverage station to corner #2 for a time. When they change the station back to corner #1, does the mouse

ACTIVITY DETECTED: Tracking multiple individual animals at once, the IntelliCage records how often they lick a water bottle, poke their noses through a hole, or visit a specific location.

COURTESY OF NOLDUS INFORMATION TECHNOLOGY; COURTESY TSE SYSTEMS

recall that's a good place to try, or does it wander to the other corners just as often in its search for drink?

COST $40,000 for one cage and software. The RFID chips are about $10 each.

PROS

- High throughput makes it possible to screen a population of mice for certain behaviors.
- Housing mice together results in lower stress for the animals, and thus improved animal welfare, says Wolfer.
- Scientists can use TSE's new Intellimaze software to design a custom cage setup, and thus any type of experiment. That way, they can publish and share their protocols, making it easy for other scientists to validate novel designs, says Knot.

CON

- Unlike the video-based systems, the main output is charts and lists, rather than detailed analyses of spontaneous behaviors such as grooming or jumping. However, video is included in PhenoWorld.

OPEN-SOURCE SOLUTION

seas.upenn.edu/~molneuro/autotyping.html

David Meaney didn't initially think he'd need a computerized tool to analyze the behaviors of mice in response to concussion. But he quickly realized that the 30 minutes to 2 hours it takes to assess a single mouse in a maze would add up. So he and his lab built software, using the computing language MATLAB, to do it for them. "[It's] faster, even better than a manual technique," says Meaney.

Their open-access software—the researchers haven't given it a name—uses top-down video data from a standard camera to score mouse behavior. The program tracks where the mouse is as well as the direction its head is pointing, which is important in certain tests to determine if the animal is looking at a novel object. The software can also tell when a mouse has adopted a standard, curled-up "freeze" posture, which the animal might do when anticipating a foot shock or other unpleasant stimulus. The authors have built in procedures to analyze data from several standard tests, such as a Y-maze or open-field test. Using the open-field procedure, one can also easily monitor a mouse's normal behaviors.

The program mostly works with one mouse, unless two are used in tests of social interactions. The software is available on Meaney's university website.

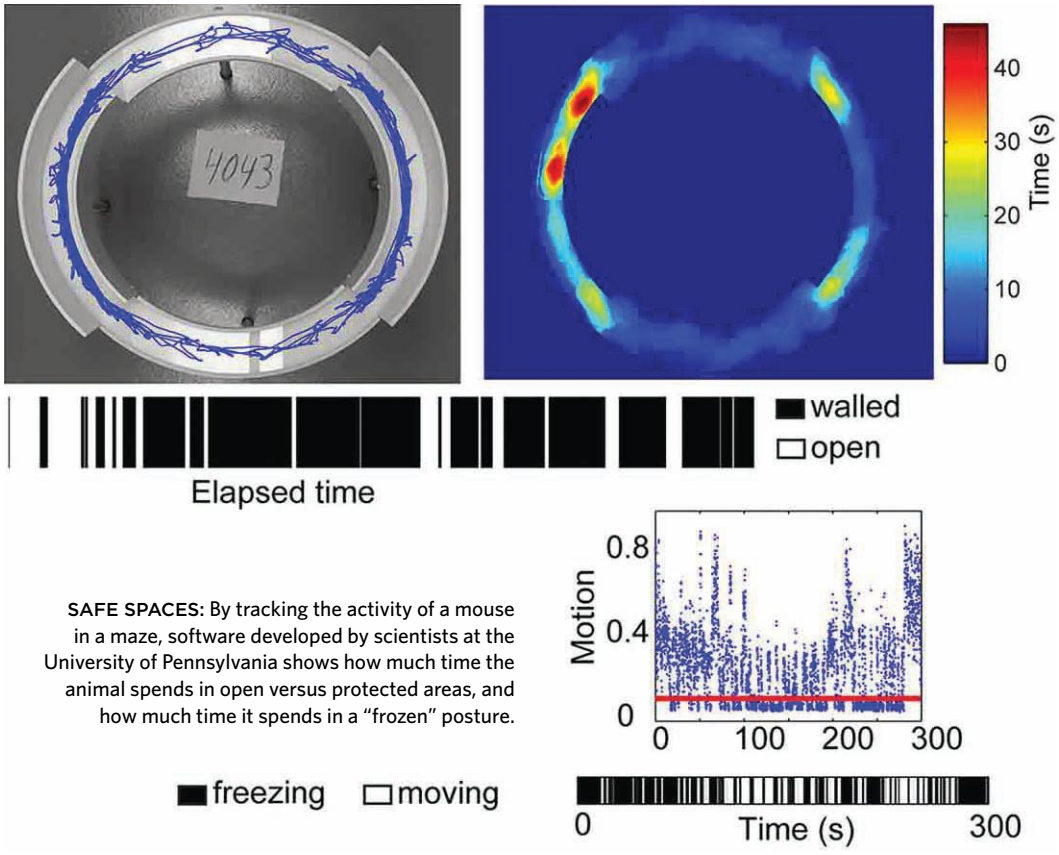
COST Free, but users will need a MATLAB license. An individual license costs $2,150 for companies, $500 for academics, and may be available via one's institution.

PROS

- No special computer skills are needed to use the program; users simply start up MATLAB, type the file name, and work via a graphical interface.
- Those who are already familiar with MATLAB can customize the program to add new tests. Even for noncoders, it's fairly easy to modify a few parameters, such as experiment duration, says Arnold Gonzalez, a research specialist at Penn's Neurobehavior Testing Core.

CONS

- The researchers developed the software using dark-colored mice on white backgrounds, so albino mice can be more difficult for the program to discern. For white mice, the Meaney lab still scores tests manually. O'Brien's group at Penn is considering dyeing the animals or using dark-colored mazes to provide the necessary contrast.
- Because the view is top-down, it can't fully distinguish certain behaviors, such as rearing.
- The software hasn't been through commercial quality control, so there can be bugs, especially if users have different computers than the designers. Updates are relatively infrequent. ■



SAFE SPACES: By tracking the activity of a mouse in a maze, software developed by scientists at the University of Pennsylvania shows how much time the animal spends in open versus protected areas, and how much time it spends in a "frozen" posture.

