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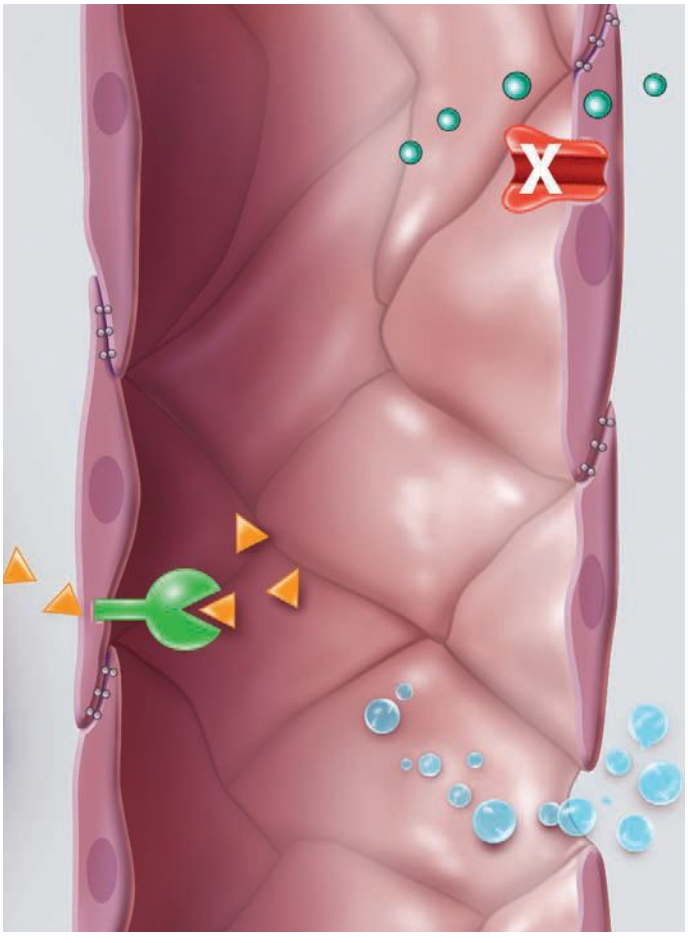

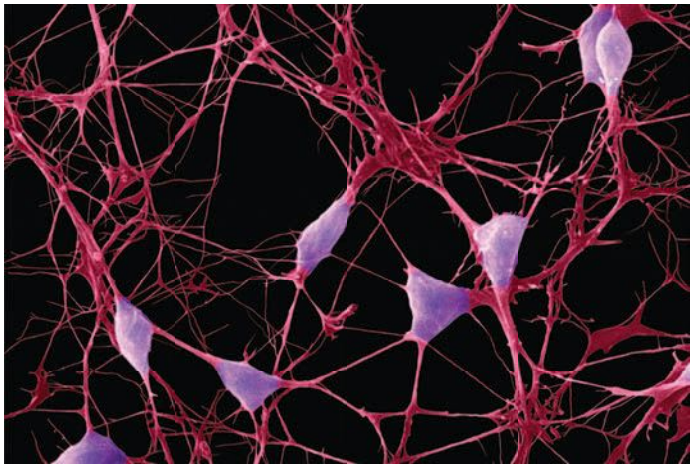

Features

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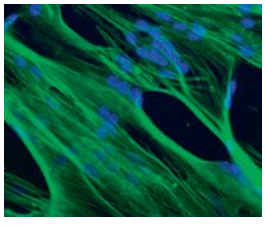

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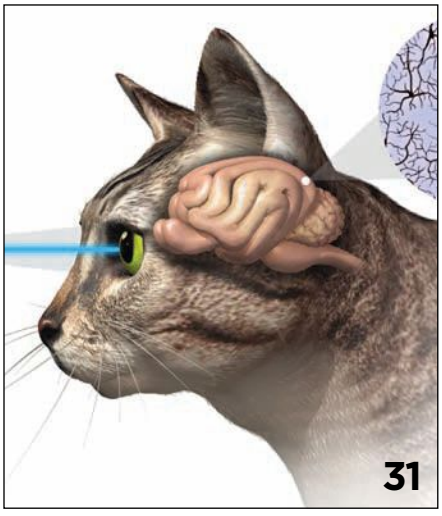
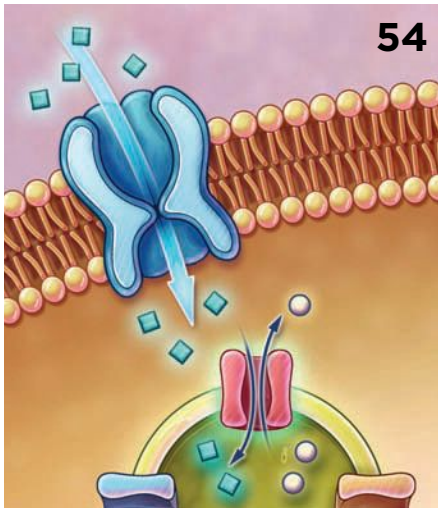




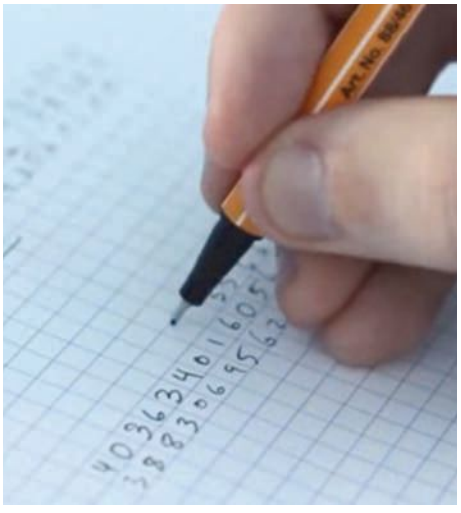
CORRECTIONS:

In the table on page 41 of "Trippy Treatments" (*The Scientist*, September 2017), two addiction studies were listed with incorrect years of publication. The study of 15 cigarette smokers was published in 2014; the study of 10 participants who underwent psilocybin-facilitated treatment for alcohol dependence was published in 2015.

In "Cage Sweet Cage" (*The Scientist*, October 2017), Brianna Gaskill was incorrectly described as an applied pathologist. She is an applied ethologist.

The Scientist regrets the errors.

Online Contents



THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Memory Master
Four-time USA Memory Champion Nelson Dellis reveals some of his memory-training tactics.

VIDEO
Fire Ant Rafts
The invasive insects weathered extreme climatic conditions by banding together and riding out Hurricane Harvey's flood waters.

VIDEO
Frog-Sucking Flies
Insects feast on amorous túngara frogs, drawn to a meal by eavesdropping on the amphibians' love songs.

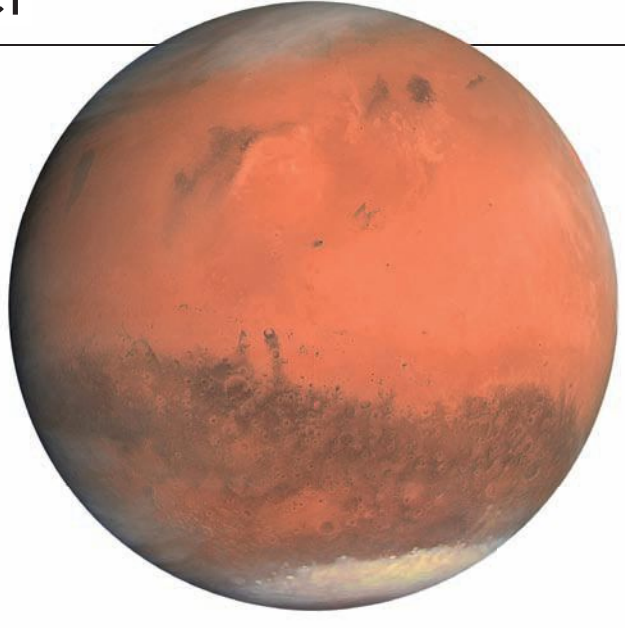
AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

Coming in December

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

- The search for life on Mars
- How the zygote takes control of its own development
- Annual Top 10 Innovations Awards
- A one-step method for making knockout stem cell lines
- Macrophage exosomes and insulin sensitivity

AND MUCH MORE



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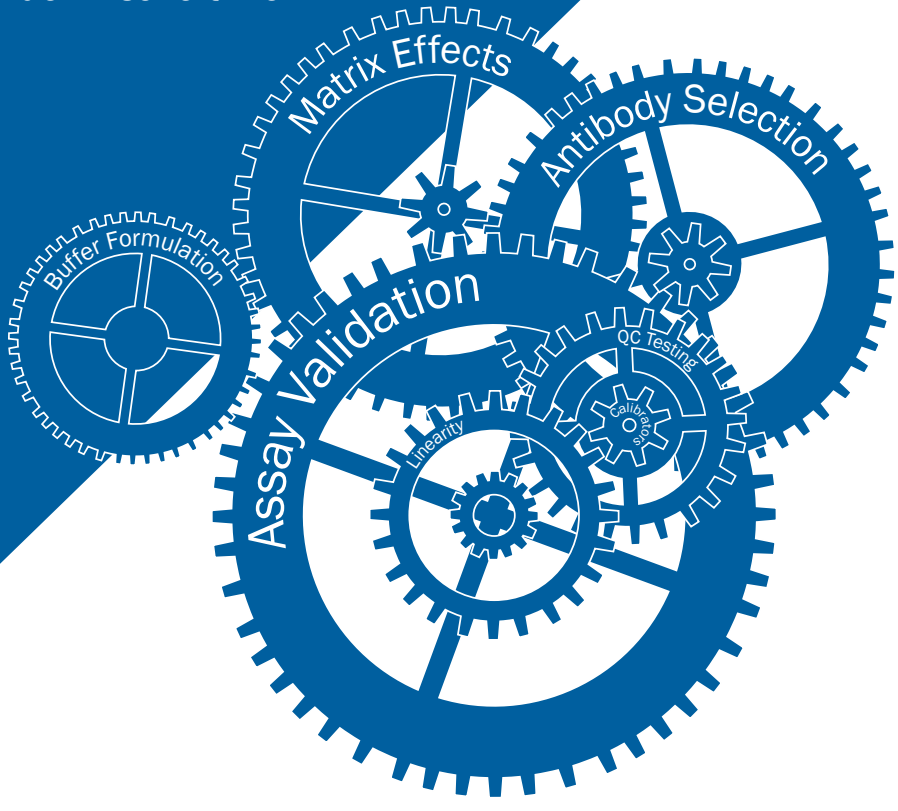

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FOLIO AWARD S 2016 • **March 2016 issue**—Winner B-to-B Full Issue • **B-to-B News Coverage**—Honorable Mention

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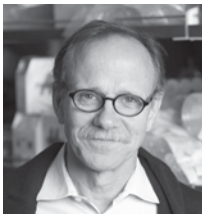

After graduating from the University of South Florida in 2006, **Sara B. Linker** spent a little more than a year in Hawaii doing high-performance liquid chromatography for a startup company. That's when she realized, rather than just doing science, "I wanted to be the one leading the projects." So she applied to graduate programs, landing in Dale Hedges's lab at the University of Miami's Hudson Institute for Human Genomics, where she studied retrotransposons and learned about bioinformatics. She earned her PhD in 2014, then accepted a postdoc at the Salk Institute for Biological Studies in the lab of **Fred "Rusty" Gage**, where she's since been working on single-cell RNAseq of individual mouse neurons to identify the transcriptional component of memory. "Rusty is one of the key people in transposon research," Linker says. "It was a wild dream to come here."

Another of Gage's postdocs, **Tracy A. Bedrosian**, was also drawn to the lab because of its work on mobile elements. Although she had originally intended to go to medical school, Bedrosian switched gears after getting involved in research on the neurobiology of stress in Huda Akil's lab at the University of Michigan as an undergraduate. She ended up at Ohio State University for grad school, working with Randy Nelson on how disrupting circadian rhythms can cause symptoms of depression in Siberian hamsters. Transitioning to Gage's lab, "I wanted to take my background in behavioral neuroscience and apply it to [genetic diversity in neurons]."

Gage's own interest in retrotransposons and somatic mosaicism was a bit serendipitous, he admits. As he recounts in "The Kaleidoscopic Brain" (page 40), a feature story coauthored by Linker and Bedrosian, it all started with a comparison of gene expression in neural progenitor cells (NPCs) and other cell types derived from them, which pointed to elements of LINE-1 retrotransposons as being most highly expressed in the NPCs. Following up on these early results, Gage and his colleagues found that retrotransposons are a major source of genetic diversity among neurons. Prior to that work, Gage had been a leader in the field of adult neurogenesis, publishing the 1998 pioneering study that demonstrated the birth of new neurons in the adult human brain. Following a PhD from Johns Hopkins University, he held faculty positions at the Texas Christian University, Lund University in Sweden, the University of California, San Diego, and is now a professor of genetics at the Salk Institute for Biological Studies. Gage says he learned early on that "being a scientist is more than just doing science; it's being a part of a community. . . . I try to give back to my scientific community for the privilege of practicing the art."

Abigail Marsh was a Dartmouth College undergrad with a few introductory psychology classes under her belt when she took a trip home to visit her family in Tacoma, Washington. But she had not yet decided to devote her life to a career in the field. It was a fateful drive down Interstate 5 that would dictate her decision. As she drove down the multilane highway around midnight, Marsh swerved to avoid hitting a dog that had run out into the road. Her car fishtailed, spun around, and ended up across the highway, facing oncoming traffic, the engine dead. "I was sure I was going to die," Marsh says. She turned on her flashers and high beams and continued trying to start the car. Then, a man appeared at her passenger door's window after having run across eight lanes of highway. The mysterious savior got into her car, managed to start it, and piloted it safely over to the shoulder. His actions left an indelible mark on her. "You can't really have an experience like that without having it dramatically affect your perception of human nature," she says. "This guy has instantly decided to risk his own life to save mine."

So Marsh made up her mind to devote her career to seeking the psychological root of such altruistic behavior. Through the rest of her undergraduate and graduate studies, she sought answers in the human brain's amygdala, studying its role in experiencing fear and in sensing fear in others. Her work has helped her understand the motivations and neuroscience behind ultimate altruists, such as kidney donors, as well as people with psychopathic traits. One key to aligning her test subjects on this spectrum, says Marsh, now a researcher at Georgetown University, is the functionality of their amygdalas. "You can learn a work-around to identify someone's emotions," she says, "but they still have to develop an emotional response."

Marsh writes about the important role that sensing and feeling fear play in living a healthy life in an essay on page 71.

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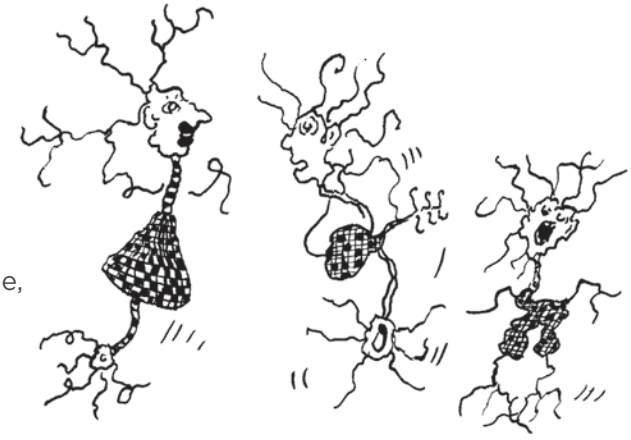
To Each His Own

Every human brain is far more unique, adaptable, and vulnerable than ever suspected.

BY MARY BETH ABERLIN



Like the entomologist in search of colorful butterflies, my attention has chased in the gardens of the grey matter cells with delicate and elegant shapes, the mysterious butterflies of the soul, whose beating of wings may one day reveal to us the secrets of the mind.

—Santiago Ramón y Cajal, *Recollections of My Life*

Based on this quote, I am pretty certain that Santiago Ramón y Cajal, a founding father of modern neuroscience, would approve of this month's cover. The Spaniard had wanted to become an artist, but, goaded by his domineering father into the study of medicine, Ramón y Cajal concentrated on brain anatomy, using his artistic talent to render stunningly beautiful and detailed maps of neuron placement throughout the brain. Based on his meticulous anatomical studies of individual neurons, he proposed that nerve cells did not form a mesh—the going theory at the time—but were separated from each other by microscopic gaps now called synapses.

Fast-forward from the early 20th century to the present day, when technical advances in imaging have revealed any number of the brain's secrets. Ramón y Cajal would no doubt have marveled at the technicolor neuron maps revealed by the Brainbow labeling technique. But the technical marvels have gotten even more revelatory.

In "The Kaleidoscopic Brain" (page 40), Rusty Gage and two of his postdocs, Sara Linker and Tracy Bedrosian, describe nonvisual methods for delving ever deeper into neurons—analyzing not just what you see, but what you get by performing genome sequencing and transcriptional, posttranscriptional, posttranslational, and epigenetic analyses on single cells. The results of such research paint neurons as tiles in a cellular mosaic. "The human brain contains approximately 100 billion neurons, and we now know that there may be almost as many unique cell types," they write. "Brain cells in particular may be as unique as the people to which they belong." To each brain, its own ecology. And a clear picture of the implications of this cellular individuality, they write, "may one day reveal to us the secrets of the mind"—Ramón y Cajal's ultimate goal.

Also in this issue—November being the month each year when *TS* focuses on advances in neuroscience—you will find Amanda Keener's feature about the characteristics of blood vessels in the central nervous system and how they act to restrict access to brain cells ("Into the Breach," page 32). "A real naive view is that the blood-brain barrier is just a wall," a neuroscientist tells her. "It is a whole series of physical properties that allow the vessels to control what goes between the blood and the brain." Creative solutions for getting useful drugs into the brain include disrupting endothelial-cell tight junctions with ultrasound waves and microbubbles, and tricking vessels' transport systems into letting target molecules through. Researchers are now testing these new methods in animal models and 3-D cultures, as well as in human patients.

More articles that underscore the brain's intricacy include a method for recording the magnetic signals that accompany nerve cell electrical discharges (page 31); optogenetic and chemogenetic methods for studying behavior in primates; how the brains of memory athletes process the prodigious amount of material they must master to win competitions (page 17); a simple eye exam for early, noninvasive detection and tracking of Alzheimer's disease (page 20); and a recent report from the MIT lab of profilee Li-Huei Tsai showing that exposing mouse models of Alzheimer's to strobe lighting reduced amyloid-β levels by half (page 56).

What a wonderful ecosystem the brain is, always beckoning scientists to map the secrets locked in its cells. I'll give Ramón y Cajal the last word: "The brain is a world consisting of a number of unexplored continents and great stretches of unknown territory." ■

Editor-in-Chief
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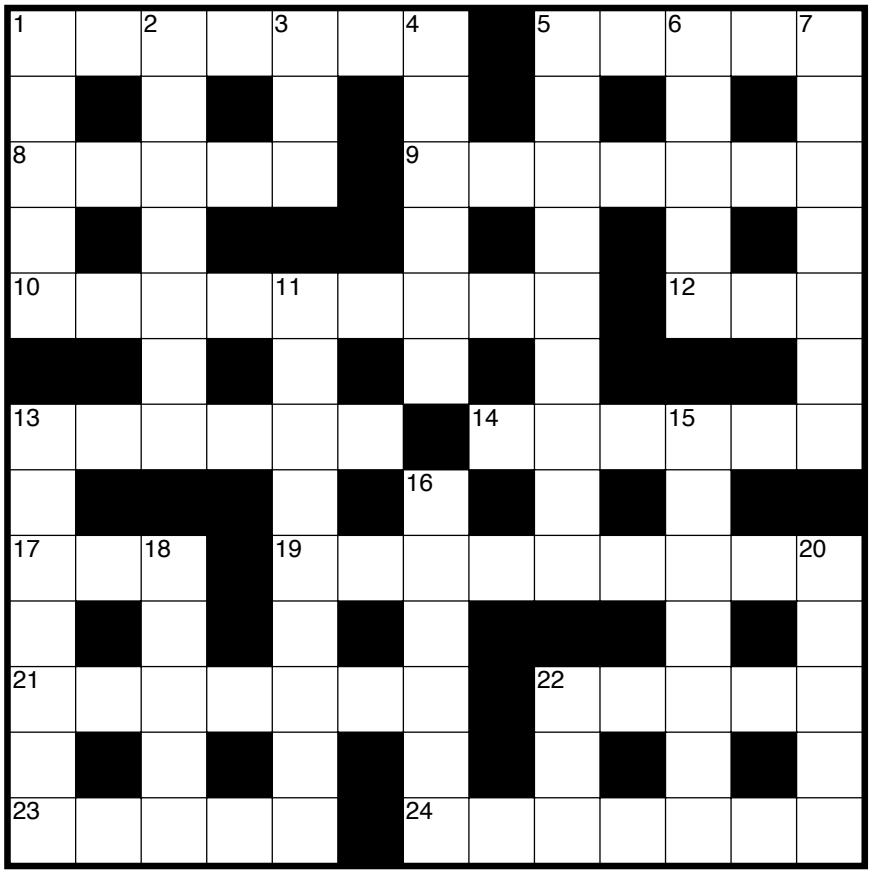
ANDRZEJ KRAUZE

Speaking of Science



BY EMILY COX AND HENRY RATHVON

ACROSS

1. Puzzle that uses the entire alphabet
5. Elementary particle that may be strange
8. What gustatory cells help you do
9. Atomic pile
10. Relating to the ear or the sense of hearing
12. Lynx or serval
13. Gryllidae family member from Disneyland
14. Hypothetical missing link (hyph.)
17. Spinneret-guided construction
19. *Brontosaurus* in action, per its name
21. Organs studied by Gabriele Falloppio
22. Where rays converge
23. Mach known for sound thinking
24. Condition treated by alprazolam

DOWN

1. Ancient "rose-red" city of Jordan
2. Cure-all of dubious scientific value
3. What ergot fungi grow on
4. Periwinkle by another name
5. One going on four
6. Reverer of Quetzalcoatl
7. Protein in fingernails and hair
11. Be like a boa
13. Mandible
15. Event defying the laws of science
16. Land of Mendeleev and Popov
18. Location of the Broca area
20. Like a kitten's tongue on your cheek
22. *Vulpes* member

Answer key on page 68

Although labor market conditions almost certainly prevent some graduates who are interested in an academic career from obtaining a faculty position, we find that a substantial share of PhD students lose interest in an academic research career for reasons other than labor market conditions.

—Economists **Michael Roach** and **Henry Sauermann,** in a recently published analysis, which found that although 80 percent of US PhD students were interested in pursuing an academic career at the start of their program, 25 percent had lost interest in academia just three years later (*PLOS ONE,* September 18)

"It's a rather interesting phenomenon. Every time I press this lever, that post-graduate student breathes a sigh of relief."

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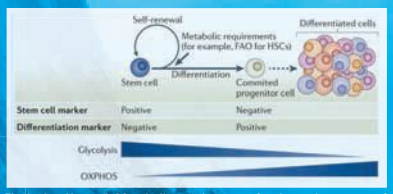

Fig 1 - Ito, K., et al. Metabolic requirements for the maintenance of self-renewing stem cells. Nat Rev Mol Cell Biol. 2014. 15: 243-56.

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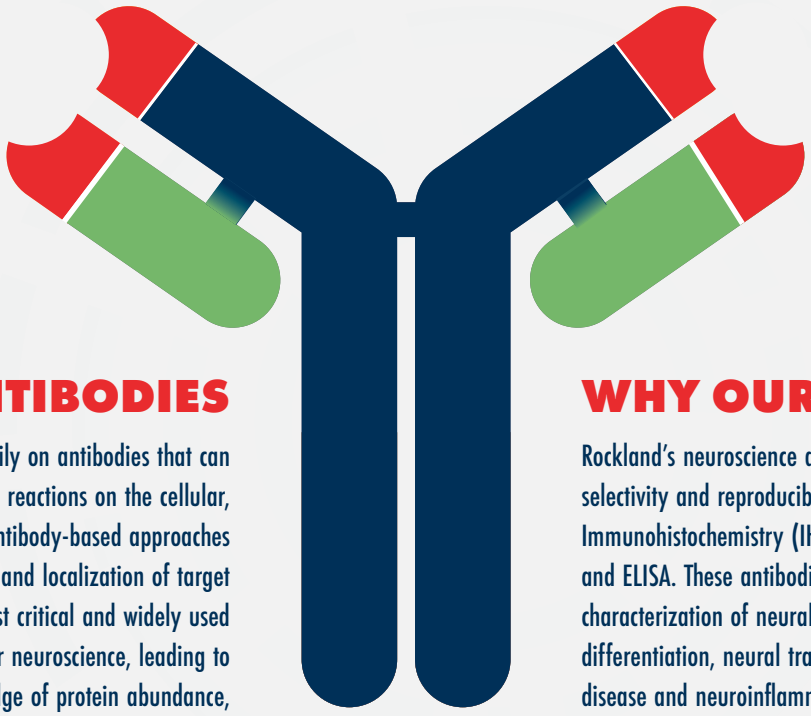

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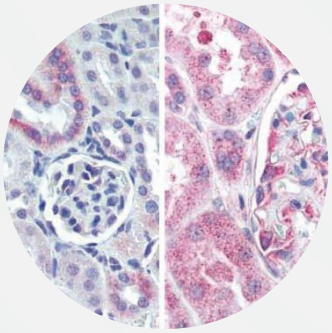

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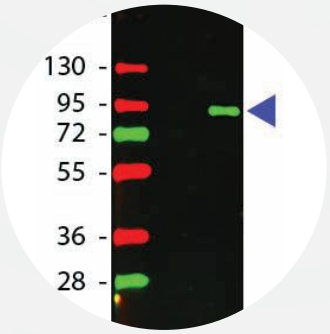

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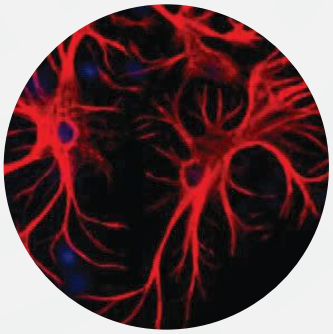

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Notebook

NOVEMBER 2017

NELSON DELLIS

MEMORY MASTER: Nelson Dellis, four-time USA Memory Champion, preparing to memorize nine decks of cards

Total Recall

After Nelson Dellis's grandmother passed away from Alzheimer's disease in the summer of 2009, he became obsessed with memory. "I had seen her whole decline, so brain health was on my mind," he says. He found out about annual memory competitions that tested people's ability to remember large volumes of data—for example, the exact order of 104 playing cards in two decks—and began to learn the strategies so-called "memory athletes" used to pull off these incredible feats.

"I found the techniques worked, and with a bit of practice, you can do a lot more than you ever thought you could," Dellis says.

He entered the 2010 USA Memory Championship in New York City and came in third. The next two years in a row, he took first. A mistake in the finals cost him the championship in 2013, but he regained the crown in 2014 and won again in 2015, making him the first and only four-time USA Memory Champion. And all it took was "a bit of practice."

Dellis says there are several strategies memory athletes use, but they're all based on the same principle: "You want to turn information you're trying to memorize into something that your brain naturally prefers to absorb"—typically, an image. "Once you have that picture, the next step is to store it somewhere—somewhere in your mind you can safely store it and retrieve it later." This place is known as a "memory palace," and it can be any place that's familiar to you, such as your house. You can then place the images you've chosen along a particular path through the memory palace, and "the path, which you know very well, preserves the order."

So when Dellis is asked to memorize two decks of playing cards for the USA Memory Championship, he assigns each card to a person—the king of hearts is his dad, the queen of hearts is his mom, and the nine of hearts is his wife, for example—and then he envisions those people along a path through one of his old apartments

BRAIN BATTLE: Two competitors square off at the 2014 Extreme Memory Tournament at Dart NeuroScience headquarters in San Diego.

NELSON DELLIS

or a childhood home. "I'm imagining those people interacting with an environment."

Dellis's improved retention does seem to depend on his cognitive strategy, rather than on any improvement to his overall IQ, says Henry "Roddy" Roediger, a cognitive psychologist at Washington University in St. Louis who has tested the four-time champ. Dellis admits, "If I'm not using a technique, my memory is as good as the next person. But . . . it's hard for me not to think that way now, just because I've trained so much."

For the past several years, Roediger has been studying the cognition of memory athletes such as Dellis, along with others who've demonstrated exceptional memory abilities, including *Jeopardy* contestants, Bible memorizers, and superior crossword puzzlers. In a project sponsored by Dart NeuroScience, Roediger and his colleagues recruited 25 memory champions from around the word, including Dellis, to complete a suite of cognitive tasks testing long-term retention, working memory, and attention. They also tested 41 *Jeopardy* contestants, 27 Bible memorizers, and 36 crossword puzzlers.

The work, which is ongoing, found that most people in the latter groups did not exhibit memorization skills on a par with memory athletes. "They are just spectacular at what they do, but they showed just a perfectly normal pattern on our tests," Roediger says. The memory athletes, on the other hand, did excel in areas beyond the specific tasks they trained on—such as remembering which polygons of a large set they had seen before—compared with both the controls and the other elite memory groups. "The memory athletes generally blow them out of the water on most tests," Roediger says.

Other researchers have also taken an interest in Dellis's brain. Roediger's wife, Washington University neuroscientist Kathleen McDermott, is interested in individual variation in brain activity during the performance of various tasks or at rest. In August, she and her colleagues published a study analyzing more than 10 hours of functional MRI (fMRI) data from 10 healthy adults (*Neuron*, 95:791-807.e7, 2017). And her group has subjected Dellis to the same treatment. The team is now completing supplementary analyses and writing a paper on the results. "At this point, all I can really say is that we have collected many hours of fMRI data on Nelson, and we will be able to compare his brain activity directly to that of the 10 extensively scanned control participants in the *Neuron* paper," McDermott writes to *The Scientist* in an email.

We're at this exciting point where we can start looking at the link between exceptional long-term memory and genetics.

—Mary Pyc, Dart NeuroScience

Dellis isn't just influencing research, though; research is also influencing him. During his time working with the scientists at Washington University, Dart NeuroScience was just opening a new headquarters in San Diego, and the company accepted Dellis's proposal to design a new memory competition. Launched in 2014 and running for three consecutive years, the tournament offered the largest prize of any memory competition—a whopping $100,000—and drew the most renowned memory athletes from around the world.

At the same time, Dellis, who has a background in computer science, teamed up with friend and fellow memory athlete Simon Orton to develop the software used in the competition. In 2014, the duo launched their own company, called Art of Memory, which offers the games online to people who want to learn the memory techniques.

And now Dellis is onto a new project with Dart: the Extreme Memory Challenge, an online memory test that aims to identify subjects with exceptional memory willing to participate in genetic testing. "We estimate we need about a million people to take this test in order for us to have enough exceptional people to have the numbers we need to be able to detect the genetic locus of exceptional memory," says Mary Pyc, a cognitive scientist at Dart and a former postdoc in Roediger's lab, where she met Dellis. As one incentive, Pyc and her colleagues had Dellis take the test and reveal his results, "so people can see how they compared to a four-time memory champion."

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So far, says Pyc, they've gotten more than 15,000 people to complete the two-day test, which takes less than 10 minutes per day. "From that, we've been able to pull out about 12 exceptionals," she says. They've mailed saliva kits to those 12 people, along with dozens of controls, and have just recently gotten the genetic data back for analysis. "We're at this exciting point where we can start looking at the link between exceptional long-term memory and genetics," Pyc says. If any genetic loci pop out, the researchers hope to use that information to create a product "that can essentially mimic what's going on in these exceptionals. . . . The end goal is to develop a drug that can help with cognitive rehabilitation."

—Jef Akst

Windows on the Brain

Neurodegenerative diseases are tough nuts to crack, not just because of the inherent difficulties of sorting through what has gone awry, and why, but also due to a dearth of biomarkers that could help spot the diseases and track their progression. This inability to easily diagnose many forms of neurodegeneration means that the diseases can't be treated early in their progression. The lack of biomarkers also hinders the certainty with which researchers running clinical trials can assess whether and how well experimental treatments of the diseases are working. A simple, noninvasive eye scan now being developed for Alzheimer's disease (AD), however, may help address both shortcomings.

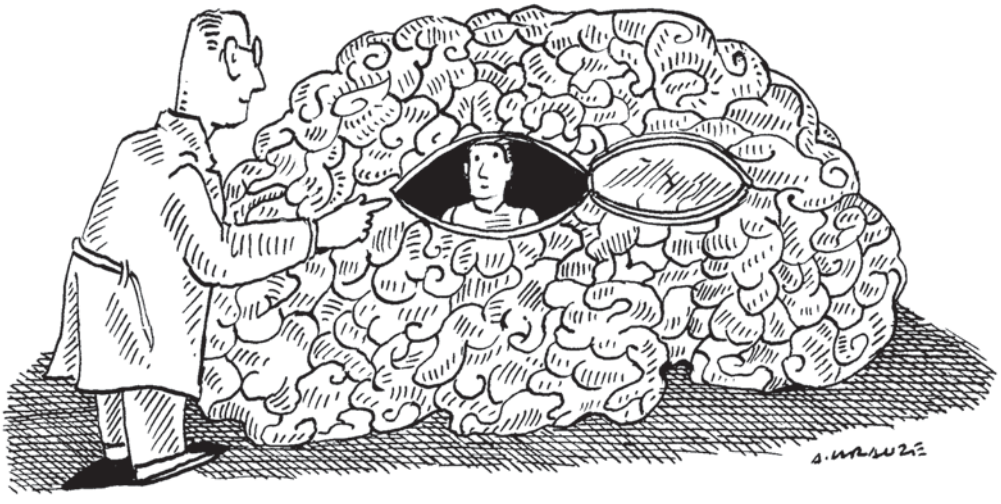
AD researchers already utilize amyloid positron emission tomography (PET), in which tracers are injected into patients' brains to make the disease's characteristic amyloid plaques detectable by PET imaging. But the scans are very expensive, spurring the continuing hunt for biomarkers. "What we now know is that the disease essentially occurs about 20 years before a patient becomes symptomatic," says Cedars-Sinai Medical Center neuroscientist and neurosurgeon Keith Black. "And by the time one is symptomatic, they've already lost a lot of their brain weight; they've already lost a significant number of brain cells; they've already lost a significant amount of connectivity." What's needed, he says, is a way to detect the disease early so it can be treated—with drugs, lifestyle interventions, or both—before it's too late.

So Black has been working with Cedars-Sinai colleague Maya Koronyo-Hamaoui and others on a different way of peering into the skull. "The retina is really part of the brain" and shares many cell types with it, explains Koronyo-Hamaoui, so it makes sense that people who have amyloid plaques in their brains might also have them in the retina. To find out whether that's the case, Koronyo-Hamaoui has led animal studies that showed the quantity of plaques in the retina correlates with levels in the brain (*NeuroImage*, 54:S204-S217, 2011).

In a new study, the researchers analyzed brains and eyes from cadavers of humans with and without AD, and found that plaques tend to cluster in a far corner of the retina, the superior quadrant, an area not typically examined by ophthalmologists.

To visualize the plaques in living people, the researchers had volunteers eat a chocolate pudding spiked with curcumin—which gives the spice turmeric its deep-yellow color—2 or 10 days prior to scanning their eyes. Previous experiments had shown that curcumin fluoresces when bound to the characteristic amyloid-β plaques of Alzheimer's, and the team selected a form of the substance with relatively high bioavailability, Koronyo-Hamaoui says.

The researchers then used a modified ophthalmoscope to look at the superior quadrant in the retinas of AD patients, and compared the readings with those of healthy volunteers. Those with the disease showed twice as much amyloid-β–linked



ANDRZEJ KRAUZE

What we would like to see is extension of this data across different stages of Alzheimer's disease, and how it relates to other biomarkers, such as amyloid imaging.

—Douglas Galasko, University of California, San Diego, School of Medicine

fluorescence in that area of the eye, the team reports (*JCI Insight*, 2:e93621, 2017).

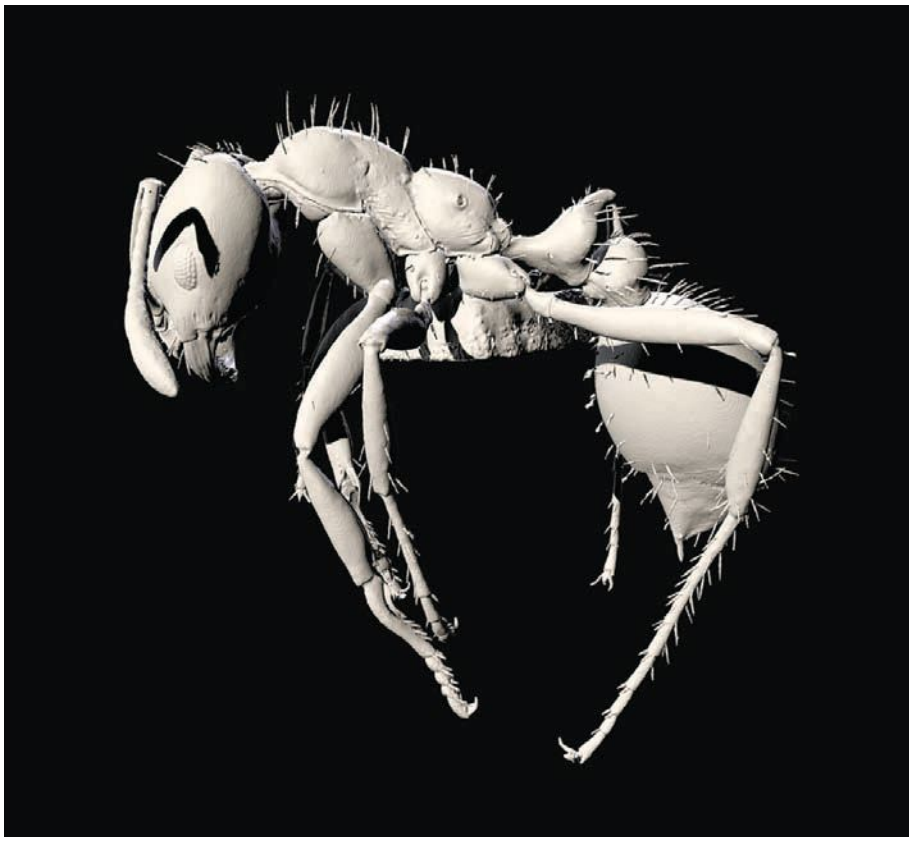
This latest study is "an interesting and novel and promising step," says Douglas Galasko, a neurologist at the University of California, San Diego, School of Medicine who was not involved in the work but has collaborated in the past with some of its authors. "What we would like to see is extension of this data across different stages of Alzheimer's disease, and how it relates to other biomarkers, such as amyloid imaging."

While previous studies from the Cedars-Sinai group have suggested amyloid-β deposition on the retina could reflect similar aggregations in the brain, "I think that this is much stronger," in part because the researchers analyzed tissue from both the human retina and the brain, says eye researcher Bang Bui of the University of Melbourne who was not involved in the work. As for the scans of living patients, "I think it's good proof of principle and certainly really exciting to take things from there, to go forward with this, and maybe to a larger clinical trial," he adds.

The development of a simple noninvasive procedure using a device that's already in wide clinical use is particularly exciting, Bui tells *The Scientist*. Koronyo-Hamaoui, Black, and two other coauthors of the study have founded a company, NeuroVision Imaging, that is now working on getting US Food and Drug Administration approval for the modified ophthalmoscope as a detector of fluorescence in the eye.

If further testing confirms the results, Black envisions people in their 50s and 60s one day getting routine eye scans for Alzheimer's disease as part of their yearly checkups. "If we could potentially stop the disease . . . I think that's a realistic possibility—that's an excellent outcome," he says. "If we could delay the onset of the symptomatic phase of the disease for 5 years or 10 years, that's also a wonderful outcome."

—Shawna Williams



Ants on Fire

INVADER: 3-D model of a red imported fire ant (*Solenopsis invicta*), a species that has spread around the world from its native territory in South America

In Hong Kong, some 1,300 skyscrapers butt up against rows of retail properties and restaurants. But because of conservation laws that mandate the preservation of 40 percent of the city's land, lush parks break up the congested landscape. Someone or something seeking constant activity intermingled with green space would thrive here.

For the last 12 to 15 years, that perfect Hong Kong transplant has been the red imported fire ant (*Solenopsis invicta*), an invasive species native to South America.

"We've created the perfect environment for them," says Benoit Guénard, an ecologist at Hong Kong University, who studies ant biodiversity.

The species invaded the United States in the late 1930s, but it didn't make its way to Asia for another 60 or 70 years, courtesy of global trade. Bustling ports in Hong Kong, mainland China, Taiwan, and Japan, which reported its first sightings this summer, have all been breached. In most places, it is too late for eradication. In others, there is time to at least try.

"If you let them establish the population," Guénard says, "then you've essentially lost the battle."

Guénard is an ant detective of sorts, separating out the "criminals" from the "good guys." Since his arrival in Hong Kong more than two years ago, he and his research associates have spotted five invasive species and helped almost double the number of known native ones, from 170 to nearly 300. And he didn't need to go far. Outside his campus office, his team found an entirely new species they named the golden tree ant (*Asian Myrmecol*, doi:10.20362/am.008016, 2016).

Another ant, it turned out, has also taken up residence on campus: red imported fire ants.

EVAN ECONOMO, OKINAWA INSTITUTE OF SCIENCE & TECHNOLOGY

"You only find something when you start looking," Guénard says. "And if you don't have anyone looking for it, then, of course, you never know."

The French native has been tasked by Hong Kong's Agriculture, Fisheries, and Conservation Department (AFCD) to map the distribution of the red ant, as well as other invasive and native ants, across the territory, and to study their effects on the local flora and fauna. So far, the spread is alarming.

Hot spots of fire ant activity on one map glow on a computer screen in his office. He points to various locations they have heavily colonized, including the Tsuen Wan and Kowloon districts. "Unfortunately, it's pretty widespread," he says. Hong Kong Island, home to the university, shows activity, too, though not as much—it is in the very early stages of invasion, he says.

Just outside Guénard's office, a lab technician sits hunched over a dissecting microscope, sorting different ant species collected around Hong Kong International Airport. The red ant is there, as well as in residential areas and farmlands.

"At this point . . . I do not think eradication is on the table," says Guénard, who plans to submit the full findings to the AFCD this spring. "[But] with the right type of management, we can suppress populations."

Hong Kong's fire ant colonies—some of which have been sighted and reported by the public—are handled using pesticides sprayed by whichever department manages the invaded patch of land. Comprehensive maps could inform a larger, coordinated effort to better track and control the ants, or, Guénard says, serve as a more immediate warning about where people should be careful. Fire ants are aggressive insects with nasty bites and stings that cause intense burning and blisters, and, in rare cases, anaphylaxis or even death.

Fire ants are also amazingly adaptive. One queen hitching a ride in a ship's cargo hold is enough to start a new colony at its destination. The species favors setting up shop on manicured lands, underneath paved surfaces, and within electrical equipment. They can ruin crops and bully native insects and plants out of their homes.

Fire ants originated in areas of Argentina prone to flooding, so reestablishing themselves after disturbances comes easily to the insects. Photos and videos taken of fire ants forming floating islands during Hurricane Harvey in Texas illustrate just how resourceful they can be. Rafts of ants can attach to a tree and wait for flood waters to recede.

An ant colony functions as its own city, having at least one ruling queen (often more), worker ants to forage for food, and larvae to digest and regurgitate the food for the benefit of all parties. While many ant species will fight off rival colonies, fire

ant colonies will join forces, making for a larger and more efficient group.

Those traits and that resilience allow them to be successful, Guénard says. "That is why understanding the ecology is important," he says, "because if you can modify that or revert or limit those kinds of aspects, you're putting yourself at an advantage."

In Japan, fire ants have been sighted at 10 ports since May. The government's response has been aggressive. Officials have beefed up monitoring at 68 ports, and pesticides were sprayed in the container yards at breached ports to eradicate the ants, says Shunya Hashiguchi of the Office for Alien Species Management in Japan's Ministry of the Environment, in an email to *The Scientist*. So far, he says, all identified red fire ants have been killed.

"Not so many ants have been discovered outside of containers at the ports," says Shigeto Dobata, an insect ecologist at Kyoto University. "So, I am optimistic about the eradication." Pouncing on it this early, before they colonize, is critical, he adds.

More than 1,600 km south of Kyoto, Okinawa is prepping itself. The lab of Evan Economo, a biologist at the Okinawa Institute of Science and Technology (OIST), is helping the government devise a control plan for when the fiery arthropod arrives.

Pesticides have not yet been sprayed at Okinawa's ports. Economo's team and his colleagues helped convince officials to move forward with an intensive survey instead at Naha, one of the international ports on Okinawa. Because ongoing surveys at five of the ports hadn't identified any fire ants, they didn't believe the ants had made their way to the island, so pesticides weren't necessary.

Early this summer, one evening after the Naha port closed, 510 bait traps were set every five meters over a grid in the international container areas. No fire ants were found, and now, every two weeks, researchers continue to collect ant samples from traps set around the entire island, in urban, agricultural, and forested areas, and bring them back to the lab to sift through and ID.

"We have been able to show that there are no signs of red ants on Okinawa yet," Economo says. "But we're trying to figure out how to make sure that we will detect it soon after it arrives."

If you let them establish the population, then you've essentially lost the battle.