FRONTIERS IN BEHAVIORAL NEUROSCIENCE, 8:349, 2014

Taking Microscopy for a SPIM

Making the most of this new live-imaging technique

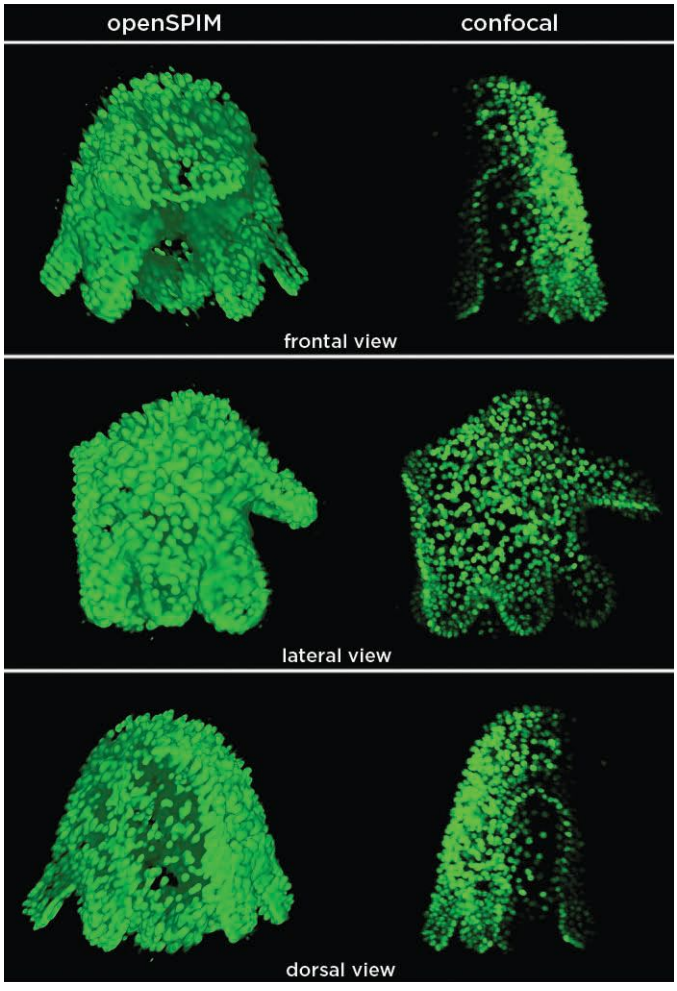
BY KELLY RAE CHI

Last summer, Elizabeth Hillman showed participants in a Cold Spring Harbor Laboratory course the new light-sheet microscope she had invented. Students pulled leeches out of a pond and imaged them wiggling under the instrument, which shoots frames so quickly that the creatures' quick movements were captured with no blur. "It was a von Leeuwenhoek moment, where we felt like we were seeing things we've never seen before," says Hillman, an associate professor of biomedical engineering and radiology at Columbia University in New York.

Since 2004, when the first paper on light-sheet microscopy (aka selective plane illumination microscopy, or SPIM) appeared (*Science*, 305:1007-09), users of the technique have coalesced into a growing community of enthusiastic biologists. Compared with confocal microscopy, SPIM takes images more gently and rapidly, which allows researchers to track biological processes in 3-D at higher resolution over longer time periods. There are many variations of SPIM, but all use laser light focused into a thin sheet and a detection arm that is oriented perpendicular to the plane illuminated by the light sheet. In most SPIM setups, only the part of the sample that is being imaged becomes illuminated—which basically allows you to optically section your sample and reduce light damage. Researchers commonly use the technique to track developing zebrafish or fruit fly embryos, but in the past few years, new variations have expanded potential applications into areas such as in vivo brain imaging in small animals, cancer diagnostics, high-throughput drug screens, and medical imaging.

A basic SPIM system can be assembled for about $50,000, though it can take a year or longer to build and optimize the machine. Using it takes some training: the mounting process is completely different than for a traditional confocal microscope. And the amount of data you can gather in a single run is on the enormously unwieldy level of terabytes.

The Scientist asked some SPIM veterans how to get started, what to expect, and what's on the horizon for this new technique. Here's what they said.



CONFOCAL COMPARE: Scientists built a light-sheet microscope to study the development of *Maritigrella crozieri*, a large flatworm found in the Caribbean. Their new SPIM setup provided more complete views of fixed larvae compared with traditional confocal microscopy. (Nuclei are stained green.)

BUILD, BORROW, OR BUY?

Currently, only a handful of light-sheet microscope platforms are commercially available. Carl Zeiss's Lightsheet Z.1, launched in 2012, is the newest dedicated system, but more are coming. The Z.1 is geared for a broad base of users studying embryos or small samples of fixed tissue. Nipam Patel, an evolutionary developmental biologist at the University of California, Berkeley, considers SPIM imaging as one tool in his toolbox. In three days, he was able to use a new Z.1 to image *Drosophila melanogaster*, *Tribolium castaneum* (red flour beetle), and *Parhyale hawaiensis* (an amphipod crustacean). "I find it very user-friendly on the hardware side. It's well designed, and it's pretty easy to get things lined up and get started with it," Patel says.

An out-of-the-box solution is cost prohibitive for many researchers, and sharing with a core facility may be impossible or impractical. Working in the lab of Max Telford at University College London, graduate student Johannes Girstmair—a biologist with no physics or bioinformatics training—recently built an instrument to track embryonic development of the flatworm

BMC DEVELOPMENTAL BIOLOGY, DOI:10.1186/S12861-016-0122-0, 2016.

Maritigrella crozieri, which is too opaque and too slow to develop to adequately live-image with confocal microscopy. It took seven months and £47,000 (a little more than $60,000) to build the machine and acquire the team's first image, although it took additional time to optimize the sample and setup. Negotiating the price for some of the instrument's parts and having others custom-made was an unexpected time drain, Girstmair says. (He detailed his experiences firsthand in *BMC Developmental Biology*, doi:10.1186/s12861-016-0122-0, 2016.) "Our microscope gives us amazing, good-quality images, even though it isn't 100 percent perfect," Girstmair says. "I think that really everybody could do it."

Girstmair is part of an enthusiastic community of SPIM do-it-yourselfers around the world. Members participate in open-access online resources such as OpenSPIM, an effort launched in 2013 that shows researchers how to build a basic light-sheet microscope and allows them to document their experiences.

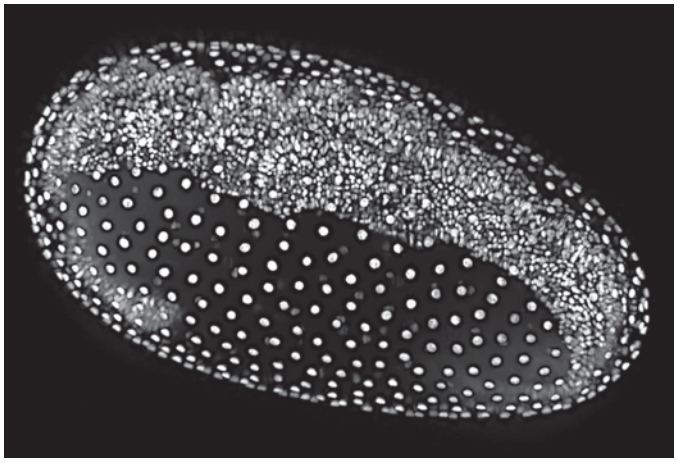
In general, it takes about a year to build your own microscope and to optimize it, says Lars Hufnagel, group leader in cell biology and biophysics at the European Molecular Biology Laboratory in Heidelberg. Even with myriad resources available online, you'll probably need help with one or more of the steps, whether from a physicist who can help you customize or tweak a home-built instrument or a bioinformatician to help with data analysis.

Regardless of your choice, you should first try out someone else's machine to determine how well it resolves your sample and whether it's worth the investment of time or money. Also, all users should understand at least the basic principles of light-sheet microscopy to get the most out of this method, says Pavel Tomancak of the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany, who co-created OpenSPIM and who runs a biannual EMBO Practical Course on the technique. For instance, knowing how to tweak the thickness of the light sheet relative to your sample will allow you to determine the resolution you need, he explains. Hillman adds, "There needs to be more in-depth training for people on the physics that's creating this image, so that we can do a better job interpreting the data."

MOUNTING THE SAMPLE

Sample-mounting techniques used for traditional light microscopy don't work for SPIM, especially if you want to image the sample from multiple sides or if you want to maintain its health over several days of imaging. The two most commonly used types of specimens, embryos of zebrafish and fruit flies, are mounted in a thin tube filled with agarose gel. Tutorials for these samples are available in the *Journal of Visualized Experiments* and on OpenSPIM. SPIM setups can also handle tissue that has been fixed and cleared with CLARITY or other techniques. (See "Synapses on Stage," *The Scientist,* November 2013.)

If your sample is completely new to SPIM, then rather than going it alone, you should bring it to a hands-on light-sheet microscopy course, such as the EMBO Practical Course. "The light-sheet community has figured out over the last 10 years how to mount various samples on these microscopes," Hufnagel says. "It's best to take advantage of the knowledge the community has accumulated."



NIPAM PATEL

FLOUR POWER: Light-sheet microscopy is allowing researchers to expand their studies of insect development. This *Tribolium castaneum* (flour beetle) embryo, for example, can be imaged from multiple directions for up to 120 hours.

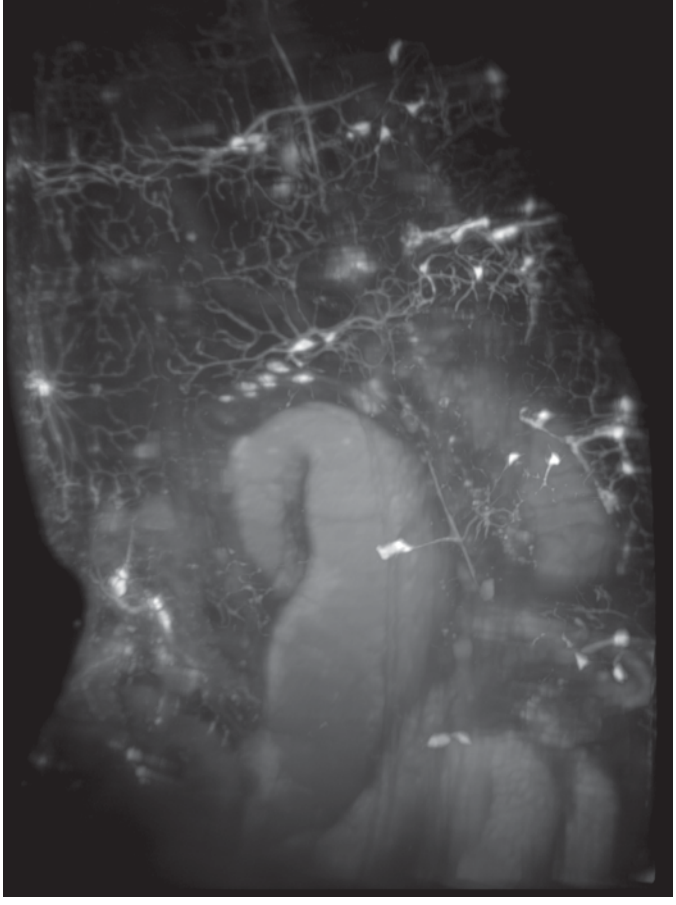
DATA STORAGE AND PROCESSING

The first time he used the Lightsheet Z.1, Patel collected four terabytes (TB) of raw data and found that he didn't have the computational power to access it. Every new user experiences this reality check. SPIM can generate more data in a single day than a confocal microscope produces over the course of a year, Hufnagel says.