—Benoit Guénard, Hong Kong University

A step on the path to that goal involved going to fire ant–infested Taiwan to study the species and test detection methods. Ant data are powerful, but up until a few years ago, little existed.

To help fill that gap, Guénard and Economo launched antmaps.org, the world's first interactive Google Maps–like ant database showing where species have invaded. "It's reconstructing the tree of life of ants," Economo says. "One big use of it is to see where ants have been spread around the world by humans, and then track them over time."

The scientists reviewed more than 9,000 papers and went back 200 years to build the map, which is continually updated by Guénard as new data emerge. A recent analysis of the data they and others published in *Nature Ecology and Evolution* (doi:10.1038/s41559-017-0186, 2017) showed that the highest numbers of invasive species, including ants, mammals, and birds, exist on islands and in the coastal regions of continents. Those highly populated and economically developed areas and active trade posts create more opportunities for species, particularly ants, to be moved around by people and ships. Current measures to reduce the spread, the authors say, are not enough.

"Some of the species that are not here, but could come here, are a major problem," Guénard says. "And some of them are even worse than the fire ant."

—Steve Graff

Flies Bugging Frogs

Communication coded for a particular kind of recipient is usually considered privileged information. But sometimes signals from a sender can also have multiple unintended receivers.

Take the túngara frog (*Engystomops pustulosus*). During the breeding season, males gather in ponds and puddles throughout Central and South America and call to attract females of their own species. Also listening: predators and parasites.

Research beginning in the 1980s has demonstrated how frog-eating bats use the calls of male túngara frogs to home in on the animals. The bloodsucking flies that feed on frogs, however, were just a scientific footnote until Purdue University biologist Ximena Bernal set her sights on them. According to Bernal, shifting her focus to the flies that prey on túngara frogs was a happy accident.

In 2002, Bernal was working on how male and female túngaras perceive mating calls for her PhD in Michael Ryan's laboratory at the University of Texas at Austin. While observing calling males in the field in Panama, she noticed they were swiping at their faces with their legs. At first, Bernal thought it was a visual signal to potential mates—a kind of dance to accompany their song. Then she played a video of the behavior on a big screen and realized the frogs were attempting to swat away tiny flies.

Bernal, who is also a research associate at the Smithsonian Tropical Research Institute, says the flies fascinated her. These "frog-biting midges" (family Corethrellidae) are about the size of fruit flies, but slimmer. Like their relatives, mosquitoes, females feed on blood. Unlike mosquitoes, which use chemical cues to find victims, the midges use the frogs' mating calls to locate essential blood meals.

"Although there was a report that these midges use sound to locate and feed on frogs, Ximena made the first scientifically rigorous study of this phenomenon," says

SHOO FLY: Biting midges (top) swarm the noses of túngara frogs (*Engystomops pustulosus*) during the breeding season, keying in on the mating calls that males emit to attract females (bottom).

XIMENA BERNAL

Ronald Hoy, who studies neurobiology and behavior at Cornell University.

To demonstrate that frog-biting midges are attracted to frog calls, Bernal set up acoustic traps—speakers topped with collection tubes—at her field site in Panama. She found that broadcasting túngara frog calls, both natural and synthesized, were sufficient to lure frog-biting midges into her traps, sometimes at the rate of more than 500 in 30 minutes. What's more, frog-biting midges preferred more-complex frog calls to simple ones.

Male frogs appear visibly annoyed by the bloodsucking flies, spending valuable energy swatting at them. But the consequences could be even more dire. Bernal's research has shown that male túngara frogs are much more likely than females to be infected with trypanosomes, a kind of blood parasite transmitted to vertebrates by a variety of blood-feeding invertebrate species. It is likely that frog-biting midges are the vectors of these parasites, and because females do not call, they do not attract the midges. Bernal suspects that infection negatively impacts the male frogs, and she is currently studying the interaction between flies, frogs, and parasites.

In further experiments, Bernal placed insect traps over caged, singing male frogs to explore how flies responded to live, rather than recorded, sounds. She found that flies, much like frog-eating bats, were most attracted to males calling at higher rates or making more-complex calls (*Ethology,* doi:10.1111/eth.12452, 2016).

"When males are thinking about attracting females, making their calls more complex might help. But it also increases their chances of getting eaten by a bat or being targeted by bloodsucking flies," says Bernal. "Both bats and flies are eavesdroppers, curtailing signal complexity in the túngara frog."

Bernal's experiments have ruled out other potential cues used by the flies to locate a host, such as carbon dioxide emitted by the frogs (*J Vector Ecol,* 40:122-28, 2015). She placed insect traps over speakers that were either silent or broadcasting frog calls and determined the number of midges attracted when carbon dioxide was also added. Very few midges were attracted to the silent traps, whether or not carbon dioxide was present. And adding carbon dioxide to the speakers playing frog calls did not increase the traps' attractiveness. It turns out that the frog's mating call is all that is necessary to attract blood-hungry midges.

To better understand the evolution of this opportunistic eavesdropping, Bernal looked at how frog-biting midges use sound in a different context: mating. She recorded the sounds of the flies' wing beats in a naturally forming mating swarm and in individually tethered males and females (*Anim Behav,* 103:45-51, 2015).

"When males and females were close together, they would change the speed at

which they fly and then match the frequencies of their upper harmonics before copulating in mid-air," says Bernal. "It's like a love song."

The wing beats of midges cover a large frequency range, from about 0.5 to 5 kHz, matching the frequency range of calls produced by túngara frogs. Bernal hypothesizes that the flies first evolved the ability to use sound in mating and then co-opted that ability in order to eavesdrop on frogs. She's currently looking at the more than 100 species of frog-biting midges to see which ones depend on sound for mating and when the ability to hear such high frequencies likely evolved.

Another piece of the puzzle is how these flies hear. Bernal is collaborating with Hoy and Ronald Miles of Binghamton University in New York to investigate how the midges' antennae respond to sound. So far, the data indicate that their antennae are very sensitive to a broad range of frequencies and seem to be able to sense sounds from several meters away. Bernal and her collaborators are also exploring the possibility that frog-biting midges have a tympanic ear. They have found what could be a tympanic ear on the fly's thorax and are currently performing experiments to determine whether it vibrates at the relevant frequencies to sense fly wing beats and frog calls.

"Dr. Bernal has made many impressive contributions to the fields of behavioral ecology, insect biology, ecology, and evolution," says T. Ulmar Grafe, a biologist at the University Brunei Darussalam. He cites her work as the inspiration behind his efforts to look for frog-biting midges in Borneo, where he has discovered eight new species.

"Ximena started with this really cool observation that these midges suck blood out of túngara frogs' noses, and she has just run with it," says Mark Bee, who studies frog communication at the University of Minnesota. "Her work highlights this idea that there are illegitimate, or unintended, receivers in communication systems; that is, animals you wish did not perceive your signal."

"The problem is, if you put a signal out into the air, it's there for anyone who can hear it," says Hoy. "What's remarkable about the frog-biting midge is that it eavesdrops across the invertebrate-vertebrate barrier. They have hijacked the song of a frog."

For something that started as a side project when she was a graduate student, frog-biting midges have proved to be a fruitful subject for Bernal. "This system reveals the complex and intriguing ways that nature works," she says. "It shows that we cannot study animals in isolation. We have to take into account their enemies, as well."

—Mary Bates

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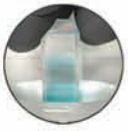

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Fast-Tracking Sexual Maturation

The brains and bodies of young female rats can be accelerated into puberty by the presence of an older male or by stimulation of the genitals.

BY RUTH WILLIAMS

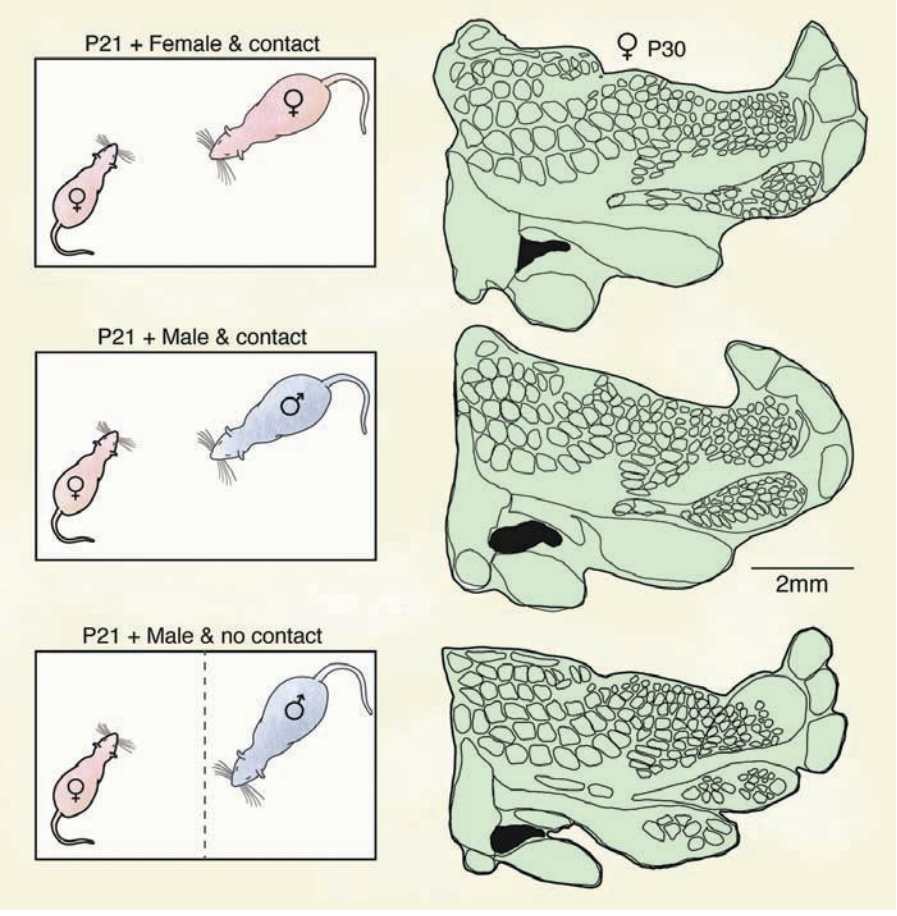
Contrary to the longstanding belief that puberty is largely controlled by hormones, new evidence shows that sexual touch is a powerful puberty promoter. Touching prepubescent female rats' genitals can cause the brain region that responds to such tactile stimuli to double in size and their bodies to show signs of puberty up to three weeks earlier than non-stimulated females, according to a report in *PLOS Biology* on September 21. The study reveals the hitherto unappreciated influence of physical sexual experience on the young brain and body.

"The dominant idea has been that puberty is controlled in the brain and in behavior by the release of hormones . . . but there has been a smattering of findings over the years that additional environmental influences effect puberty and the onset of sexual behavior," says Dan Feldman of the University of California, Berkeley, who was not involved in the study. This new work "suggests that maybe this is true and that actual tactile stimulation can be something that accelerates the onset of puberty," he adds.

Puberty in mammals is a period of dramatic changes not just to the body, but to behavior and brain function. Indeed, one of the most pronounced changes, recently observed in both male and female rats, is the doubling in size of the genital cortex, which is a part of the larger somatosensory cortex—the brain area associated with physical sensation.

But these brain, behavior, and body changes are not simply an age-dependent process. Mammalian puberty can also be under strong social control. In an earlier study, exposure to male pheromones and physical interaction with males was shown to accelerate puberty in young female mice, for example.

Michael Brecht, a neuroscientist at the Bernstein Center for Computational Neuroscience in Berlin, who with colleagues had previously reported the puberty-associated expansion of the rat genital cortex, wondered whether this dramatic brain change might occur in response to physical interactions, and if so, whether it might be linked to the male-induced accelerated puberty found in female mice.



ACCELERATED DEVELOPMENT: In prepubescent 21-day-old female rats, the genital cortex region (black) of the somatosensory cortex exhibits accelerated growth when the young female is exposed to touch and contact with a sexually mature male (right, center panel). After nine days of exposure, the genital cortex of the young rat is the same size as that of a fully mature sexual female.

To find out, he and his team housed prepubescent female rats (21 days old) either with an older male rat or in a cage where an older male could be seen, heard, and smelled, but not touched. These conditions were designed to distinguish the effects of direct tactile stimulation from exposure to pheromones only.

PLOS BIOLOGY, 15:E2001283, 2017

They found that after co-housing the females with a male for one week, the now 30-day-old females had genital cortices the size of fully sexually mature females (50 days old), while those females not in contact with the male had only mid-sized cortices. Co-housing with a male also accelerated the physical signs of puberty in the females—namely, an increased uterine weight and vaginal opening—compared with those in the noncontact cages.

To figure out just what it is about physical contact that might accelerate puberty, the team examined whether touch alone, without a male, could recapitulate the effects. Stroking the young female rats' genitals with a small brush held by one of the researchers produced similarly accelerated genital cortex expansions and physical signs of puberty.

The team went on to show that inhibiting the activity of neurons in the genital cortex using a locally applied neurotoxin prevented both neurological and physical signs of puberty in young female mice co-housed with older males. The authors interpret this result to mean that neural activity in the genital cortex may not only expand in response to a sexual stimulus, but be necessary for puberty to progress.

These first sexual experiences, I think, change the brain in a very profound way that we are only beginning to understand.

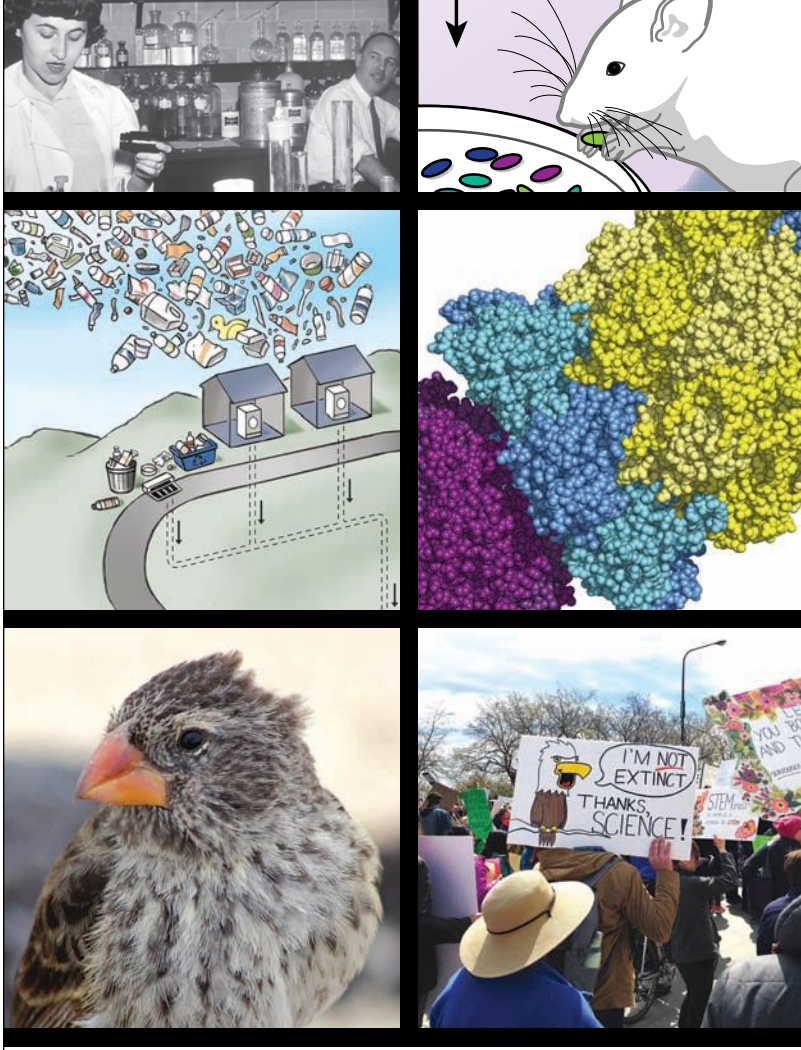
—Michael Brecht, Bernstein Center for Computational Neuroscience in Berlin

"I think [our work] puts more emphasis on sexual touch as a regulator of brain development and of puberty," says Brecht. "These first sexual experiences, I think, change the brain in a very profound way that we are only beginning to understand," he adds. That said, Brecht's team found that sex hormones were still essential for genital cortex expansion in puberty.

"It's a potentially important paper," says Barry Komisaruk, a psychologist at Rutgers University, "because it's showing that sensory stimulation and hormonal activity can influence the structure and function of the brain."

This new work "provides insight into the possible mechanisms underlying the widely observed, but poorly understood, phenomenon of puberty occurring about a year earlier in girls who have been sexually abused," says psychiatrist Jay Giedd of the University of California, San Diego. The caveat, of course, is that these studies were in rats and such experiments would obviously be impossible to conduct in humans. Nevertheless, Giedd adds, "it would be really amazing if this mechanism only occurred in rats." (C. Lenschow et al., "Development of rat female genital cortex and control of female puberty by sexual touch," *PLOS Biology*, 15:e2001283, 2017.) ■

A version of this story was published at the-scientist.com on September 21, 2017.



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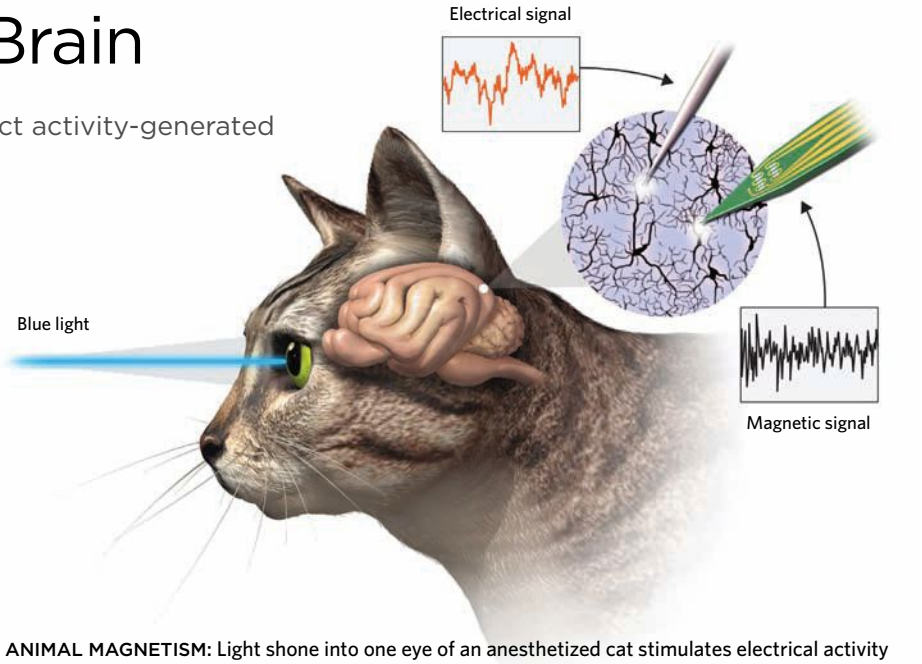
The Magnetic Brain

Micrometer-size magnetrodes detect activity-generated magnetic fields within living brains.

BY RUTH WILLIAMS



ANIMAL MAGNETISM: Light shone into one eye of an anesthetized cat stimulates electrical activity in the visual cortex. A magnetrode (green) inserted less than a millimeter into the visual cortex detects the magnetic fields created by this electrical activity. Insertion of an electrode adjacent to the magnetrode allows researchers to gather and compare electrical current and magnetic field data at the same time.

The brain is often described in terms of its wiring, connections, and circuits, and such language is not merely an analogy to a building's electrical infrastructure. Neurons control the flow of charged ions—receiving, perpetuating, and discharging currents—to perform their essential functions.

Analyzing the brain's electrical activity to gain insights into its function can be achieved with electrodes either placed upon the scalp—as in electroencephalograms (EEGs)—or inserted into the brain. But electrical currents also produce magnetic fields, and detecting these fields can offer several advantages over voltage measurements, says Myriam Pannetier-Lecoeur of the French Alternative Energies and Atomic Energy Commission.

For example, while electrical fields and voltage measurements are distorted by the insulating or conductive properties of surrounding tissues, magnetic fields are not. Furthermore, electrodes are unable to detect the direction of an electrical current flow, making the source of neuronal activity hard to pinpoint. Magnetic sensors, on the other hand, can determine both the intensity and direction of a magnetic field and, by inference, the underlying current flow. Lastly, for measuring voltages, a second reference electrode is required, which can complicate interpretations, whereas magnetic recordings need just one detector.

Like voltages, however, magnetic fields become weaker the farther away a detector is located, explains Pannetier-Lecoeur. So she and her colleagues have developed the first magnetic probe for insertion directly into the mammalian brain.

To create magnetic detectors small enough to insert into the brain, yet powerful enough to detect magnetic fields, the team used a new technological approach called spin electronics. This allowed them to engineer needle-shape probes just 150 μm wide—an impossibility with previous magnetic sensor technologies. Inserting these probes, called magnetrodes, into the visual cortex of anesthetized cats, the researchers could monitor the brain's magnetic activity in response to light stimulation of one eye.

Although the signal-to-noise ratio of Pannetier-Lecoeur's magnetrodes isn't yet perfect, notes Lauri Parkkonen of Aalto University in Helsinki who was not involved in the work, "spin electronics is a field that is advancing rapidly," so improvement in sensitivity "I think is likely."

Riitta Hari, also of Aalto University, adds: "This is an interesting proof-of-concept paper . . . that can open a new important research line in neurophysiology." (*Neuron*, 95:1283-91, 2017) ■

AT A GLANCE

IN-BRAIN ACTIVITY DETECTOR	HOW IT WORKS	LOCALIZATION OF ACTIVITY SOURCE?	IN VIVO SIGNAL DISTORTION?	SPECIAL REQUIREMENTS
Electrodes	Two electrodes (one signal and one reference) are needed, at least one of them inserted into the cortex. The difference in voltage between the two is measured.	Multiple electrodes (with their reference electrodes) can improve mapping of the activity source.	Yes, the properties of nearby tissues can interfere with the signal.	None
Magnetrode	One magnetrode is inserted into the cortex to measure the intensity and orientation of the magnetic field.	Multiple magnetrodes can be used to accurately pinpoint the activity source.	No, magnetic fields are not distorted by tissues.	To improve magnetrode sensitivity a magnetically shielded area would be required, which may be costly to construct.

INTO THE BREACH

To treat neurological diseases, researchers are developing techniques to bypass or trick the guardian of the central nervous system—the blood-brain barrier.