The ideal data storage setup is a high-speed Internet connection that hooks up the microscope to large servers, where the files could be accessed by powerful computers. Researchers commonly use RAID storage (redundant array of independent disks) that they keep in their lab. To process the data you'll need state-of-the-art computers with at least 128 GB of memory, says Girstmair. Moving the data can be cumbersome; you'll need to establish a plan for backing it up.

One simple strategy to limit the amount of data you store is to gather the minimum number of images you need for your experiment, says Holly Aaron, director of the Molecular Imaging Center at the University of California, Berkeley's Cancer Research Laboratory, which has two Z.1s. Everyone thinks they want the highest-resolution data possible, she says, until they find themselves unable to work with it on their laptops or computers. Having to wait for a shared workstation to conduct analyses will slow you down, she adds. Another option is to delete data after processing it, though that's not always feasible or ideal because the processing algorithms are not perfect, Tomancak says. Still another is to compress the data as it comes off the microscope, he adds.

Raw SPIM data is routinely processed using an open source platform called Fiji (fiji.sc). Commercial packages such as Bitplane's Imaris (bitplane.com/imaris) and arivis's Vision 4D (arivis.com/en/imaging-science/arivis-vision4d) are developing their own solutions, but these platforms are not cheap, Tomancak



A HIGH-POWER LANDSCAPE: A new kind of light-sheet microscope called SCAPE allows researchers to rapidly image fruit fly larvae. The researchers can immobilize the larvae and slow down their light sweeps to capture high-resolution details of individual neurons.

says. Avoid the bottomless rabbit hole of reconstruction and deconvolution, Hillman says; stay focused on your ultimate measurement goal, whether it's counting cells or looking at ratios of one signal to another.

WHAT'S NEW IN SPIM

SCAPE (SWEPT CONFOCALLY ALIGNED PLANAR EXCITATION). Developed by Hillman and her group, this new imaging technique is geared for capturing fast processes. It works so quickly that large motions, such as a zebrafish's heartbeat, can be captured in a rapid series of frames and stitched together to make a movie. In the intact brains of living mice, SCAPE can image to depths of 200–300 microns, nearly as deep as two-photon microscopy, but with less phototoxicity. Hillman is using it to look at 1-mm-long fruit fly larvae as they crawl and is collaborating with others on a diverse set of animal models. SCAPE boasts a simpler setup than conventional light-sheet microscopy: a light sheet that rapidly sweeps the sample and a single, stationary objective lens for detection (*Nat Photonics,* 9:113-19, 2015). In theory, it's possible to assemble a SCAPE microscope based on the original paper, but the group has since developed an improved version, she says. Hillman has recently struck a licensing deal with a major microscope company and will be working to bring SCAPE to the market within the next few years, she says. Her group is also developing another version of the instrument that is geared for clinical uses, such as endoscopy. And they are working on a two-photon version of SPIM for research use that will be able to image even more deeply.

LATTICE LIGHT-SHEET MICROSCOPY. Pioneered by 2014 Nobel Laureate Eric Betzig and his group at the Howard Hughes Medical Institute's Janelia Research Campus, lattice light-sheet is ideal for imaging live cells and embryos at fast speeds with dramatically lower phototoxicity (*Science,* 346:1257998, 2014). Betzig recently used the technique to observe membrane changes in dividing cells (*Mol Biol Cell*, doi:10.1091/mbc.E16-03-0164, 2016). Researchers can apply to bring their samples to Janelia Research Campus's imaging facility, and some scientists are building their own machines, though doing so requires expertise in optics, says Reto Fiolka, an assistant professor of cell biology at the University of Texas Southwestern Medical Center.

meSPIM (MICROENVIRONMENTAL SPIM). In a recent study, Fiolka and his colleagues quickly imaged a ball of cancer cells with subcellular resolution in three dimensions (*Dev Cell,* 36:462-75, 2016). Classical SPIM or lattice light-sheet microscopy wouldn't have worked for this study, because light sheets as thin as he needed cannot extend over a sufficiently long range. Presently, meSPIM is still too challenging to set up for most labs, Fiolka says, but he and his collaborator Gaudenz Danuser are working to expand its applications and make it more widely accessible.

MuVi-SPIM AND InVi-SPIM (MULTIVIEW SPIM AND INVERTED MICROSCOPE SPIM). Hufnagel and his EMBL collaborators have commercialized some of the institute's microscopes by creating a company called Luxendo, which has received venture capital funding and is now demo'ing its instruments at conferences and in individual labs. Multi-view, or MuVi-SPIM gives four different views, eliminating the need to rotate the sample. Another of Luxendo's instruments, InVi-SPIM, is an inverted microscope designed to image early-stage development of mouse embryos or other samples that do not survive the sample-preparation process required for traditional light-sheet microscopes (*Nat Methods,* 13:139-42, 2016). ■

OTHER LIGHT-SHEET INSTRUMENTS:

Lightsheet Z.1
Carl Zeiss

Ultramicroscope
LaVision BioTec

Single light sheet arrangement (iSPIM)
Applied Scientific Instrumentation

Dual light sheet arrangement (diSPIM)
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TheScientist

#Science

Tweeting, blogging, and other forms of online communication are enabling an era of open and accelerated research.

BY BEN ANDREW HENRY

In early June 2011, at a bioinformatics conference outside of Cambridge, U.K., University of British Columbia epidemiologist Jennifer Gardy watched a Twitter storm take place. "The world's tiny population of genomic epidemiologists is sitting in this lecture hall," she remembers thinking—and they all seemed to be firing off tweets as fast as they could compose them. Just weeks earlier, reports had surfaced that a new and deadly variant of *E. coli* was infecting people in parts of Germany. While the epidemiologists were gathered together in the hall, researchers from the Beijing Genomics Institute (BGI) announced on Twitter that they had just publicly released an unassembled sequence of the strain's genome. By sheer coincidence, the geneticists at the conference had just been discussing whole-genome sequencing as a forensic tool in epidemiology. Suddenly, they were handed an open case.