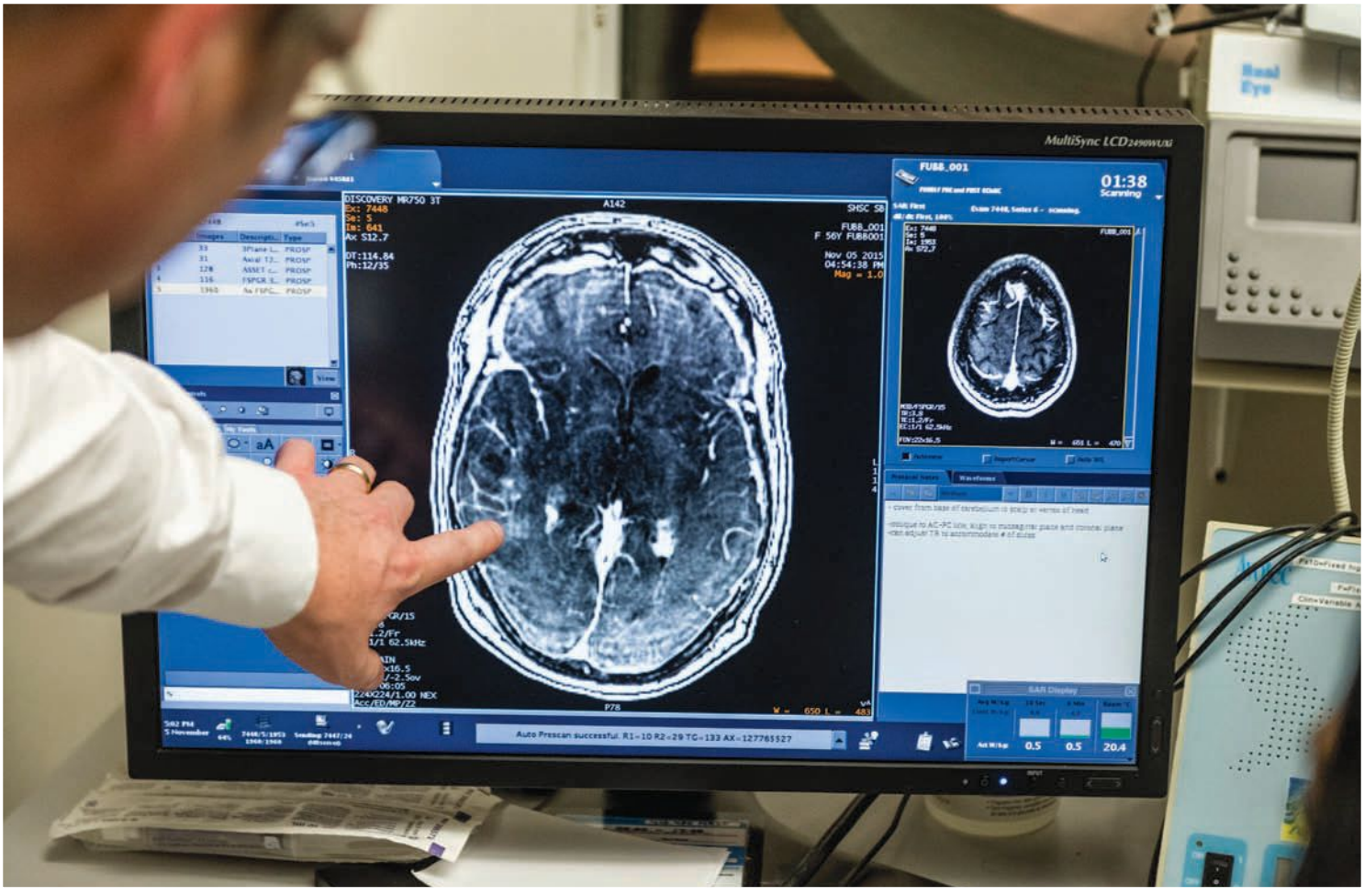
BY AMANDA B. KEENER

On a fall day in 2015 at Sunnybrook hospital in Toronto, a dozen people huddled in a small room peering at a computer screen. They were watching brain scans of a woman named Bonny Hall, who lay inside an MRI machine just a few feet away. Earlier that day, Hall, who had been battling a brain tumor for eight years, had received a dose of the chemotherapy drug doxorubicin. She was then fitted with an oversized, bowl-shape helmet housing more than 1,000 transducers that delivered ultrasound pulses focused on nine precise points inside her brain.

Just before each pulse, her doctors injected microscopic air bubbles into a vein in her hand. Their hope was that the microbubbles would travel to the capillaries of the brain and, when struck by the sound waves, oscillate. This would cause the blood vessels near Hall's tumor to expand and contract, creating gaps that would allow the chemotherapy drug to escape from the bloodstream and seep into the neural tissue. Finally, she received an injection of a contrast medium, a rare-earth metal called gadolinium that lights up on MRI scans. Now, doctors, technicians, and reporters crowded around to glimpse a series of bright spots where the gadolinium had leaked into the targeted areas, confirming the first noninvasive opening of a human's blood-brain barrier (BBB).

"It was very exciting," says radiology researcher Nathan McDannold, who directs the Therapeutic Ultrasound Lab at Brigham and Women's Hospital in Boston and helped develop the technique that uses microbubbles and ultrasound to gently disturb blood vessels. Doctors typically depend on the circulatory system to carry a drug from the gut or an injection site to diseased areas of the body, but when it comes to the brain and central nervous system (CNS), the vasculature switches from delivery route to security system. The blood vessels of the CNS are unlike

DOUG NICHOLSON/SUNNYBROOK



those throughout the rest of the body. Their basic units—endothelial cells—are endowed with a suite of unique properties that prevent passage of more than 90 percent of small-molecule drugs and nearly all biologics through or between the cells. Certain proteins seal spaces between cells, for example, and molecular pumps oust unwanted molecules that make their way into the endothelial cells before those substances have a chance to migrate through the blood-vessel cells and into the CNS.

More security lies just outside the vasculature. As is the case for most blood vessels throughout the body, the endothelial cells are surrounded by a layer of extracellular matrix proteins and supported by cells called pericytes, which control blood vessel development. But in the CNS, the density of pericytes is nearly 100 times higher than elsewhere in the body. CNS endothelial cells are further covered by the pseudopods, or false feet, of neural cells called astrocytes. Both pericytes and astrocytes provide an extra barrier and influence the expression of genes, such as those encoding components of tight junctions, that make CNS blood vessels so selective.

That day at Sunnybrook hospital, however, the focused ultrasound technique proved successful in breaching this formidable barrier. "We worked on this for 15 years doing animal studies, and we're at the point where it's ready to go to the clinic," says McDannold.

Although Hall's procedure was a milestone, and could pave the way for targeted, noninvasive drug delivery to the brain, it was hardly the first time the BBB had been opened for a medical purpose. For decades, physicians have used hyperosmotic solutions that cause endothelial cells to shrink throughout the brain-adjacent vasculature, opening gaps that allow drugs to pass through. Procedures that bypass the BBB by directly injecting a drug into brain tissue or into cerebral-spinal fluid (CSF) using catheters have also been in use since

A real naive view is that the BBB is just a wall. It is a whole series of physical properties that allow the vessels to control what goes between the blood and the brain.

—Richard Daneman
University of California, San Diego

TRIAL RUN: In 2015, brain tumor patient Bonny Hall (opposite page) underwent an experimental procedure aimed at delivering the chemotherapy drug doxorubicin directly to a tumor in her brain. The helmet framing her head delivered ultrasound pulses to nine locations her brain, immediately after scientists (Yuexi Huang pictured) injected microscopic air bubbles into a vein in her hand. Using gadolinium as a marker, the resulting MRI scans (above) showed that the approach was successful: the ultrasound caused the microbubbles to oscillate, expanding the blood vessels near Hall's tumor and allowing the chemotherapy to cross the blood brain barrier and enter the neural tissue.

DOUG NICHOLSON/SUNNYBROOK

the 1990s. But researchers such as McDannold are looking for less invasive and more-precise ways to get in. Some want to open the BBB at specific locations and defined times to treat conditions such as brain tumors; others aim to leave it intact while delivering daily treatments for diseases such as Alzheimer's. To achieve these goals, researchers are developing a diverse set of strategies, each designed to circumvent or exploit the unique features that give the BBB its strength and selectivity.

Sneaking past the guards

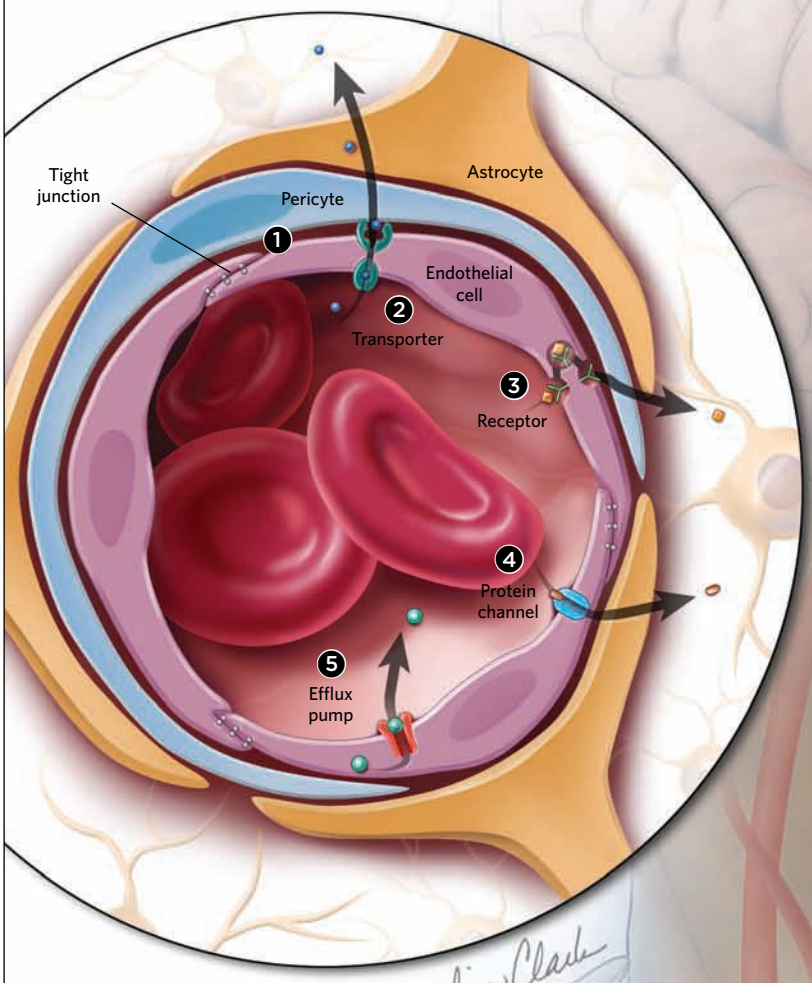
To design ways to breach the BBB, researchers first have to understand it. Biomedical engineer Peter Searson has spent decades meticulously acquiring the necessary technologies and methodologies to reverse-engineer a three-dimensional facsimile of the human BBB, complete with fluid flow to represent the shear force supplied by blood. (See "Designing In Vitro Models of the Blood-Brain Barrier," *The Scientist*, September 2016.) He and his team at Johns Hopkins University have gradually increased their model's complexity, first deriving the required cell types from human induced pluripotent stem cells, then determining the most appropriate cell culture conditions to achieve the desired phenotypes. In 2015, for example, they finally worked out a way to culture human astrocytes in a 3-D gel matrix without activating the cells' stress response, which would alter the expression of certain genes in the astrocytes and other BBB cell types.[1] The next step is to culture stress-free astrocytes and pericytes together.

"We're just about at the point now where we can start combining everything we've learned," Searson says. Still, he doesn't think this complex model will be complete for at least another decade or two. That's because the BBB is more than just a couple of extra layers around blood vessels.

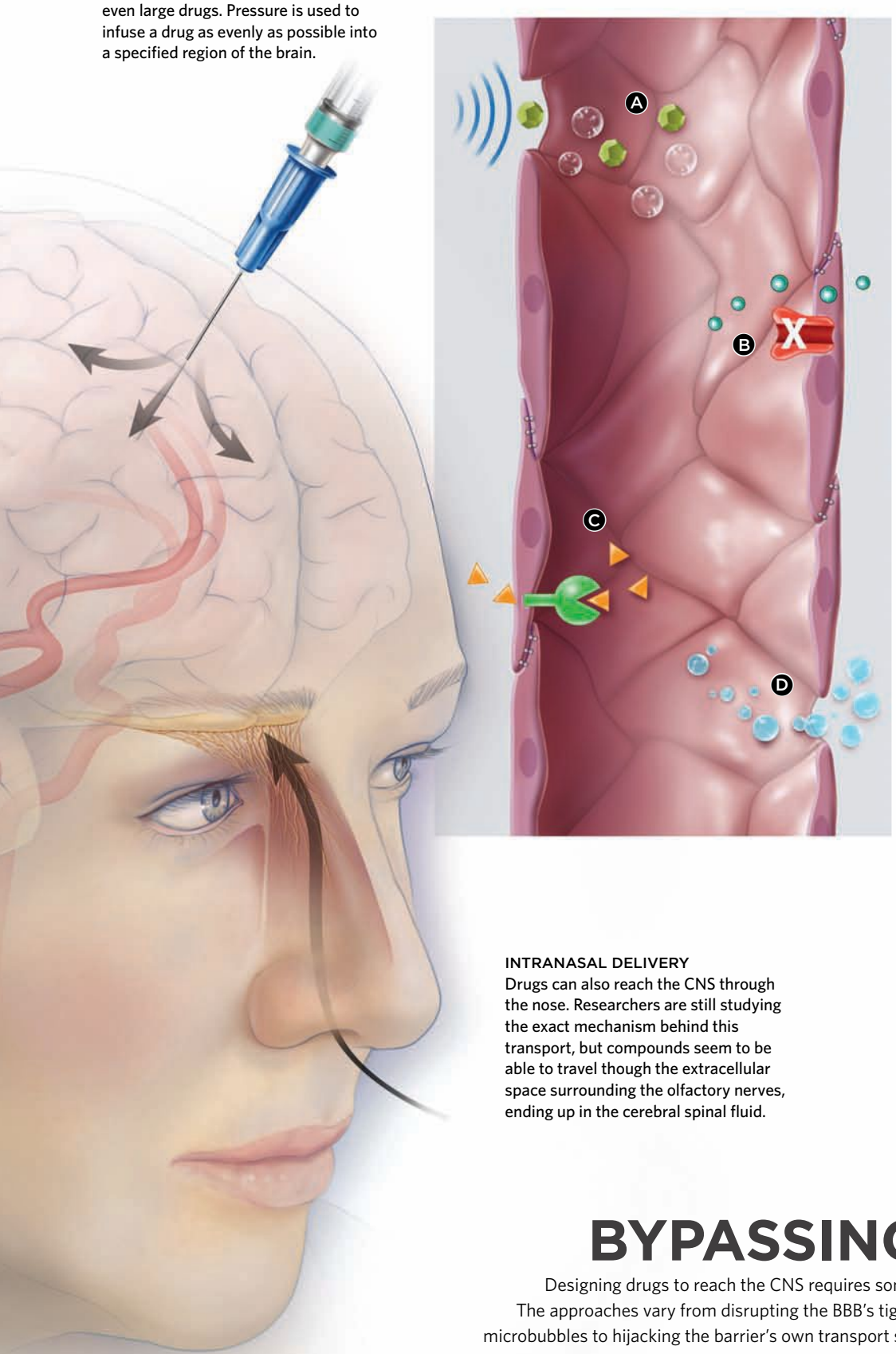
"A real naive view is that the BBB is just a wall," agrees Richard Daneman, a neuroscientist at the University of California, San Diego. "It is a whole series of physical properties that allow the vessels to control what goes between the blood and the brain."

A FORMIDABLE BARRIER

The blood-brain barrier (BBB) is a collection of specialized cells and proteins that control the movement of molecules from the blood to the central nervous system (CNS). The blood vessel endothelial cells of the BBB are cemented together by protein structures called tight junctions ❶, preventing diffusion of most molecules between cells. BBB endothelial cells display transporters ❷, receptors ❸, and channels ❹ that facilitate selective transport of vital nutrients into the CNS. They also possess efflux pumps, such as P-glycoprotein, that expel most small, amphiphilic molecules that are soluble in the blood and in cell lipid membranes ❺. Pericytes and astrocyte pseudopods serve as an additional physical barrier between the blood vessel and brain tissue, and support the expression of endothelial cell genes required to maintain the BBB.



CONVECTION-ENHANCED DELIVERY
Catheters placed in the brain through a hole in the skull allow the passage of even large drugs. Pressure is used to infuse a drug as evenly as possible into a specified region of the brain.



A OPENING THE BBB
After injecting microbubbles into a patient's bloodstream, researchers apply low-energy ultrasound waves that cause the bubbles to swell and contract. This oscillation weakens the BBB and reduces the abundance of tight junction proteins, reversibly opening the barrier to allow the delivery of a drug from the blood to a targeted region of the brain.

B PASSING THE PUMPS
Amphiphilic compounds—those that are soluble in the blood and in cell lipid membranes—can reach the CNS by entering and exiting BBB endothelial cells. Most of these molecules are expelled from the endothelial cells back into the bloodstream through efflux pumps. To get around this barrier, researchers alter drugs to avoid binding to such pumps or use compounds that hinder pump activity.

C TRAVERSING RECEPTORS OR TRANSPORTERS
Drug designers can tailor compounds to bind one of the many receptors or transporters that BBB endothelial cells use to supply the brain with nutrients and essential molecules such as amino acids. The drug could be covered with a natural substrate of one of these receptors or engineered to bind both a BBB endothelial cell receptor and its target within the CNS.

D OSMOTIC DIURETICS
When solutions of diuretics are injected into the carotid artery, they draw fluid out of BBB endothelial cells, causing the cells to shrink. This leaves gaps between the endothelial cells, granting small and large molecules indiscriminate access to the entire brain for minutes to hours.

INTRANASAL DELIVERY
Drugs can also reach the CNS through the nose. Researchers are still studying the exact mechanism behind this transport, but compounds seem to be able to travel though the extracellular space surrounding the olfactory nerves, ending up in the cerebral spinal fluid.

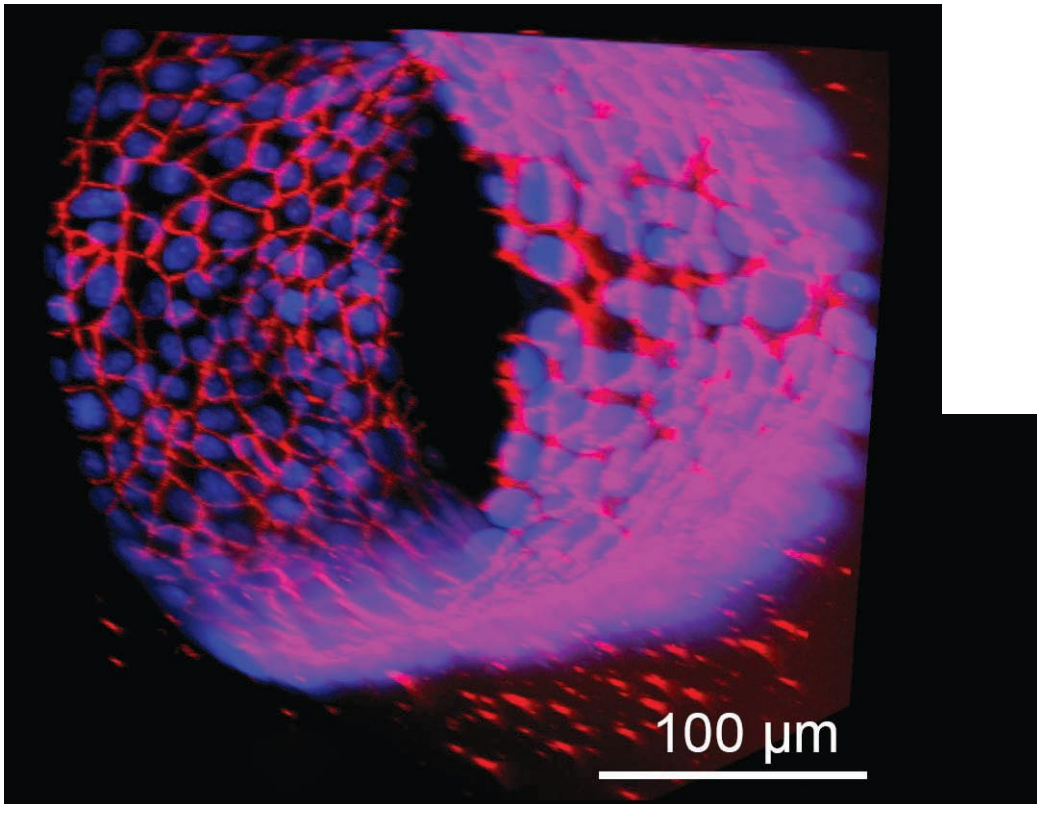
BYPASSING THE BBB

Designing drugs to reach the CNS requires some creativity on the part of researchers. The approaches vary from disrupting the BBB's tight junctions with ultrasound waves and microbubbles to hijacking the barrier's own transport systems. Each technique comes with its benefits and drawbacks, making it appropriate for some patients or drug types but not others.

One of the most prominent of those properties is the presence of tight junctions, protein structures that button up the membranes of neighboring endothelial cells near their blood-facing, or luminal, ends. In most areas of the body, nutrients reach organs by slipping between blood vessel endothelial cells. Tight junctions in the brain vasculature restrict the flow of molecules from the blood to the CNS. "Even water molecules can't make it through these cracks," says Ronald Cannon, a molecular biologist at the National Cancer Institute.

Tight junctions seem to be where ultrasound and microbubbles work their magic. In 2008, Sunnybrook biophysicist Kullervo Hynynen and his colleagues showed in rats that, in the presence of microbubbles, focused ultrasound waves not only increased leakiness in brain vessels for up to four hours, but also reduced the abundance of the tight junction proteins occludin, claudin-5, and zona occluding (ZO)-1. As a result, a large molecule called horseradish peroxidase, which usually can't get past the BBB, started to slip in between blood vessel endothelial cells and into the rats' brains.[2]

McDannold, who was a graduate student in Hynynen's lab at Brigham and Women's Hospital in the 1990s, says there's still a lot of work to do to understand the mechanisms behind the focused ultrasound procedure—and to demonstrate its safety—but the concept has come a long way. "The idea of using ultrasound to disrupt the BBB goes back to the 1960s," he says. "At that time, it would either be associated with damage in the brain or it would not be reproducible." He and Hynynen spent several years trying to apply focused ultrasound in a safe and controlled manner, but they kept running into the problem of damaging the tissue with heat produced by the sound waves. Then, in 2001, they had the idea to try microbubbles, commercially available as a tool to light up blood vessels in MRI images. "To be honest, the first experiment we did, we caused massive damage in the brain," McDannold says of their initial tests on rabbits. "That's when we realized these bubbles are really focusing the effect of the ultrasound right on the vessel walls," allowing the researchers to dramatically reduce the ultrasound's acoustic power while still achieving the BBB breach.[3]



Since then, both McDannold and Hynynen have continued to refine the procedure in animal models and to detail its mechanisms. In a study published last year, McDannold's team used focused ultrasound combined with microbubbles to deliver anti-HER2 antibodies to the brains of 10 rats with breast cancer tumors that had metastasized to the organ.[4] The antibody slowed brain tumor growth in four of the rats that received the ultrasound treatment. "We didn't see [slowed growth] at all without the ultrasound," says McDannold.

Hynynen's group also used the method in a mouse model of Alzheimer's disease and showed that simply opening the BBB in the hippocampus was enough to reduce amyloid-β plaque levels and improve the animals' spatial memory.[5] They found that opening the barrier allows endogenous anti–amyloid-β antibodies into the brain, leading astrocytes and microglia to gobble up more of the toxic protein.[6]

The technique is also being tested in humans. In addition to a Phase 1 brain cancer trial using Bonny Hall's treatment that began in 2015, this year researchers at Sunnybrook began testing the safety of using focused ultrasound to open the BBB in the frontal lobes of patients with Alzheimer's disease. And McDannold is working toward getting US Food and Drug Administration (FDA) approval for human trials in the U.S.

In the meantime, McDannold, Hynynen, and others are developing ways to monitor sound waves that bounce off the microbubbles and adjust the ultrasound power mid-treatment to prevent blood vessel damage. So far, Hynynen's team has shown they can do this in rodent models and rhesus macaques. If the technique can be proven safe for humans, especially when used repeatedly, McDannold says, there's a long list of conditions that may benefit from such treatment. "If you knew exactly where you wanted [drugs] to go in the brain, we could direct them, but that's a long ways away."

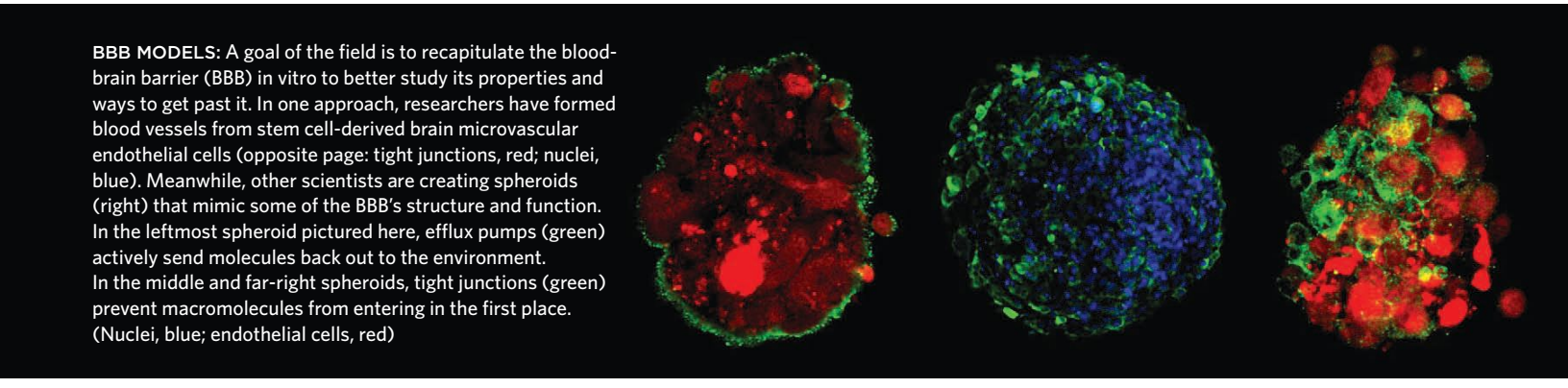
IMAGE TAKEN BY MORIAH KATT

Over, under, around, and through

For now, the microbubble-and-ultrasound method is simply too risky for most patients. "The brain has the BBB there specifically to protect it from foreign compounds—it's a defense mechanism," says Choi-Fong Cho, a neuroscientist at Brigham and Women's Hospital. For non-deadly diseases, "we want to keep the BBB intact while we deliver our drugs so that we don't risk affecting the brain in other ways."

when cultured alone, these cells quickly lose their identity and stop expressing tight junctions. Recognizing a need for a simple and accurate screening platform, Cho and her colleagues have developed a spheroid model that allows mouse endothelial cells, pericytes, and astrocytes to interact in a 3-D matrix, preventing the endothelial cells from losing their identity. (See "Image of the Day: Brain Barrier Balls," posted on the-scientist.com June 7, 2017.) The cells

two membrane receptors characteristic of the BBB, and a functional efflux pump called permeability glycoprotein (P-gp). Expression of P-gp is a helpful feature because small molecules that do breach an artificial BBB often fail miserably in animal models, where the pump spits them right back into the blood vessel. P-gp is the most common of the BBB efflux pumps, which collectively bind and export 60 percent to 70 percent of small-molecule drugs. "It's a



BBB MODELS: A goal of the field is to recapitulate the blood-brain barrier (BBB) in vitro to better study its properties and ways to get past it. In one approach, researchers have formed blood vessels from stem cell-derived brain microvascular endothelial cells (opposite page: tight junctions, red; nuclei, blue). Meanwhile, other scientists are creating spheroids (right) that mimic some of the BBB's structure and function. In the leftmost spheroid pictured here, efflux pumps (green) actively send molecules back out to the environment. In the middle and far-right spheroids, tight junctions (green) prevent macromolecules from entering in the first place. (Nuclei, blue; endothelial cells, red)

Fortunately, many carefully crafted small drugs can cross the BBB without any disruption. "When you're thinking about how to get a molecule across, you have to think about [the BBB's] endogenous properties," says Daneman. After all, he says, "the goal [of the BBB] isn't to keep [all] molecules out of the CNS," but to regulate which are allowed to pass and when. If a molecule is soluble in both blood and in lipids, for example, it can dissolve into a cell's lipid membrane and work its way through the cell and out the other side to reach the brain. Molecules such as oxygen, alcohol, and most anesthetics do this all the time, as do nearly all current CNS drugs. There are many ways to tinker with a drug's charge and lipophilicity, but the success of such tailoring is hit-or-miss depending on the compound, says Cho. "There's no secret recipe to make something that crosses the BBB."

Companies rely on screening drugs in vitro, often using a canine kidney cell line because, like the BBB, kidney cells express tight junctions. Researchers can also obtain endothelial cells from human brain tissue removed during surgery, but

Researchers have spent decades meticulously acquiring the necessary technologies and methodologies to reverse-engineer a three-dimensional facsimile of the human BBB.

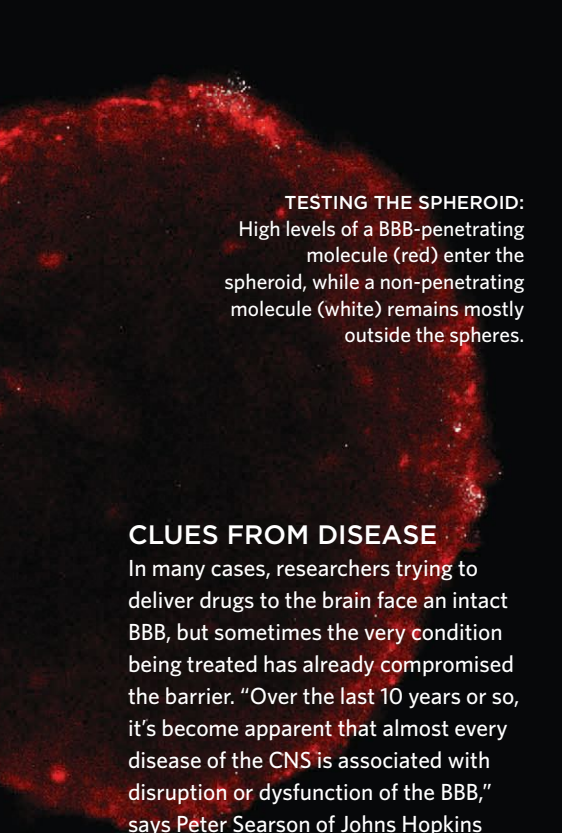
tend to self-assemble so that the astrocytes are in the center of each sphere, with pericytes on the outside and endothelial cells in between.[7] Although the layering is different from what is seen in vivo, the spheroids faithfully recapitulate some BBB functions. Cho's team showed that while the spheroids let carbon dioxide, oxygen, and alcohol into their centers, they exclude a sugar molecule called dextran, just as would be expected by the BBB.

Importantly, the endothelial cells in the spheroids produce tight junction proteins,

big problem," says Cannon. "It's one of the reasons why drugs don't make it to market."

In a study published in April, Cannon and his colleagues reported that they could temporarily inhibit P-gp's activity.[8] Using capillaries cut out of rat brains, they tested agents for their ability to increase or decrease P-gp's activity, and found that a phospholipid called lysophosphatidic acid (LPA) and the antidepressant amitriptyline can together reduce the ability of P-gp to pump a fluorescent substrate into the capillaries' interior. The effect occurred within minutes of exposure to the drugs, and disappeared almost as fast once the drugs were removed. "That reversibility is important," says Cannon, so that the BBB can resume its normal function after allowing the drug into the brain.

Cannon's team tested both LPA and amitriptyline on brain capillaries from a rat model of amyotrophic lateral sclerosis (ALS), in which P-gp expression is increased, and found that injecting the drugs into the animals' carotid arteries increased the amount of substrate that made it across the BBB and into the brain,



TESTING THE SPHEROID: High levels of a BBB-penetrating molecule (red) enter the spheroid, while a non-penetrating molecule (white) remains mostly outside the spheres.

IMAGE BY CHOI-FONG CHO

CLUES FROM DISEASE

In many cases, researchers trying to deliver drugs to the brain face an intact BBB, but sometimes the very condition being treated has already compromised the barrier. "Over the last 10 years or so, it's become apparent that almost every disease of the CNS is associated with disruption or dysfunction of the BBB," says Peter Searson of Johns Hopkins University. The extent to which BBB leakiness in disease helps drugs get into the brain is variable and debatable, but it has already opened doors to potential ways to disrupt the barrier on purpose.

In July, for example, an international team of researchers published a study on a type of antibody, isolated from the cerebrospinal fluid of patients with an optic nerve inflammatory disease, that made cultured BBB endothelial cells more permeable to the sugar dextran. In mice receiving injections of the antibody, proteins that are normally sequestered to the blood ended up in brain tissue, suggesting that such antibodies could aid in drug delivery to the CNS. (*Sci Transl Med,* 9:eaai9111, 2017).

Searson says his ultimate goal is to build BBB models using induced pluripotent cells from diseased individuals to understand how various neurological conditions compromise the barrier and how it might be repaired. Each day, that goal gets closer as researchers understand more about the intricacies that define the BBB—intricacies that Searson's team is striving to mimic in cell culture. It's a challenge, but he is not giving up anytime soon. "It's what I like to tell my students: if it was easy, someone else would have already done it," he says.

indicating that the treatment reduced the pump's activity. Cannon says he hopes others could make use of the properties of these compounds to revisit drugs that cross the BBB but have been shelved because they get immediately removed from the brain by P-gp. Used clinically, drugs that interrupt efflux pump activity could be helpful in getting drugs into the brain without the risks of disturbing the integrity of the BBB.

Trojan horses

Many of the BBB's specialized features are not designed to keep molecules out, but to bring them into the CNS. The brain needs sugar, certain amino acids, and electrolytes, says Cannon, "and we have transport systems set up for that." Specifically, BBB endothelial cells have channels, protein transporters, and receptors that chaperone nutrients right through endothelial cells, all of which allow the passage of vital molecules such as ions, sugars, and amino acids into the CNS. In some instances, a therapy may be a natural substrate of one of these transporters—that's the case for the Parkinson's drug L-dopa, an amino acid that uses the large neutral amino acid transporter type 1 to get into the brain, where it is converted into dopamine. But in theory, researchers could dress up any drug to be recognized by a transporter or receptor to trick the brain into taking it up.

For example, it's possible to decorate nanoparticle-based drugs with a transporter's natural substrate. A company in the Netherlands called 2-BBB Medicines developed a technique that incorporates the antioxidant glutathione into a liposome membrane, allowing the liposome and its drug cargo to be taken into endothelial cells through a glutathione transporter. (See "Penetrating the Brain," *The Scientist,* November 2013.) Using these glutathione-spiked liposomes to encase doxorubicin, the researchers got nearly five times more of the chemotherapeutic drug into the brains of treated rats[9] and the technique limited brain tumor growth in mice.[10] The company is now testing the liposomes in people with brain tumors and as a means to deliver a steroid across the BBB of people with multiple sclerosis and other inflammatory CNS diseases.

Other pharmaceutical companies are trying to attach therapeutic compounds to receptors that actively bear cargo into, across, and out of cells through a process called transcytosis, allowing the drugs to "piggyback" their way into the CNS. A popular target for this approach, the transferrin receptor, brings iron into the CNS by transporting iron's carrier protein, transferrin. Rather than mimic iron or transferrin, which would throw off the cells' iron homeostasis, many research groups have turned to antibodies that glom onto the receptor outside of the transferrin-binding pocket. Some of the antibodies tote along a drug, while in other cases the antibody itself is the drug, designed to bind both the transferrin receptor and a disease target. For example, a team at Genentech has tested an antibody that binds the transferrin receptor and β-secretase, an enzyme that cleaves an amyloid-β precursor. The antibody accumulated in the brains of cynomolgus monkeys and reduced amyloid-β levels in the animals' CSF and brain tissue.[11]

Northwestern University chemist Chad Mirkin is taking a completely new route into the brain. Twenty years ago, Mirkin's team developed a unique class of gene therapy drugs called spherical nucleic acids (SNAs), which consist of densely packed DNA or RNA arranged on the surfaces of gold nanoparticles. Mirkin says that when his team began injecting the SNAs into mice, they realized the particles quickly spread throughout the body, including the brain. "We have the reverse problem that everyone else has," he says. "They go everywhere."

In a study published in 2013, his group demonstrated that in mice with brain tumors SNAs crossed the BBB and accumulated in the cancerous tissue, where they delivered siRNAs to knock down expression of the oncogene *Bcl2L12*.[12] Now, a mere four years later, clinical researchers at Northwestern are teaming up with the National Cancer Institute to test this approach in people with glioblastoma. Back in the lab, Mirkin's team found that cell cultures of astrocytes internalize SNAs using a group of receptors called scavenger receptors that bind to a variety of ligands

with repetitive patterns, but he says they haven't uncovered the precise route that the particles take through the BBB.

As another strategy to sneak drugs into the brain, some scientists are taking advantage of a class of viruses that naturally penetrate the BBB. In 2009, Brian Kaspar, who studies gene therapy at Ohio State University, and colleagues reported that a strain of adeno-associated virus called AAV9 they injected into the bloodstreams of mice crossed the BBB and reached the animals' spinal cords, accumulating in the motor neurons of newborns and astrocytes of adults.[13] "We don't know exactly which receptor it binds to cross," says Harvard Medical School microbiologist Casey Maguire. "But we do know it goes across [the BBB] by transcytosis." As a result, AAV9 can get through cultured BBB endothelial cells without disrupting the cells' characteristic features, such as tight junctions, Maguire's group showed recently.[14]

The 2009 study spurred numerous other researchers to begin using AAVs to traverse the BBB, and Kaspar, in addition to his position at Ohio State, now serves as the chief scientific officer of AveXis, a company that launched in 2010 to develop the use of AAV9 to deliver the *SMN* gene to the motor neurons of children with spinal muscular atrophy type 1. Earlier this year, AveXis released the results of an ongoing Phase 1 trial, which showed that most of the 15 children in the trial are reaching motor milestones—such as sitting unassisted—that patients with spinal muscular atrophy type 1 usually don't reach.

Good as those results are, says Maguire, the evidence now points to other viruses as better drug-delivery vehicles for crossing the BBB, at least in mouse models. "What's coming out of the pipeline now makes AAV9 look not efficient at all," he says. Specifically, researchers are evolving viruses with qualities that make them ideal for triggering their uptake into the brain. They do this by injecting a virus into animals, isolating whatever virus gets into the brain, then repeating the process again and again. Recently, a team led by Viviana Gradinaru at Caltech isolated a strain of AAV9 that reached brain and spinal cord tissues of mice 40 times more efficiently than the original version of the virus.[15]

Other groups are making use of immune cells that naturally traverse the BBB during inflammation. (See "Unlikely Allies," *The Scientist,* November 2016.) For example, a team of researchers from MIT and the University of North Carolina at Chapel Hill recently constructed nucleus-size polymer "backpacks," which they attached via antibodies to mouse macrophages before injecting the cells into the bloodstreams of mice with brain inflammation.[16] The macrophages were attracted to the inflammatory environment in the brain and brought along the backpacks. The researchers also described proof-of-concept studies in cell cultures that showed that the backpacks could be constructed to carry and release an antioxidant enzyme, suggesting the approach could potentially be used to deliver protein therapeutics to the brain.

So whether it's using immune cells, viruses, or nanoparticles studded with natural substrates of BBB transporters or receptors, drugs may be able to sneak their way past the formidable barrier without ever disturbing its normal function. Such approaches may be key to delivering therapies to the brain without the safety issues associated with more-invasive or damaging techniques, but they come with the trade-off of losing the ability to target drugs to precise locations. "In some diseases, you might want the drug everywhere; in others you might want it specifically localized to specific brain regions," says Daneman. He adds that there is still a lot of work to be done to determine how much drug each technique can get into the CNS and how long different treatments will last. "All these things will determine whether a strategy will work." ■

Amanda B. Keener is a freelance science writing living in Denver, Colorado.

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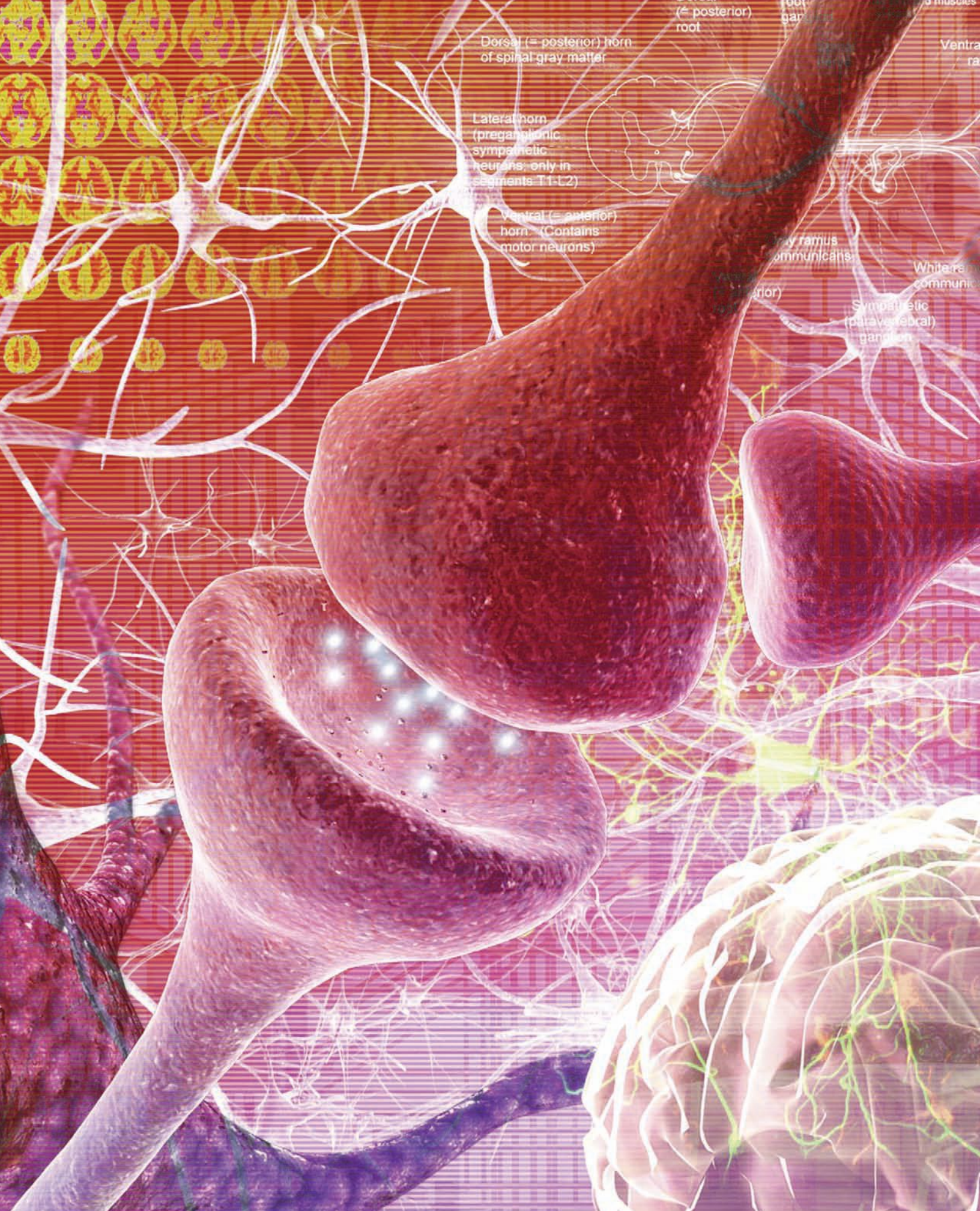

(= posterior) root
Dorsal (= posterior) horn of spinal gray matter
Lateral horn (preganglionic sympathetic neurons; only in segments T1-L2)
Ventral (= anterior) horn. (Contains motor neurons)
ramus
communicans
Sympathetic (paravertebral) ganglion

The Kaleidoscopic Brain

No two neurons are alike. What does that mean for brain function?

BY SARA B. LINKER, TRACY A. BEDROSIAN, AND FRED H. GAGE

For years, neurons in the brain were assumed to all carry the same genome, with differences in cell type stemming from epigenetic, transcriptional, and posttranscriptional differences in how that genome was expressed. But in the past decade, researchers have recognized an incredible amount of genomic diversity, in addition to other types of cellular variation that can affect function. Indeed, the human brain contains approximately 100 billion neurons, and we now know that there may be almost as many unique cell types.

Our interest in this incredible diversity emerged from experiments that we initially labeled as failures. In 1995, we (F.H.G. and colleagues) found that a protein called fibroblast growth factor 2 (FGF2) is important for maintaining adult neural progenitor cells (NPCs) in a proliferative state in vitro. We could only expand NPCs by culturing them at high density, however, so we could not generate homogeneous populations of cells.[1] Five years later, we identified a glycosylated form of the protein cystatin C (CCg) that, combined with FGF2, allowed us to isolate and propagate a very homogeneous population of NPCs—cells that would uniformly and exclusively differentiate into neurons.[2] We compared gene expression of this homogeneous population of cells to that of rat stem cells and the oligodendrocytes, astroglia, and neurons derived from the NPCs. To our surprise and disappointment, the top nine transcripts that were unique to the NPC-derived population were all expressed components of long interspersed nuclear element-1, also known as LINE-1 or L1— an abundant retrotransposon that makes up about 20 percent of mammalian genomes.