Gardy was sitting in the front row next to the University of Birmingham's Nick Loman, who helped organize the meeting, the Wellcome Trust conference on Applied Bioinformatics and Public Health Microbiology. "We see the tweet from BGI go up, and Nick immediately grabs it," Gardy recalls.

Loman sent the data to a server of his that assembles genomes, stitching short reads together into a coherent whole to be analyzed using any number of bioinformatics techniques. He then wrote a blog post and sent a tweet, setting the assembled genome loose online. Over the remainder of the conference, during presentations and on coffee breaks in the lobby, researchers cracked open their personal genomic toolboxes to investigate the genome. They and other researchers from around the world shared and discussed their findings with each other in real time on Twitter. Even after the conference, analyses of the *E. coli* genome continued to roll in, so prolifically that Loman set up a wiki to collect the information. Less than two months later, Loman and others published a paper based in part on this crowdsourced genomic analysis (*N Engl J Med*, 365:718-24, 2011).

"Twenty-four hours after the release of the genome, it had been assembled; two days after its dissemination, it had been assigned to an existing sequence type," the paper read. "Five days after the release of the sequence data, we had designed and released strain-specific diagnostic primer sequences, and within a week, two dozen reports had been filed on an open-source wiki."

Twitter, at the time, had grown into an established social media platform, but scientists were still warming to the idea of having meaningful technical exchanges using 140-character missives. To some, though, this tool for networking and sharing showed

enormous promise for scientific research. Now, scientists in fast-moving and data-driven fields are finding that such online forums enable real-time dissemination of their work. Sharing research on social media also fits with the community's growing interest in accelerating scientific publishing, as evidenced by a spate of new preprint servers.

"Twitter is the place where I actually hear people discussing science," says Richard Sever, executive editor at *Cold Spring Harbor Perspectives* and cofounder of the life-science preprint server bioRxiv. "That's been an eye-opener for some people. . . . Suddenly, serious people, serious scientists, particularly in genomics, started joining. And serious discussions are happening."

Community research and review

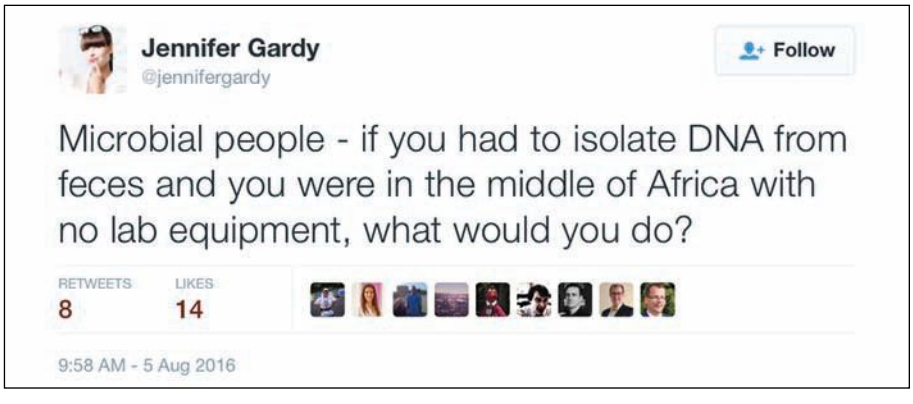
Microbial genomics was "a real leader in data sharing and in open, transparent, regular communication," says Gardy. After all, a single research group could only sequence so many genomes. "If you want to understand something about your organism—what's making it pathogenic, what's causing resistance—the only way to get at those answers is to compare your bacterial genome with other bacterial genomes . . . sequenced by other groups," she says.

The field also led the scientific community when it came to adopting social media platforms for research. "When tools like Twitter came along, it just seemed like a natural extension," says Gardy. Some researchers who assess outbreaks of disease using genomics hope that the whole process will one day be carried out online in real time, she adds. "The second a genome sequence goes up onto the Web, there's no reason why you can't have a cadre of people around the world analyzing that and posting their results in a common space."

As interest in open access and data sharing continues to grow, some are even eyeing social media as a tool for science publishing. Sever, who recently helped launch bioRxiv with the aim of accelerating the pace of biology publishing, sees social media as part of a rapid information dissemination pipeline. When a preprint goes up, "you may immediately see some reaction on Twitter, you may see some responses . . . in the comment section on bioRxiv, then you might see someone write a blog post about it." As readers share the work, they scrutinize it and compare their reactions—generating what amounts to crowdsourced peer review.

Sever argues that crowdsourcing should not replace traditional peer review entirely, and still believes that a paper should undergo that process after being made available as a preprint. In some cases, feedback about a preprint from an online discussion helps authors to strengthen their manuscript with added experiments, analyses, or discussion before submitting to a journal, bolstering their chances of acceptance and streamlining the traditional review process.

Other scientists believe open, online review methods can and should supplant the age-old process. In addition to sidestepping the problems of anonymous review and the slow pace of traditional scientific publication, social media brings more voices into the discussion. "Peer review is two, three if you're lucky, people reading a paper, and they're certainly not trying to [repeat] any of the data analysis," says Gardy. When a set of data makes the rounds on Twitter, "mistakes that would have gone unnoticed in a traditional system come to the surface very quickly and get corrected."



Sounding board

In early August, Gardy tweeted a question to her nearly 6,000 followers: "If you had to isolate DNA from feces and you were in the middle of Africa with no lab equipment, what would you do?" The question was not hypothetical.

Gardy had just spoken to Jer Thorp, a data scientist who specializes in visualization. Thorp is involved in the Okavango Wilderness Project, and travels with his colleagues along the Okavango River in southwest Africa performing wildlife surveys and taking photographs, as well as measuring the heart rates of team members, all while uploading their data online for others to explore. Thorp was hoping to add genomic data to the project, but getting usable DNA samples with limited power and no access to a laboratory was proving challenging. So he asked Gardy for ideas, and she asked Twitter.