Most mammalian L1s have lost the ability to jump around the genome. However, the average human genome is estimated to contain 80–100 retrotransposition-competent L1s (RC-L1s), and about 10 percent of these elements are classified as highly active, or "hot." The mouse genome contains even more—at least 3,000 RC-L1s. In 2005, we provided evidence that L1s can jump around in adult rat NPCs in vitro and in the brains of transgenic mice.[3] Retrotransposition events were also detectable in non-neurogenic areas of the adult mouse brain, which, given that retrotransposons should only be able to jump within dividing cells, indicated that the events had occurred during neuronal development. These surprising findings initiated more than a decade of research by us (and now many others) demonstrating that neuronal genomes are quite dynamic, with retrotransposon-based plasticity driving genomic diversity within a single individual—what's known as somatic mosaicism.

Today we know that de novo L1 retrotransposition events are just one mechanism driving this mosaicism in neurons, along with recombination, aneuploidy, copy number variants, and other structural changes to the genome. Layered on top of this genetic diversity are epigenetic and transcriptional variations, as well as posttranscriptional and posttranslational modifications that can set apart different subsets of cells. Recent technological advances have enabled a highly resolved characterization of the extent of cellular diversity in the brain, showing that there is far more heterogeneity within a given cell type than previously appreciated.

Research has also begun to examine how somatic mosaicism might drive functional differences in individual neurons. Such neuronal diversity may help explain the origin of personality in humans and interindividual behavioral variations in other animals. Anecdotally, siblings, even monozygotic twins, often have

remarkably different personalities even at young ages, despite sharing genes and environments. Diversification of neurons arising from somatic gene mutations or subtle molecular and environmental differences may help explain the origin of cognitive and behavioral individuality. The findings thus far highlight the importance of moving away from a blanket definition of "cell types" that are assumed to behave in a stereotyped manner toward a more nuanced view of neurons that includes the multidimensional combination of transcriptome, epigenome, and genome when attempting to understand the impact of a given cell state.

New technology, more cell types

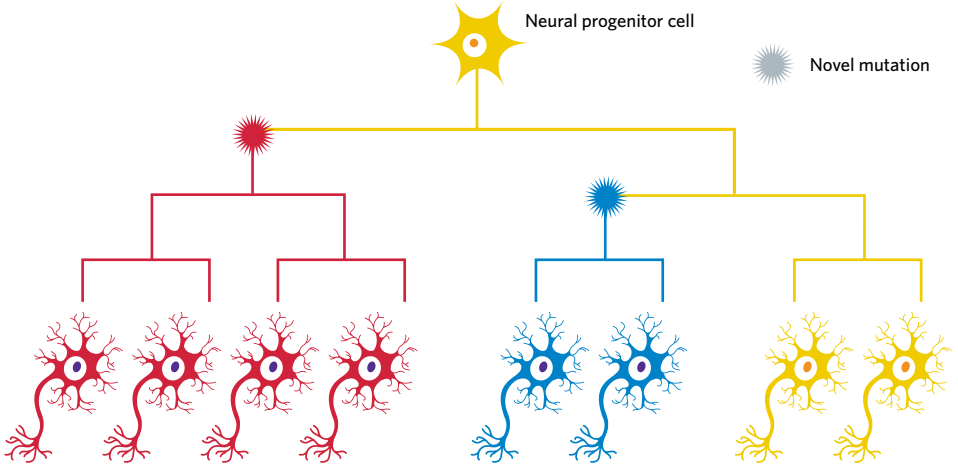
The cell is arguably the minimal functional unit of an organism, yet to this day we can define only a small proportion of the total possible mammalian cell types. Even if we were to restrict our search to one species, to one organ, or to even one subregion of an organ, the set of functionally distinct cell states that exists is likely far beyond what is measurable. This is stunning, given that it has been more than a century since the Golgi stain enabled Santiago Ramón y Cajal to visualize individual neurons and to provide the first description of neuronal subtypes that led to the early models of neural connectivity and, subsequently, to the prevailing doctrine that the neuron is the standard functional unit of the nervous system.

Since that time, genetic studies, electrophysiology recordings, and anatomic analyses have all greatly expanded the pool of known neuronal cell types. Early morphological, functional, transgenic, and staining approaches relied on identifying cell type–specific surface markers that are often rare and, in many cases, novel. Recently, there has been a shift in methodology to high-throughput approaches that are enabled by a timely combination of three distinct disciplines. The first is the advent of high-throughput molecular techniques that allow researchers to capture and prepare thousands of cells for individual RNA, DNA, and epigenetic analyses. The second advance results from the falling cost of sequencing, which makes it monetarily feasible to convert all of those single-cell preparations into information that is computer-readable. Finally, advances in data science and machine learning enable these large data sets to be distilled down to meaningful biological information. With such an approach, researchers can now focus their attention on new questions, such as how heterogeneity at the cellular level might translate into individual behavioral differences.

Sure enough, the recent surge in identifying cell types has inspired projects such as those of the Allen Institute for Brain Science and the Chan-Zuckerberg Initiative to catalog every cell type within the human brain and body, respectively. Just as the Human Genome Project provided the world with a reference map that has been at the core of nearly every subsequent human genetics experiment, a reference map of cell types has a similar potential to buoy future scientific progress by defining a high-resolution standard with which to compare individual cell variation. For example, complex-disease studies can use such a reference to bridge the gap between the identification of disease-associated genes and the functional consequences of those genes. By combining the results from single-cell profiling with disease-associated gene lists, researchers have been able to classify cell types as "highly vulnerable" for a given disorder.[4] Earlier this year, for instance, Nathan Skene of the Karolinska Institute and colleagues demonstrated that cell types such as CA1 pyramidal neurons, striatal medium spiny neurons, and cortical interneurons are particularly enriched for genes associated with schizophrenia.[5] Further clarification of cell-type diversity and the drivers of individualized differences in cell states will undoubtedly lead to a greater understanding of what underlies variation in neural circuits across individuals.

SOMATIC MOSAICISM

Although it was once assumed that all cells within an organism shared an identical genome, researchers now know this not to be true. Genetic variation can arise at any time, and these changes will be passed down to future generations of cells. Thus, mutations that occur early in development will lead to larger cell populations that carry the change, whereas mutations that occur in terminal cell lineages will be contained in relatively few cells.



Complexity of the brain

Taking advantage of these modern methods, researchers are now aware of an incredible amount of genetic and transcriptional diversity within the mammalian brain. Last year, for example, within a small cross-section of the mouse visual cortex, Bosiljka Tasic of the Allen Institute and colleagues used single-cell RNA sequencing to identify 49 transcriptional cell types, approximately 70 percent of which had not been previously described.[6] These types have since been further resolved by single-cell epigenetic profiling, such as DNA methylation mapping.[7]

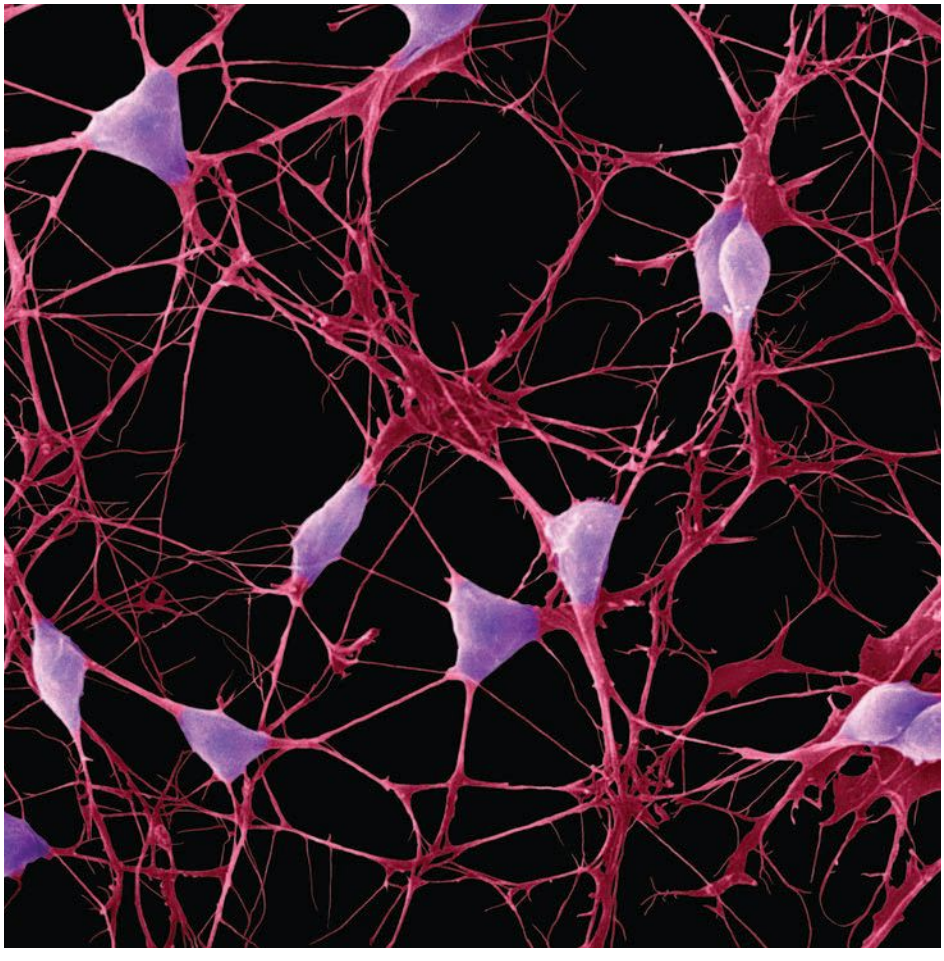
In addition to identifying many new cell types, these studies have reported an equally important finding that shifts our understanding of cellular complexity—namely, the characteristics that define a given cell type are far more plastic than we previously appreciated. For example, although disparate cell types can be clearly differentiated by the presence or lack of certain marker genes, additional sources of variability broadly separate neurons that would before have been described as a single cell type. For example, in the cornus ammonis 1 (CA1) region of the hippocampus, a region important for learning and memory, it is now becoming increasingly clear that there is a gradient of transcriptional identity associated with the cell's position along the dorsal-ventral axis.[8,9] This finding supports previous reports that dorsal and ventral CA1 neurons have different electrophysiological properties and play independent roles in memory encoding.[10,11]

As researchers continue to identify more and more neuronal subtypes, a major question is what generates and maintains this diversity. One answer to this has turned out to be genetics. While it was traditionally believed that every cell in the body contained identical genetic material, recent evidence has revealed that individual neurons actually differ significantly due to somatic DNA mutations and rearrangements, including those caused by the movement of L1 and other retrotransposons. Somatic mutations can occur both during development and in adulthood. Early progenitor cells that accumulate somatic mutations may give rise to many progeny, which also carry the same mutation, whereas a later progenitor, like an NPC in a neurogenic niche of the adult brain, may only give rise to a few progeny, limiting the spread of that particular mutation. This process could represent a lifelong flexibility of the brain, potentially making it more adaptable to changing demands.

The types of mutations that occur in the brain are diverse—including aneuploidy, single nucleotide polymorphisms (SNPs), copy number variants (CNVs), and mobile element insertions—and vary by cell type. For example, human cortical cells contain megabase-scale CNVs,[12] while NPCs contain hot spots for DNA translocations.[13,14] Depending on the specific location and nature of a somatic mutation, it could have substantial effects on cell function by altering gene expression or generating novel protein content—for example, by introducing a new promoter or splice site.

Particularly interesting somatic mutations are those arising from mobile element insertions, such as L1 and Alu retrotransposons, which exist at more than 500,000 and 1 million cop-

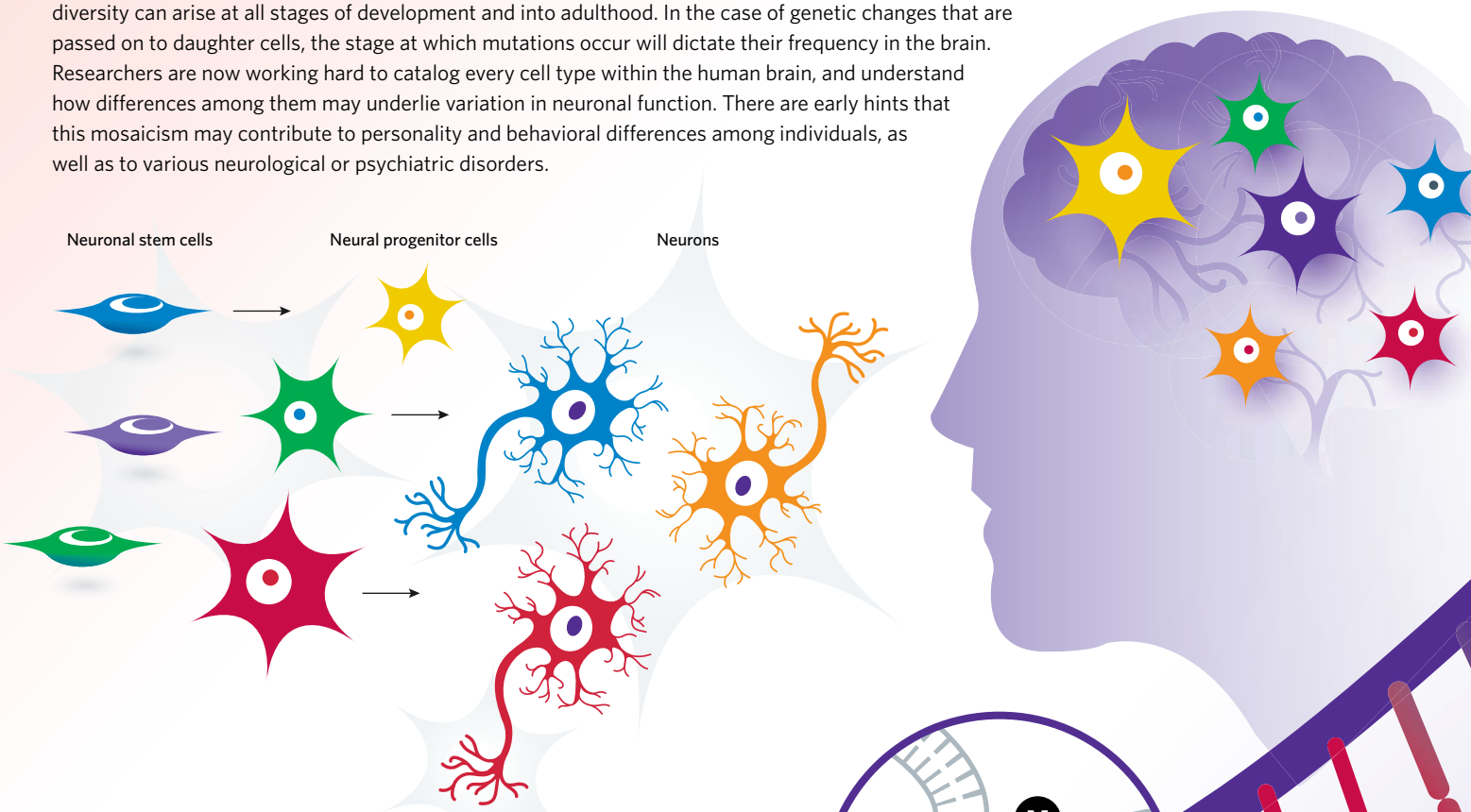
The human brain contains approximately 100 billion neurons, and we now know that there may be almost as many unique cell types.



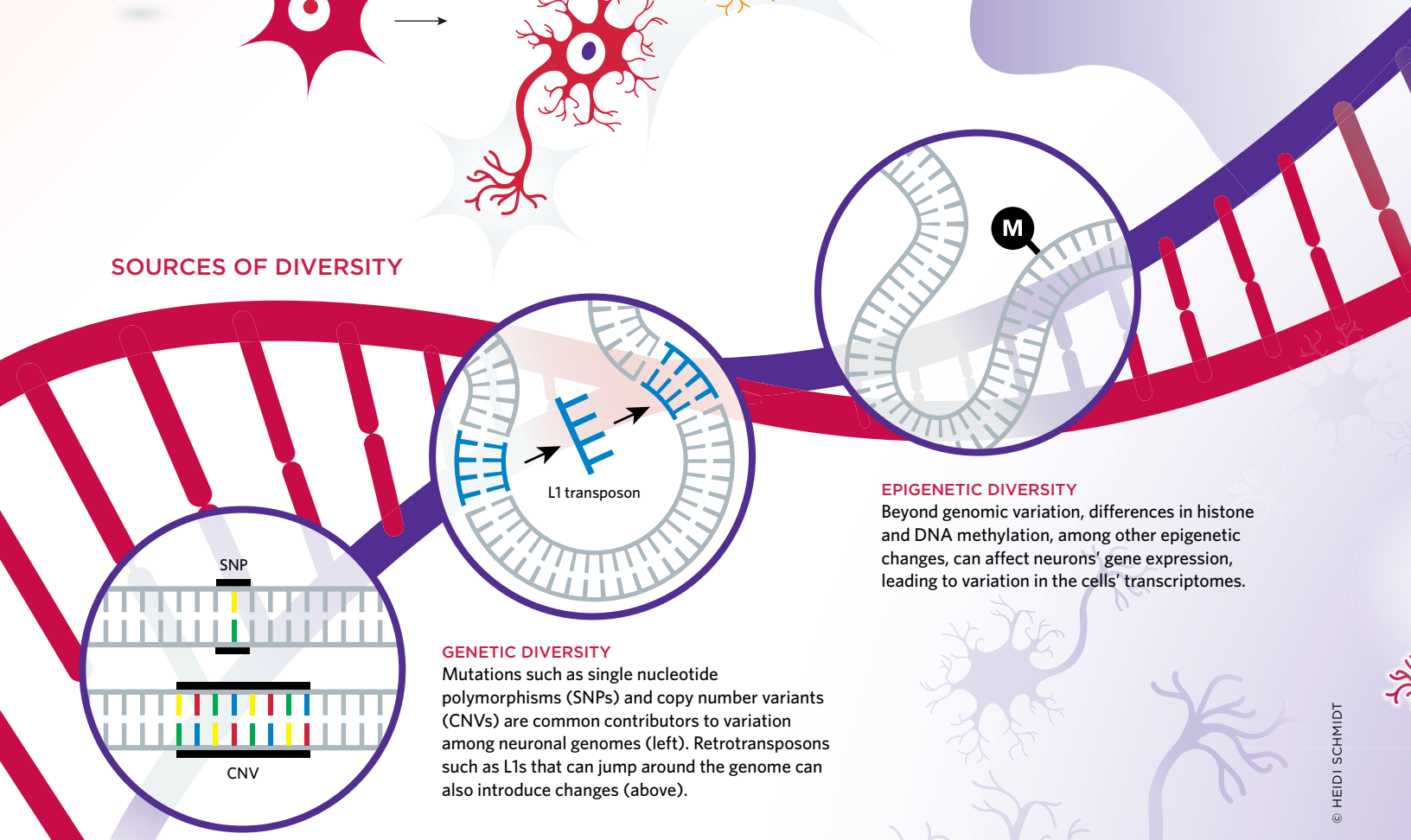
BRAIN-WIDE WEB: In additional to genomic and epigenomic differences among neurons, the cells undergo molecular and morphological changes based on factors in the local environment, providing an additional layer of diversity in the brain.

DISCOVERING DIVERSITY

Of the 100 billion or so neurons in the human brain, there may be no two that are alike. Recent advances in single-cell omics and other techniques are revealing variation at genomic, epigenomic, transcriptomic, and posttranscriptomic levels. Such diversity can arise at all stages of development and into adulthood. In the case of genetic changes that are passed on to daughter cells, the stage at which mutations occur will dictate their frequency in the brain. Researchers are now working hard to catalog every cell type within the human brain, and understand how differences among them may underlie variation in neuronal function. There are early hints that this mosaicism may contribute to personality and behavioral differences among individuals, as well as to various neurological or psychiatric disorders.



SOURCES OF DIVERSITY

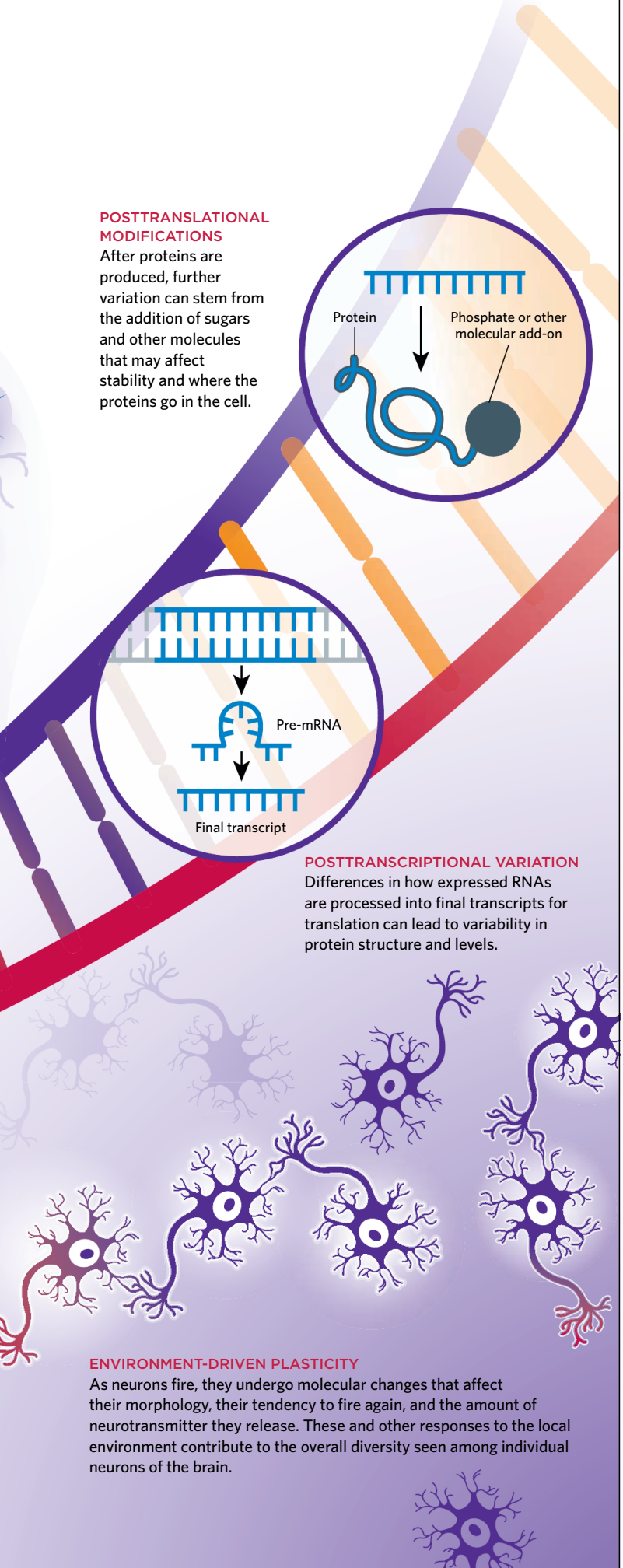


EPIGENETIC DIVERSITY

Beyond genomic variation, differences in histone and DNA methylation, among other epigenetic changes, can affect neurons' gene expression, leading to variation in the cells' transcriptomes.