"If I run into a genomics or bioinformatics problem, I know that I can go to Twitter, tweet about it, and because I've got enough bioinformaticians and computational biologists following me, probably within a few minutes I'll get an answer," she says. Indeed, in response to tweeting about DNA collection in undeveloped Africa, Gardy got responses that ran the gamut from technical (an exchange over storage solutions) to facetious ("really tiny tweezers") to meta ("probably just tweet asking for help"). But she also got her answer: a fellow genomicist with experience in field sampling knew how to turn a drill into a low-tech centrifuge for extracting a DNA sample.

Gardy relayed the information back to Thorp, who is now testing the idea in the field. If the Okavango team gets the approach to work, they'll be able to share their data with the same population of genomicists who responded to Gardy's question.

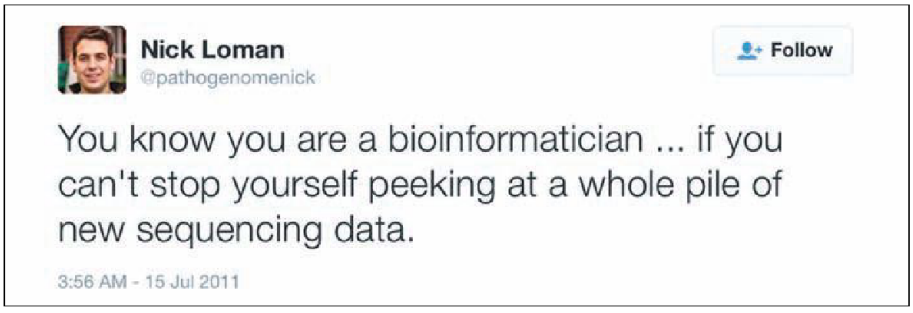
This type of casual crowdsourcing has become a regular activity for many scientists who have cultivated a responsive Twitter following. "Instead of me sitting here for two days trying to hammer through a bit of R

code that is making no sense to me, I can send it out to the community" for a solution, says Gardy. As ecologist Andrea Kirkwood of the University of Ontario Institute of Technology put it, bouncing questions off Twitter "is part of my job. It's just one tool in my toolkit."

Extra! Extra!

Of course, beyond helping scientists do, share, and discuss their own research, social media remains a core source of science news for both scientists and the public. In 2015, the Pew Research Center reported that nearly half of American Association for the Advancement of Science (AAAS) members use social media such as Twitter, Facebook, and LinkedIn to talk about science or receive science news.

Although she regularly scans the literature for her research, Kirkwood says she encounters papers through tweets and blog posts that she might otherwise never have found. A new paper usually receives only a fleeting moment in the digital sun, if any, but during that window of time researchers across very different fields might catch a glimpse. "I've found that I've actually picked up a lot of information that's useful to my research," Kirkwood says. She adds that Twitter has even improved her teaching, because "I can stay on top of current trends and controversies" that anchor her lectures in relevant social context.



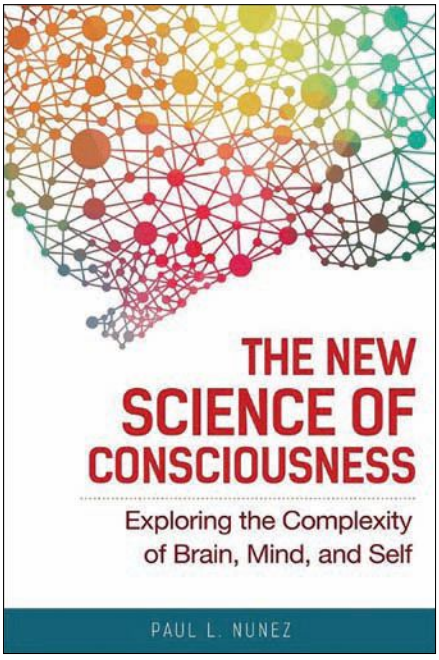
Establishing the use of social media as a mainstay in science departments remains a distant notion, however. Some researchers simply have no inclination to share their work on Twitter, Sever notes. "The predominant criterion for selecting people to be scientists is that they are good at science," not necessarily that they are avid communicators, he says. "There are great scientists who can write clearly and eloquently . . . and there are great scientists who cannot."

But others would contend that social sharing and scientific progress are made for each other, as passionate researchers can't help but jump onboard a budding crowdsourced project. As Loman tweeted a few months after the *E. coli* event, "You know you are a bioinformatician . . . if you can't stop yourself peeking at a whole pile of new sequencing data." ■

Minding the Mind

The black box that is human consciousness is grudgingly yielding its secrets thanks to the cross-disciplinary work of researchers.

BY PAUL L. NUNEZ



Prometheus Books, November 2016

Ever since I switched my research focus from theoretical physics to neuroscience many years ago, my professional life has focused on the "easy problem" of consciousness—exploring relationships between brain activity and mind. So-called signatures of consciousness, such as increased blood oxygen or electrical activity patterns in different brain regions, are recorded using several different imaging methods, including electroencephalography (EEG) and functional magnetic resonance imaging (fMRI).

The "hard problem"— how and why neural activity produces our conscious awareness—presents a much more profound puzzle. Like many scientists and nonscientists alike, I have a long-running fascination with the mystery of consciousness, which serves as the inspiration for my latest book, *The New Science of Consciousness*.

A new approach to studying consciousness is emerging based on collaborations between neuroscientists and complexity scientists. Such partnerships encompass subfields of mathematics, physics, psychology, psychiatry, philosophy, and more. This cross-disciplinary effort aims to reveal fresh insights into the major challenges of both the easy and the hard problems. How does human consciousness differ from the apparent consciousness of other animals? Do we enjoy genuine free will or are we slaves to unconscious systems? Above all, how can the interactions of a hundred billion nerve cells lead to the mysterious condition called consciousness?

I recruit a number of analogies and metaphors, showing how brain behavior can be compared to the collective behaviors of other large-scale systems. Our global social system, for example, harbors novel features such as wars and economic depressions that emerge though they are absent from the small component parts. Similarly, many scientists believe that consciousness emerges from brain networks. But how is this possible?

My approach to both the easy and hard problems rests on the following conceptual framework, which is consistent with mainstream science: (1) human brains and minds are correlated; that is, many consciousness signatures have been discovered; (2) human brains and those of many other species are genuinely complex systems; (3) brains, like other complex systems, consist of nested hierarchies of subsystems that operate at different levels of organization (spatial scales); (4) accordingly, signatures of consciousness are observed over a wide range of scales; (5) multiple conscious, unconscious, and semiconscious entities coexist within each human brain; (6) interactions between these subsystems contribute substantially to making the human brain human.

This conceptual framework supports an idea called "the multiscale conjecture," which posits that consciousness manifests at multiple levels of brain organization, from confined neural networks to large brain regions, and no single scale need be special. Thus, the various dynamic patterns of information observed as consciousness signatures may all contribute to the mind. In this view, consciousness is rooted in the dynamic patterns of multiple interacting scales.

Although fully consistent with peer-reviewed research, the multiscale conjecture allows room for both reductionist and nonreductionist interpretations. I approach the consciousness challenge with questions about a category beyond ordinary information—that is, ultra-information—defined broadly to include ordinary information, hidden physical processes, and consciousness. Thoughts, emotions, self-awareness, memory, and the contents of the unconscious are, by definition, categories of ultra-information whether or not these mental processes also involve ordinary information. This idea is fully consistent with modern physics, which tells us that some kinds of information are fundamentally unknowable. For example, all interpretations of quantum mechanics rely on the existence of some sort of hidden reality that we can never observe directly, but that nevertheless influences the familiar world that is available to our senses. This hidden reality has been given a number of fancy labels, including wavefunction collapse, many worlds, multiverse, implicate order, coherent histories, and more. By my definition, this hidden world contains ultra-information. Many have speculated that consciousness is somehow related to such hidden reality, but it appears that nobody really knows how this might occur. ■

Paul L. Nunez is an emeritus professor of biomedical engineering at Tulane University and heads the small consulting firm Cognitive Dissonance LLC. Read an excerpt from The New Science of Consciousness *at the-scientist.com.*

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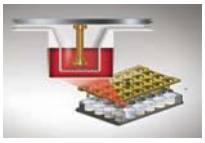

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In a recent study, Henn and coworkers used the CytoSMART™ System for the remote monitoring of human Mesenchymal Stem Cells (hMSC) in combination with the Xvivo System X3 for cell handling and incubation under strict hypoxic conditions. The researchers found that the hMSC yields were higher when kept in optimal conditions, compared to cells that were interrupted for handling and inspection.

To learn more about Henn's and coworkers' findings, download their White Paper at: www.lonza.com/cytosmart

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Two-Photon Microscopy, 1990s

BY ALISON F. TAKEMURA

In the 1980s, neuroscientists were facing an imaging problem. They had developed a new way to detect neuronal activity with calcium dyes, but visualizing the markers proved challenging. The dyes fluoresced in the presence of calcium ions when illuminated with ultraviolet (UV) light, but it was difficult to build UV lenses for confocal microscopes—instruments that allowed scientists to peer hundreds of micrometers deep into the brain. To make matters worse, because biological tissue scatters light so effectively, confocal scopes required excessive light intensities, which caused irreparable damage to samples. "You basically burned your tissue," says Winfried Denk, director of the Max Planck Institute of Neurobiology in Martinsried, Germany.

The time was ripe for a gentler option, and Denk developed two-photon excitation microscopy in 1990. Instead of using a single photon to excite a calcium dye, scientists could use two photons and half the illumination energy—red or infrared lasers, instead of ultraviolet. The scatter of such low-energy rays caused far less damage to surrounding tissue.

The technology had another advantage. To excite a molecule, both photons had to reach it simultaneously. This meant the laser could only excite a tiny patch of tissue where its photons were most concentrated, giving scientists a new level of precision.

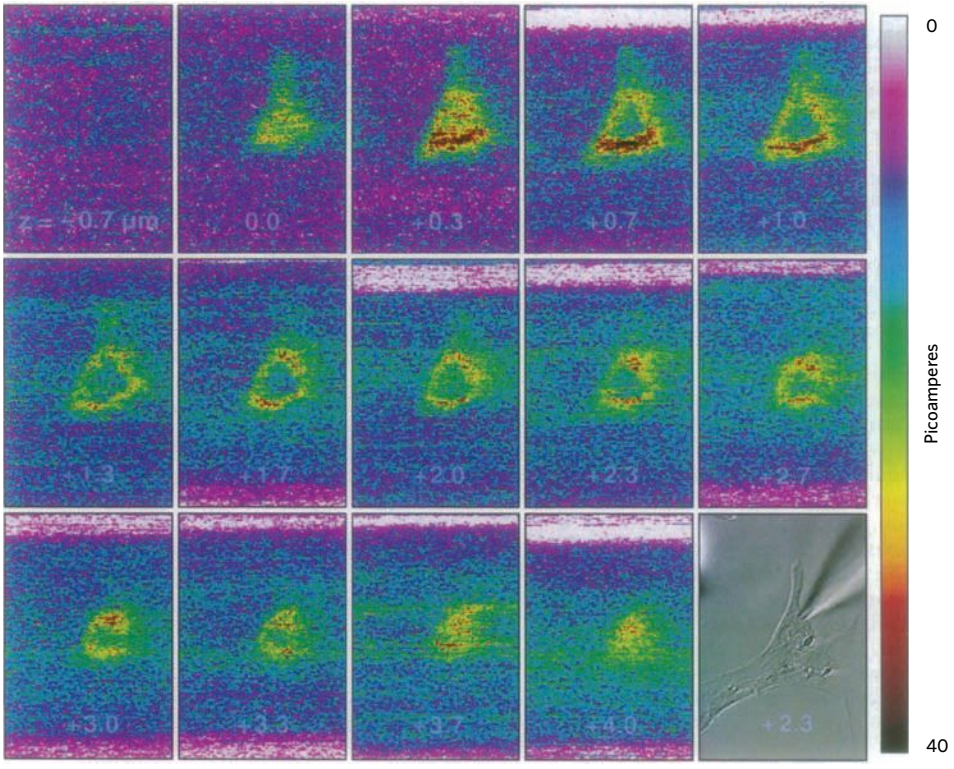
In 1994, Denk demonstrated the utility of two-photon microscopy by mapping receptors embedded in the cell membrane of a mouse brain-tumor cell. To locate the receptors, he exposed the cell to a solution containing an inactive, "caged" receptor agonist. Hitting the agonist with two red photons was like springing a lock, liberating the molecule to bind receptors in its immediate vicinity and alter the cell's electrical potential (see image).