GENETIC DIVERSITY

Mutations such as single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) are common contributors to variation among neuronal genomes (left). Retrotransposons such as L1s that can jump around the genome can also introduce changes (above).



ies in the human genome, respectively. L1 retrotransposons are unique in that they encode all of the protein machinery necessary for their own replication and mobilization. When expressed, L1 mRNA forms a ribonucleoprotein complex that moves into the nucleus, where it uses its own endonuclease to nick the genome, making room to reverse transcribe its mRNA into DNA and insert it into a new genomic location. Although still open to debate, this process appears to occur an average of once for every two cells of the human brain, creating the potential for tremendous genomic diversity through this mechanism alone. And given the length of many genes involved in neuronal function, such as synaptic density proteins and cadherins, such insertions are likely to occur in neuronal genes.

Intriguingly, unlike random DNA damage events, L1-linked mutations are driven by a protein that has been coevolving with the human genome for millions of years. If it is found that the impact of L1 insertions can modify downstream neuronal function, this could hint at an advantageous role for these mobile elements, which were once considered parasites. Although researchers have explored this role of L1 in the setting of cancer for many decades, it has only recently been considered in the context of normal human variability. (See "Wrangling Retrotransposons," *The Scientist,* March 2015.) On the other hand, L1-induced mutations are also just now being probed for their possible contribution to neurological disease.

Other sources of neuronal diversity

A number of other mechanisms, including chromatin structure or DNA methylation, further modulate cellular diversity, reversibly altering gene expression on a cell-by-cell basis. Closed chromatin may also protect the DNA from acquiring certain permanent mutations, such as mobile element insertions, by restricting accessibility. These and other epigenetic modifications can also dictate allelic expression, potentially leading some cells to express genes from one parent's allele whereas other cells use the opposite allele. Parental expression bias may change across different brain regions and ages, further adding to the brain's cellular diversity.

Additional variation among cells can also emerge posttranscriptionally, such as via alternative splicing of transcripts, which can increase the repertoire of unique proteins available in a cell. And other types of RNA editing by specialized editing enzymes can cause nucleotide substitutions, frameshifts, or structural changes that alter the protein product. Posttranslational modifications that can affect those proteins' stability and localization further distinguish one neuronal subtype from the next.

Beyond such internal mechanisms of variation, environment-driven plasticity lends yet another layer of complexity to the brain. The brain is capable of remarkable remodeling in response to experience. Signals originating from the environment can cause both widespread and localized adaptations. At the level of individual cells, structure and function are continually changing with the environment in a dance of lifelong brain plasticity, and some experiences, such as stress or physical exercise, affect the growth,

survival, and fate of newborn neurons in neurogenic regions of the brain.[15] (See "Brain Gain," *The Scientist,* December 2015.) Considering that each neuron in the human brain makes 5,000 to 200,000 connections with other neurons, changes at the synapse level could have effects on multiple brain circuits and downstream behavioral or cognitive phenotypes.[16] Emerging evidence suggests that experience could also induce structural variants in the genomes of individual neurons by increasing transposon activity or creating DNA breaks that permit transposon insertions or recombination events, further contributing to somatic mosaicism of the brain.[17,18]

The genetic, molecular, and morphological diversity of the brain leads to a functional diversification that is likely necessary for the higher-order cognitive processes that are unique to humans.

Cell-to-cell differences tied to experience, such as genetic variants or variation in the local microenvironment of the cell, can be amplified by cascading molecular responses that ultimately influence functional properties. For example, electrical stimulation received by a neuron will induce molecular changes at the synapse that affect its own tendency to fire an action potential, and molecular feedback at the synapse will affect the amount of neurotransmitter released when it communicates with another cell. These differences constitute a portion of the diversity that exists among individual neurons of the same cellular subtype and help explain how so many functional differences exist among individual cells in the brain.

Functional implications of neuronal diversity

Traditionally, cells are defined by the tissue to which they belong as well as their particular functional role or morphology. This classification represents a developmental trajectory that begins early in embryogenesis and is hardwired into each cell. But other differences among cells are more subtle. Multi-dimensional analyses of gene expression and other metrics have revealed remarkable heterogeneity among cells of the same traditional "type." Cells exist in different degrees of maturation, activation, plasticity, and morphology. Once we begin to consider all of the subtle cell-to-cell variations, it becomes clear that the number of cell types is much greater than ever imagined. In fact, it may be more appropriate to place some cells along a continuum rather than into categories at all.

Brain cells in particular may be as unique as the people to which they belong. This genetic, molecular, and morphological diversity of the brain leads to functional variation that is likely necessary for the higher-order cognitive processes that are unique to humans. Such mosaicism may have a dark side, however. Although neuronal diversification is normal, it is possible that there is an optimal extent of diversity for brain function and that anything outside those bounds—too low or too high—may be pathological. For example, if neurons fail to function optimally in their particular role or environment, deficits could arise. Similarly, if neurons diversify and become too specialized to a given role, they may lose the plasticity required to change and function normally within a larger circuit. As researchers continue to probe the enormous complexity of the brain at the single-cell level, they will likely begin to uncover the answers to these questions—as well as those we haven't even thought to ask yet. ■

Sara B. Linker and Tracy A. Bedrosian are postdoctoral research fellows in the Laboratory of Genetics at the Salk Institute for Biological Studies, where Fred H. Gage is a professor and Vi and John Adler Chair for Research on Age-Related Neurodegenerative Disease.

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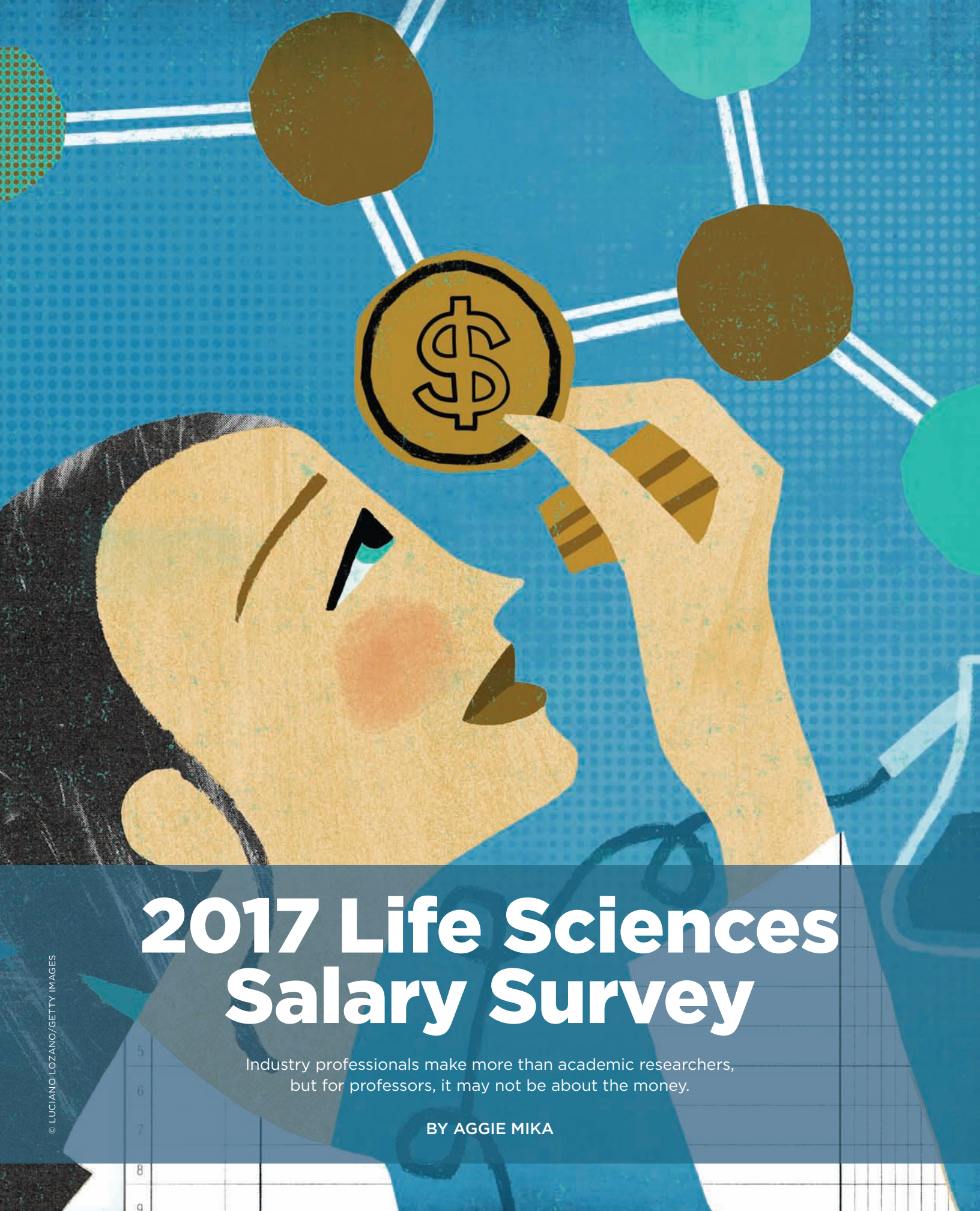


2017 Life Sciences Salary Survey

Industry professionals make more than academic researchers, but for professors, it may not be about the money.

BY AGGIE MIKA

As part of this year's Life Science Salary Survey, more than 2,500 life science professionals from around the world answered our questions about their job status, compensation, feelings of job satisfaction and security, the inclusion of women and minorities in their workplace, and more. The survey results are in, and highlight some intriguing trends in workplace culture and income across sector, specialty, rank, and gender.

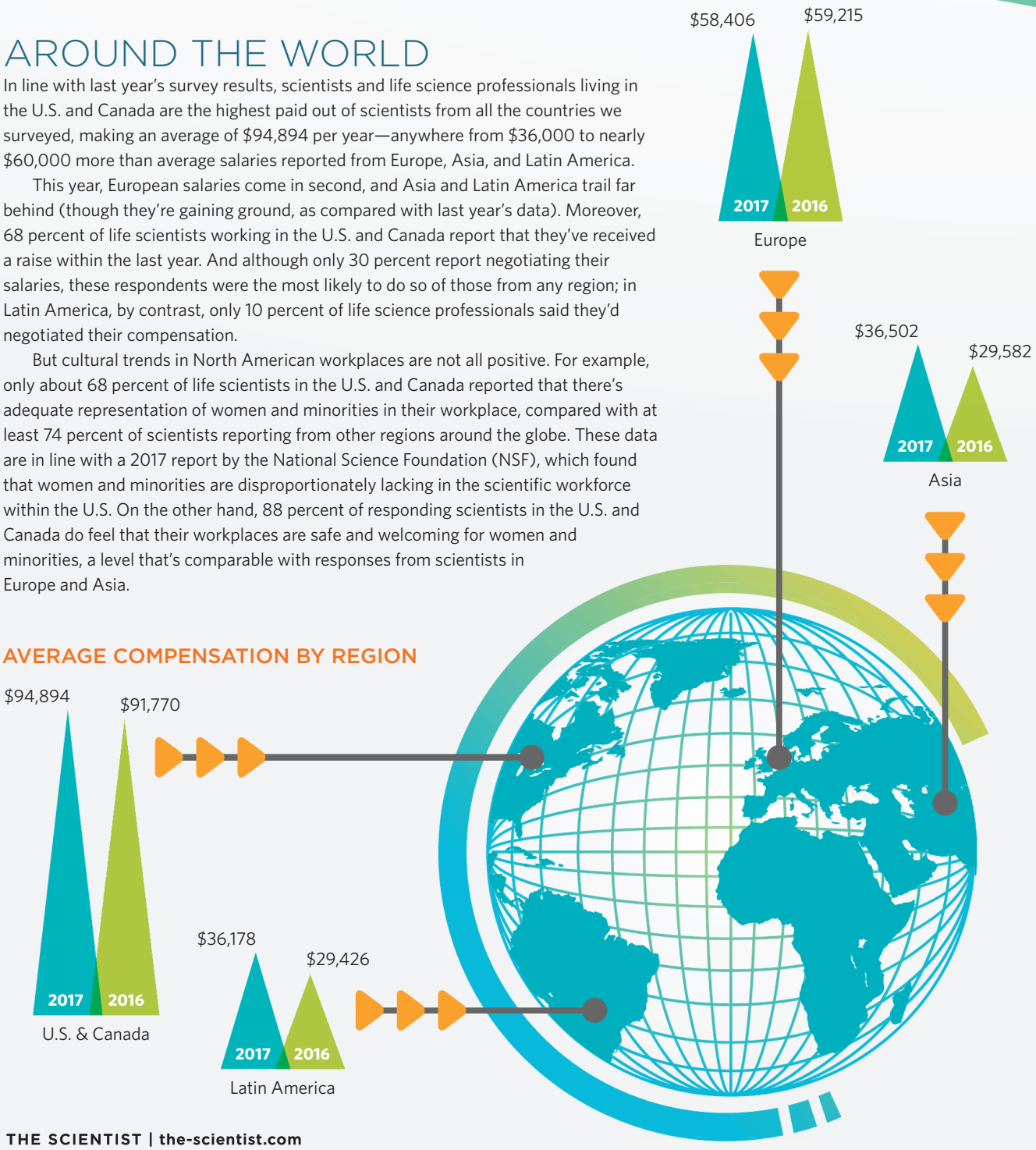
AROUND THE WORLD

In line with last year's survey results, scientists and life science professionals living in the U.S. and Canada are the highest paid out of scientists from all the countries we surveyed, making an average of $94,894 per year—anywhere from $36,000 to nearly $60,000 more than average salaries reported from Europe, Asia, and Latin America.

This year, European salaries come in second, and Asia and Latin America trail far behind (though they're gaining ground, as compared with last year's data). Moreover, 68 percent of life scientists working in the U.S. and Canada report that they've received a raise within the last year. And although only 30 percent report negotiating their salaries, these respondents were the most likely to do so of those from any region; in Latin America, by contrast, only 10 percent of life science professionals said they'd negotiated their compensation.

But cultural trends in North American workplaces are not all positive. For example, only about 68 percent of life scientists in the U.S. and Canada reported that there's adequate representation of women and minorities in their workplace, compared with at least 74 percent of scientists reporting from other regions around the globe. These data are in line with a 2017 report by the National Science Foundation (NSF), which found that women and minorities are disproportionately lacking in the scientific workforce within the U.S. On the other hand, 88 percent of responding scientists in the U.S. and Canada do feel that their workplaces are safe and welcoming for women and minorities, a level that's comparable with responses from scientists in Europe and Asia.

AVERAGE COMPENSATION BY REGION



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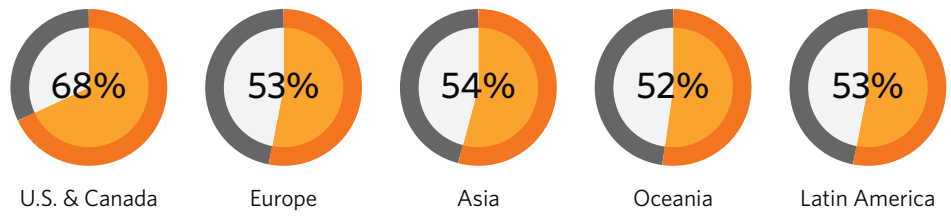
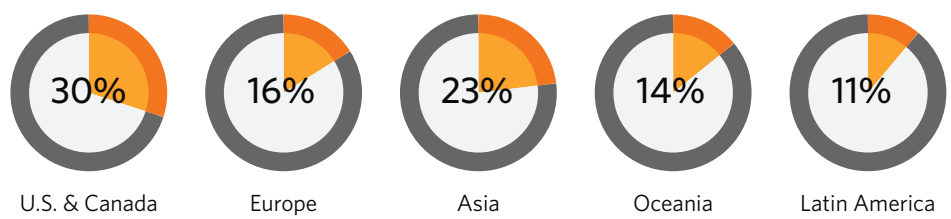
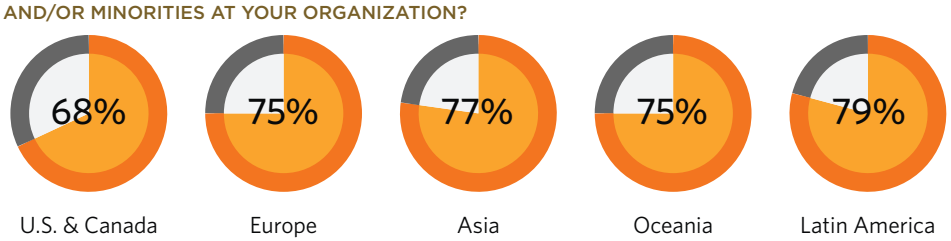


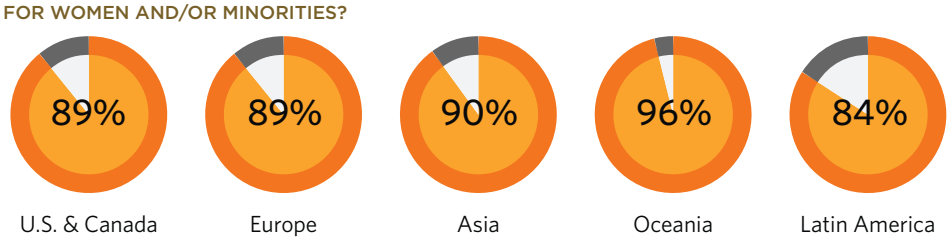
DID YOU NEGOTIATE YOUR SALARY?



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?



Scientists and life science professionals living in the U.S. and Canada are the highest paid out of all of the countries we surveyed. But cultural trends in North American workplaces are not all positive.

ON INDUSTRY AND THE IVORY TOWER

US academic respondents to *The Scientist's* survey report salaries consistent with last year's. Also consistent with last year's data is the considerable bump in average yearly wages from associate professor to professor—almost a $50,000 difference. Bargaining power is relatively low until one reaches a certain level of seniority, says labor economist Michael Roach of Cornell University.

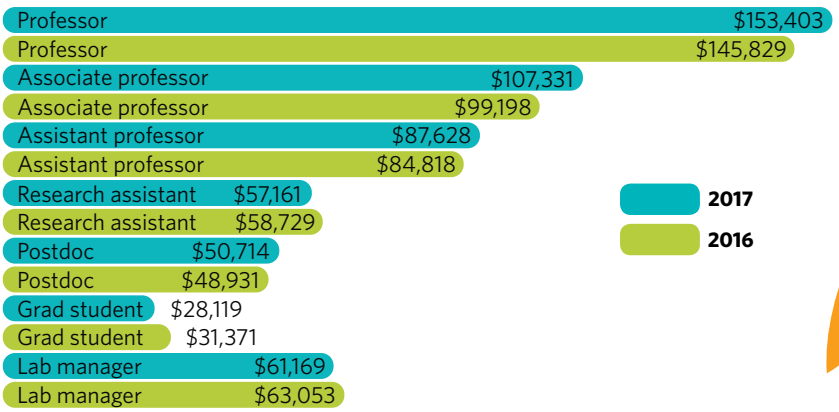
Averaging across different positions within sectors, industry professionals make more than other life scientists in the U.S., pulling in $125,936 per year, compared with the $86,021 of academics and $97, 525 of those in other sectors such as government. "I've seen even higher wages [in industry] than what's been reported in this recent survey," says Roach, referring to his own data that are yet to be published. In his surveys, he and colleagues home in on the private sector, examining wage differences between big companies and start-ups, for instance. His data, however, include PhD students from chemistry, physics, and engineering, in addition to the life sciences.

But regardless of field, industry salaries are higher. "It's always been that way," says Roach. He's not aware of any systematic analysis probing the primary drivers of such differences in pay scale, but believes there could be multiple forces at play. For starters, in academia, "there's a high demand for faculty positions," he explains, driven by the flood of applicants and the lack of available jobs. This environment is highly competitive, and universities have the advantage. "If people try to make demands for very high salaries, then universities will go on to the next most able person," he says. "They're really not going to give in. . . . This helps keep wages from getting too big."

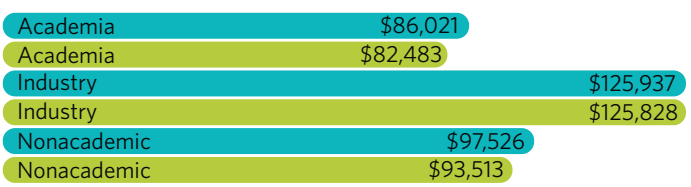
But the lower salaries aren't necessarily a turn-off for most faculty candidates, Roach adds. "The stereotype is that faculty are not in it for the money. A lot of people get into science and graduate research out of a love of science."

On the industry side, however, the demand for candidates is greater. "There's a lot of competition in the private sector for highly trained STEM workers, especially PhDs, which drives down the unemployment rate and drives high salaries," Roach explains. In addition to higher salaries, PhDs in the private sector also enjoy lower unemployment, according to his unpublished data.

AVERAGE COMPENSATION IN US ACADEMIA



AVERAGE COMPENSATION IN U.S. BY SECTOR



WOMEN IN SCIENCE

There were far more male than female respondents within senior levels in US academia, especially amongst full professors, which, according to economist Shulamit Kahn of Boston University's Questrom School of Business, reflects a larger trend. "There are a ton more men at the full-professor level," she says.

Roach agrees and says that the difference in the number of male and female respondents is unlikely to be due to a response bias. "I think that there are a lot fewer women at the full-professor rank. . . . It's not at all surprising that you see more equality in the lower

ranks." For instance, in the U.S. the number of respondents is well-matched by gender among less-senior academics, such as graduate students, postdocs, and even assistant professors.

He cautions against interpreting these data to mean that there are forces that "favor men and push women out," however. He adds that he believes this discrepancy is improving, noting that greater gender equality within lower academic ranks in *The Scientist*'s survey results should eventually translate to more-equal numbers at the senior level. "The practices around recruiting graduates, mentoring graduates, and hiring graduates have changed over time, to where we do have greater equality now than we did 20 years ago," says Roach. "Come back and do this 10–15 years from now, and I would expect that the number of full professors could be very equal as well."

The Center for Science, Technology & Economic Policy Director Donna Ginther agrees. "There are more male professors in the senior ranks because women were less likely to obtain PhDs 20–30 years ago," she writes in an email to *The Scientist*.