But two-photon microscopy's greatest strength—and the basis for its enduring popularity—proved to be its ability to visualize neurons in living brain tissue without killing the cells. In 1995, Denk and Rafael Yuste, working together at AT&T Bell Labs, imaged neurons from a slice of rat cortex. The duo's experiments demonstrated that activating both cells on either side of a synapse strengthened their connection more than stimulating either cell on its own (*Nature*, 375:682-84, 1995).

Two-photon microscopy combined with calcium imaging "completely changed my research," says Yuste, now a professor at Columbia University who studies cortical circuitry. "It changed everybody's research." These days, he says, hundreds of groups use the technique.

Before two-photon's debut, scientists had been exploring the brain using slices only micrometers thick, recalls György Buzsáki, a neuroscientist at New York University School of Medicine. "Now you could look at the living brain with the same precision that you had done before in slices," Buzsáki says.

Yuste says he's proud of what he and Denk accomplished. "But now I go to meetings where everyone is presenting two-photon calcium imaging, and no one knows, or remembers, or cites our work," he says, laughing. ■



LOCAL ACTION: Winfried Denk captured a brain-tumor cell from a mouse responding to receptor activation. Each panel represents a different depth, with the upper left starting below the cell. A tightly focused two-photon laser scanned back and forth across the cell, locally photolyzing a solution of "caged" receptor agonists outside the cell. The freed agonists bound nearby nicotinic acetylcholine receptors on the cell surface, opening ion channels in the membrane and thus generating electrical current (detected by an electrode within a pipette, lower right). Each pixel represents a single current measurement: the greater the current, the more densely packed the receptors on the cell surface.

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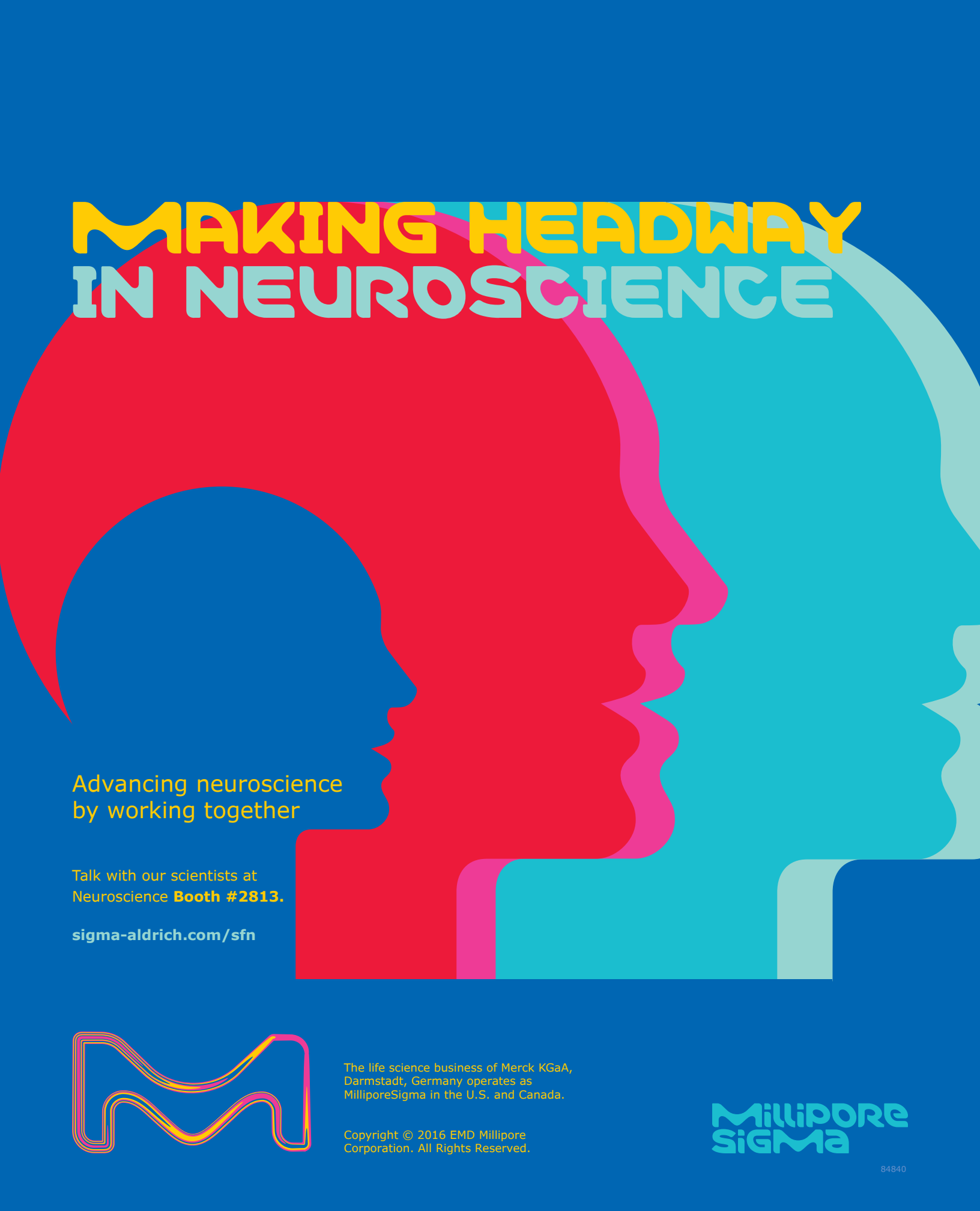

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