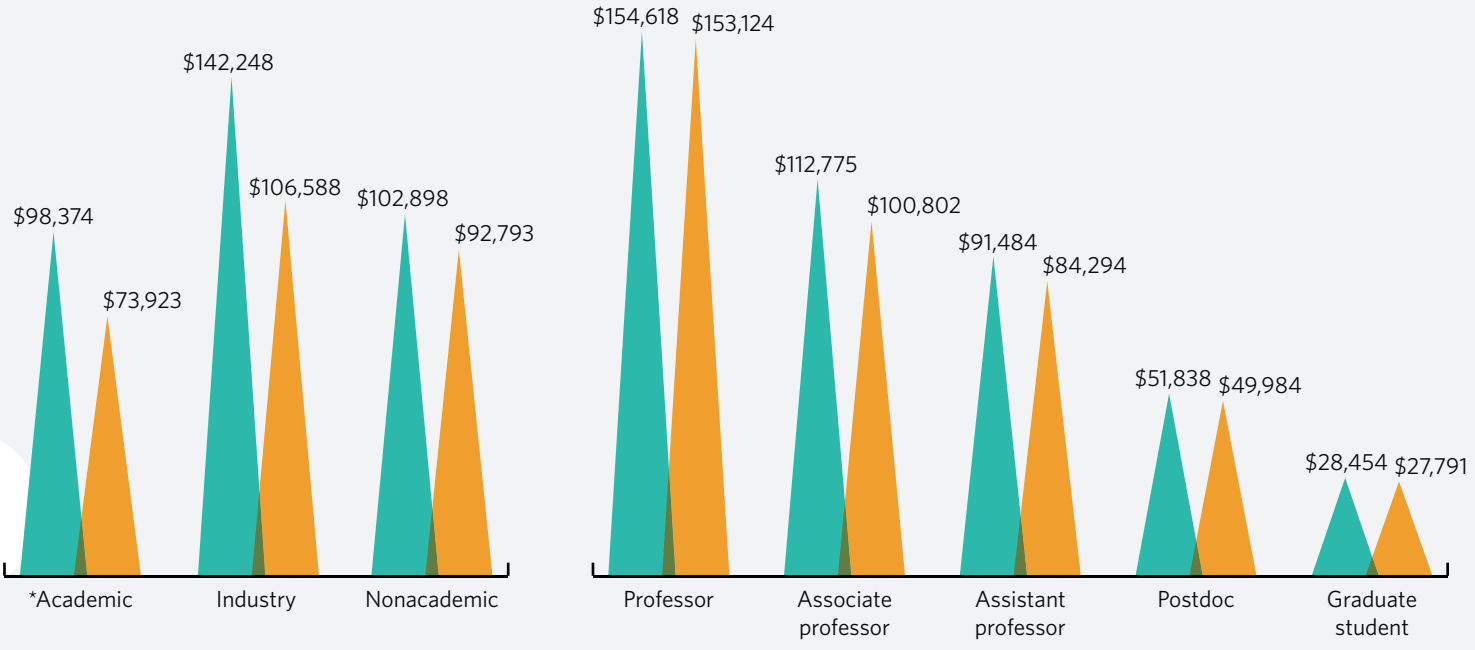
Gender differences in salary also appear to be improving. In a soon-to-be-published study, Kahn says she and her colleagues found a roughly 17 percent difference in pay between men and women in the sciences. But, she says, most of those salary differences were accounted for by a variety of factors, including pay gaps by sector. For instance, those who work in industry get paid more, and in general, there are fewer women in industry.

However, Roach notes women working in industry tend to make less money than men. "We see significant differences in wages between men and women in the surveys that we do." In his recent survey of industry scientists, "men still make significantly more than women."

According to Ginther, a similar gap doesn't exist for academia. Her previous data "showed no evidence of a gender salary gap in academia in biomedical fields," she writes (*Psychol Sci Public Interest,* 15:75-141, 2014). Indeed, when broken down by rank, *The Scientist*'s data showed very little difference between the salaries of men and women in US academia, with female full professors averaging less than $1,500 less than their male counterparts. Gender differences in pay scale become much smaller "if you start comparing apples to apples," says Kahn, who has seen similar trends in her own data.

NUMBER OF US RESPONDENTS

AVERAGE COMPENSATION IN U.S.



*Note: The gender gap in academia is greatly diminished when the data are broken down by position.

IF YOU'RE HAPPY AND YOU KNOW IT

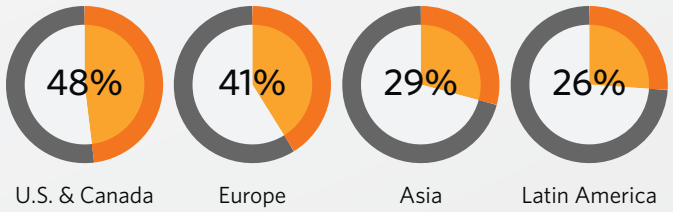
Respondents to *The Scientist*'s survey also rated their feelings of satisfaction and security in their current roles and salaries. And around the globe, responses varied widely.

Overall, no region's scientists reported high satisfaction with their compensation. In the U.S. and Canada, 47 percent of respondents said they were satisfied with their wages, and only 41 percent felt that their skills and experience matched their salaries, suggesting that more than half of life scientists in North America feel underpaid. And those numbers were even lower in Europe, Asia, and Latin America. But that doesn't necessarily mean that these life scientists are unhappy.

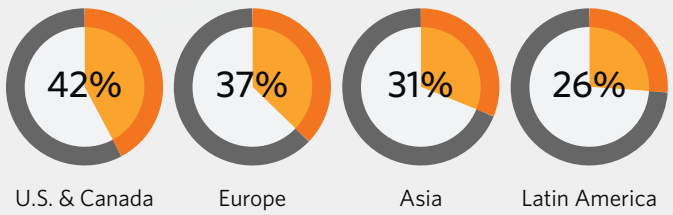
According to Roach, there are "nonfinancial work factors that drive work satisfaction." From the hundreds of interviews Roach has conducted with respondents to his own surveys, one thing that stands out as a primary driver of job satisfaction is the freedom to satisfy one's research goals and interests. "We see in our research that salary is not typically a driving factor, as it might be in other kinds of careers." In the U.S. and Canada, nearly 85 percent of respondents reported feeling stimulated in their current roles, suggesting that a large majority experience intellectual gratification.

"Intellectual challenge and a fit and interest with the type of work that you're doing is typically pretty important," says Roach. "It's really much more [about] doing exciting and interesting things."

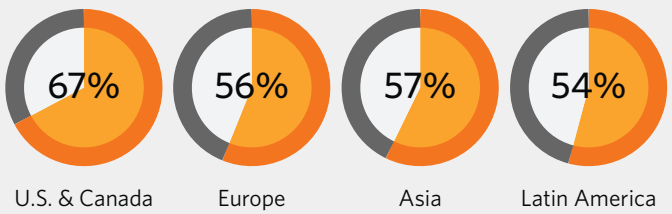
ARE YOU SATISFIED WITH THE OVERALL COMPENSATION (SALARY AND BENEFITS) YOU RECEIVE?



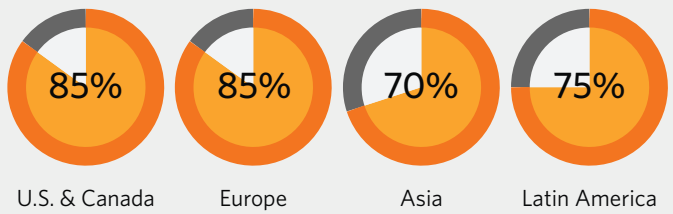
DO YOU FEEL THAT YOUR SALARY IS COMMENSURATE WITH YOUR SKILLS AND EXPERIENCE?



DO YOU FEEL THAT YOUR JOB ALLOWS A GOOD WORK-LIFE BALANCE?



DO YOU FEEL STIMULATED BY YOUR WORK?



SURVEY METHODOLOGY

The Scientist collected data via a Web-based survey, which was open from March 16 to July 14, 2017. Participation in the survey was promoted by email and advertising to readers of *The Scientist* and visitors to the-scientist.com. The responses were filtered to eliminate duplicate or misleading answers, and to eliminate reported salaries greater than $1 million or less than $10,000 for the U.S., Canada, Europe, and Oceania, and less than $1,000 for Asia, Latin America, and Africa. We received usable responses from 2,558 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 cash compensation. All international salaries were converted to US dollars using the conversion rates of July 17, 2017, and analyses were done using the US-equivalent amount. For year-over-year comparisons, data from previous surveys were converted into USD using the conversion rates from July 17, 2017, and reanalyzed according to this year's methodology. The data reported are averages of the total compensation reported for a given category.

Not every participant provided all of the information requested. If the participant provided income data plus information concerning at least one demographic characteristic, the response was included in the study. The result of this decision is that the total number of cases varies among the analyses. For average salaries, all categories reported received a minimum of 50 responses; for other questions, all categories reported received a minimum of 20 responses.

TS WEBINARS

COMING**SOON** | Microbiome-Centric Human Health: A Call for Systems Biology

With 100 times the number of genes contained in the human genome, and an array of different cell types and functions, our microbiome arguably constitutes an additional human organ system. Research to date has implicated microbial activity in autoimmune disease, cancer, and the obesity epidemic. Because it is a major source of variability across people, understanding and altering an individual's microbiome is both a challenge and novel avenue for personalized medicine and nutrition. For a detailed look at the progress made toward understanding the host-microbiome interplay and the efforts undertaken to achieve a steady state of mutualism for a larger human health benefit, *The Scientist* is bringing together a panel of experts who will share their research, summarize the state of the science, and discuss the next steps in developing personalized microbiome-based therapies. Attendees will have the opportunity to interact with experts, ask questions, and seek advice on topics related to their research.

ERAN ELINAV, PhD
Professor, Department of Immunology
Weizmann Institute of Science, Israel

ERAN SEGAL, PhD
Professor, Department of Mathematics and Computer Science
Weizmann Institute of Science, Israel

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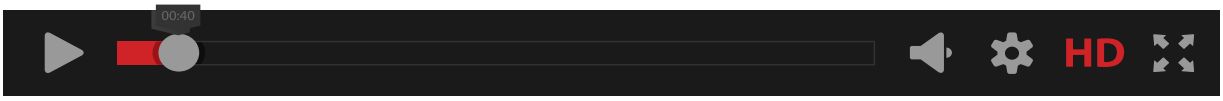
In the absence of new diagnostic tests for Parkinson's disease (PD), the diagnosis has long been one of exclusion, ruling out other causes of tremor, bradykinesia, and rigidity. With the dawn of biomarker-based molecular diagnostics, a new race has begun to identify molecular signatures of disease pathology in noninvasively derived tissue samples, including blood, urine, and saliva, as well as radiographic or magnetic scans. Scientists have begun to sort through the molecular traces associated with PD patients to find telltale signs of disease onset and progression. *The Scientist* brings together a panel of experts to share their experience with biomarker discovery and validation, as well as their predictions for this as-yet-untapped market.



WATCH NOW! **www.the-scientist.com/parkinsonsbiomarkers**

BRIT MOLLENHAUER, MD
Assistant Professor, Department of Neuropathology
University Medical Center, Goettingen, Germany;
Paracelsus-Elena-Klinik, Kassel, Germany

HUGO VANDERSTICHELE, PhD
Cofounder and Chief Integration Officer,
ADx Neurosciences
Founder, Biomarkable bvba

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- Procedures for identifying diagnostic markers
- Real-life examples of PD biomarkers currently under study

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The Literature

NEUROSCIENCE

Dialing Up Dopamine

THE PAPER

J.I. Aguilar, "Neuronal depolarization drives increased dopamine synaptic vesicle loading via VGLUT," *Neuron*, 95:1074-88.e7, 2017.

Psychiatrist Zachary Freyberg thought he knew the basics of dopamine signaling. When a dopamine neuron fires, vesicles containing the neurotransmitter migrate to the cell membrane, where they fuse and release their cargo into the synapse, all in the course of about a millisecond. But a chance observation by Freyberg a few years ago revealed a new dimension to this critical aspect of neural communication.

At Columbia University, beginning in 2009, Freyberg had helped develop a technique to observe dopamine signaling in living *Drosophila* brains. The method used molecules of FFN206, Freyberg says, which "behave like dopamine, but unlike dopamine, they're fluorescent and therefore can be readily visualized."

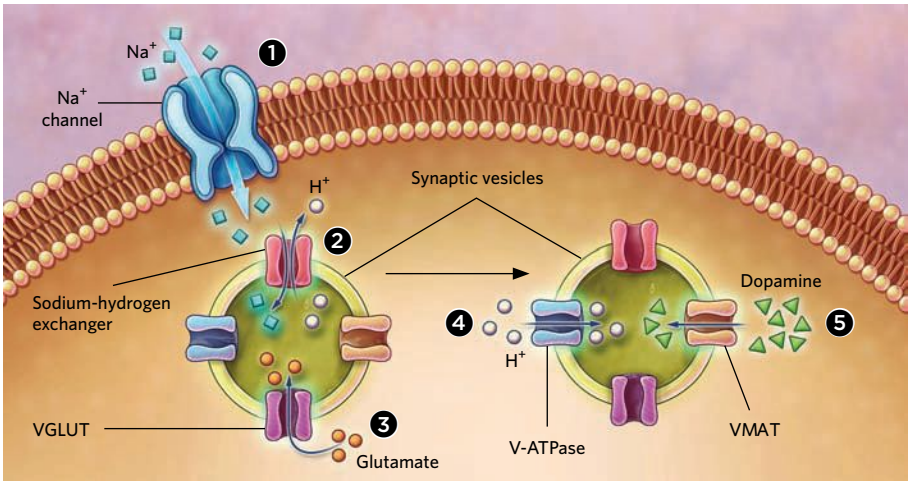
He and his colleagues expected that when neurons were artificially stimulated with potassium chloride, vesicles would transport FFN206 to the cell membrane and release it into the synapse. That did happen—but the fluorescence indicated something else was going on, too. "You'd expect the dopamine signal to go down," Freyberg, now at the University of Pittsburgh, explains. "Instead, it was going *up* before going down." The vesicles, the team realized, were loading extra cargo before fusing with the membrane—a contradiction of the textbook view that vesicles' dopamine levels were fixed.

To investigate further, the researchers looked for signals associated with the boost in vesicle content. They found that before fusion but after cell membrane depolarization—a sign of neuronal activity—the pH inside vesicles dropped. "For dopamine, it's the pH of the vesicles that creates the driving force for loading," Freyberg explains, with more-acidic conditions promoting loading.

However, it was unclear what triggered the extra acidification. The researchers suspected chloride, a negatively charged ion often involved in establishing proton gradients. But experiments didn't back that theory up. So the team turned to glutamate, a neurotransmitter that is also negatively charged. "When we blocked the entry of glutamate into these dopamine vesicles, they no longer acidified more, and no longer loaded more in response to activity," Freyberg says.

The researchers observed similar processes in mice, and in a new paradigm, suggest how this unexpected role for glutamate links neuronal activity to dopamine vesicle content across species. "It's showing a mechanism by which presynaptic neurons can be regulated," says Thomas Hnasko, a neurobiologist at the University of California, San Diego. "Most people think about plasticity in the brain as a postsynaptic phenomenon. . . . This is all really quite novel."

Freyberg is now investigating how these mechanisms fine-tune the amount of dopamine sent across the synapse, and their effects on neuronal communication in normal and diseased brains. "It's as if we've been thinking all our lives that when you turn on a light, you just flip a switch, and it's on or off," he says. "But what this suggests is that neurons are capable of a great deal more subtlety." —Catherine Offord



PUMPING IT UP: Researchers have found that synaptic vesicles releasing dopamine across neuronal synapses in fruit flies and mice can dynamically adjust the neurotransmitter content in response to neuronal firing. And they propose a mechanism to explain how. When the axon terminal depolarizes, sodium ions flow into the cell ❶. The increased sodium ion concentration activates sodium-hydrogen exchangers in the vesicle membrane that transport one sodium ion into the vesicle for every proton out ❷. This action increases the difference in electrical charge across the vesicle membrane, activating a transporter protein, VGLUT, which pumps another neurotransmitter, negatively charged glutamate, into the vesicle ❸. The resulting buildup of internal negative charge triggers the pumping of more protons into the vesicle, increasing the pH gradient across the membrane ❹. Finally, this drop in pH inside the vesicle triggers the loading of more dopamine into the vesicles via the VMAT protein ❺.

NEURAL SLUMP: The brain is less responsive to rewards received in the afternoon, compared with morning or evening, a study suggests.

NEUROSCIENCE

Daily Perks

THE PAPER

J.E.M. Byrne et al., "Time of day differences in neural reward functioning in healthy young men," *J Neurosci*, 37:8895-900, 2017.

AFTERNOON DELIGHT?

People report being happiest in the early afternoon. One idea is that the brain's mood-influencing reward system varies diurnally for evolutionary reasons. According to this hypothesis, "at certain times of day, we're more likely to want to engage with the environment," says psychologist Jamie Byrne of Swinburne University of Technology in Australia. As hunters with poor night vision, we'd have "our best chance of catching Bambi . . . at about two in the afternoon."

CASH FOR BOLD

To look for diurnal changes in reward functioning, Byrne and colleagues had 16 men guess the correct value of a concealed card in exchange for cash toward a prize. The researchers used blood oxygen level-dependent (BOLD) fMRI to monitor participants' neural responses during this task at three times: 10 a.m., 2 p.m., and 7 p.m.

SENSORY SURPRISE

Counterintuitively, correct guesses elicited more activity in the brain's reward regions, particularly the left putamen, in the morning or evening than in the afternoon. Byrne notes that while the results conflict with the standard evolutionary theory, they parallel modern humans' familiar afternoon dip in motivation. "We think it's about prediction error. Since the brain expects to get rewards at 2 p.m., it's not really surprised when it does get them. When you get those rewards at 10 a.m. or 7 p.m., the brain is figuring out what's happening."

TIMELY RESPONSES

Why these fluctuations occur is unclear, University of Cambridge neuroscientist Wolfram Schultz writes in an email. But the results suggest that "maybe future studies should mention at what time of day they did such measurements," he notes, "and keep the same time of experimentation when averaging data from different test subjects."

—Catherine Offord

FAMILY RESEMBLANCE: A new study reveals clues to how most siblings of people with bipolar avoid the disease.

NEUROSCIENCE

Wiring Differences

THE PAPER

G.E. Doucet et al., "The role of intrinsic brain functional connectivity in vulnerability and resilience to bipolar disorder," *Am J Psychiat,* doi:10.1176/appi.ajp.2017.17010095, 2017.

TIES THAT DON'T BIND

Bipolar disorder often runs in families, but genetics alone don't determine whether one develops the disease, says Sophia Frangou of the Icahn School of Medicine at Mount Sinai. She and her colleagues wondered why siblings of affected people, despite having a slightly higher chance of developing mental illness, typically don't.

IN SYNC

Using fMRI, Frangou's group previously found that, compared with the brains of bipolar patients, certain regions within healthy siblings' brains responded more synchronously during memory and emotional processing tasks. To find out whether this reflects differences in brain organization, postdoc Gaelle Doucet imaged 78 people with bipolar disorder, 64 of their unaffected siblings, and 41 healthy, unrelated controls, all while they were doing nothing.

COMPENSATORY CONNECTIONS

The sensorimotor network, which is important for integrating sensation and movement, was poorly connected in both bipolar patients and their healthy siblings compared with the control group. But, Frangou says, healthy siblings demonstrated stronger connections even than controls within the default mode network, "the backbone of the brain," which contributes to recall and self-reflection—activities unrelated to specific tasks. It's possible that the default mode network is "stepping up . . . and somehow regulating the sensorimotor network," says Ellen Leibenluft of the National Institute of Mental Health.

LITTLE TO DO WITH LUCK

Frangou emphasizes that avoiding disease is a matter of adaptive neural mechanisms. "We have to stop thinking about why people become unwell and question why people who have all the risks to become unwell stay well," she says.

—Aggie Mika

PROFILE

Flickers of Hope

Li-Huei Tsai began her career in cancer biology, then took a fearless leap into neuroscience, making singular breakthroughs along the way.

BY ANNA AZVOLINSKY

In 1991, Li-Huei Tsai was a postdoctoral fellow in Edward Harlow's cancer biology laboratory at Massachusetts General Hospital Cancer Center in Boston. She was working on mammalian orthologs of yeast cyclin-dependent kinases, which regulate cell cycle transitions and are important in tumors, where these enzymes can be mutated and deregulated. She had already cloned and characterized almost an entire family of genes for these kinases, and the gene for one protein in particular, Cdk5, stood out. "Even though this kinase was structurally similar to mitotic kinases, in all of the human and murine cell lines available at the time, there was no Cdk5 activity," says Tsai, now a professor of neuroscience at MIT. "Others in the lab told me it was probably a pseudogene, but I didn't give up. Instead, I started a big, crazy effort."

While everyone in her lab was working on cancer cell lines, Tsai decided to systematically dissect out every mouse tissue and organ and perform an in vitro protein kinase assay to test for Cdk5 activity. If Cdk5 was active as a kinase, it would attach a radioactive phosphate to a test substrate, a histone H1 protein that's a component of chromatin in eukaryotes.

I learned to think outside the box and not to be constrained by any dogma.

"There are few times in my life when there has been a defining moment, and this was one. It was late at night, and I was exhausted, waiting for the autoradiograph to come out of the developer. I didn't expect much because all of my other experiments had been negative. Instead, I couldn't believe my eyes!" Tsai recalls. On the film, she saw strong kinase activity associated with only one tissue—the brain. "I experienced what was beyond joy. I had believed in my pursuit, and I had found my answer." Cdk5 appeared to be active only in the adult murine brain and in the embryonic mouse nervous system, Tsai found.

To figure out what was special about Cdk5 kinase activity in the brain, in 1994 Tsai identified and cloned the gene for the regulatory subunit of Cdk5, p35, which is expressed exclusively in brain tissue—but only in mature, nondividing neurons and not in dividing neuronal progenitors—and is responsible for the tissue-specific activation of Cdk5.

As she finished her postdoc and began to look for faculty positions, Tsai decided to leave cancer research behind and start her own lab concentrating on brain development and neuroscience. "In my job talks, I proposed to make transgenic mouse models and knock out p35 in mice," she says. She was offered a position as an assistant professor at Harvard Medical School and set up her own lab there in 1994.

"Only recently did my postdoc colleagues tell me that, at the time, they thought I was crazy to give up my cancer research and venture into an entirely new area in which I had limited experience," Tsai says.

Here, Tsai recalls the stress of being a child processing her grandmother's dementia; dealing with snow for the first time; and her lab's recent results showing that Alzheimer's disease–associated brain plaques can be dispersed by exposing neurons to flickering light.

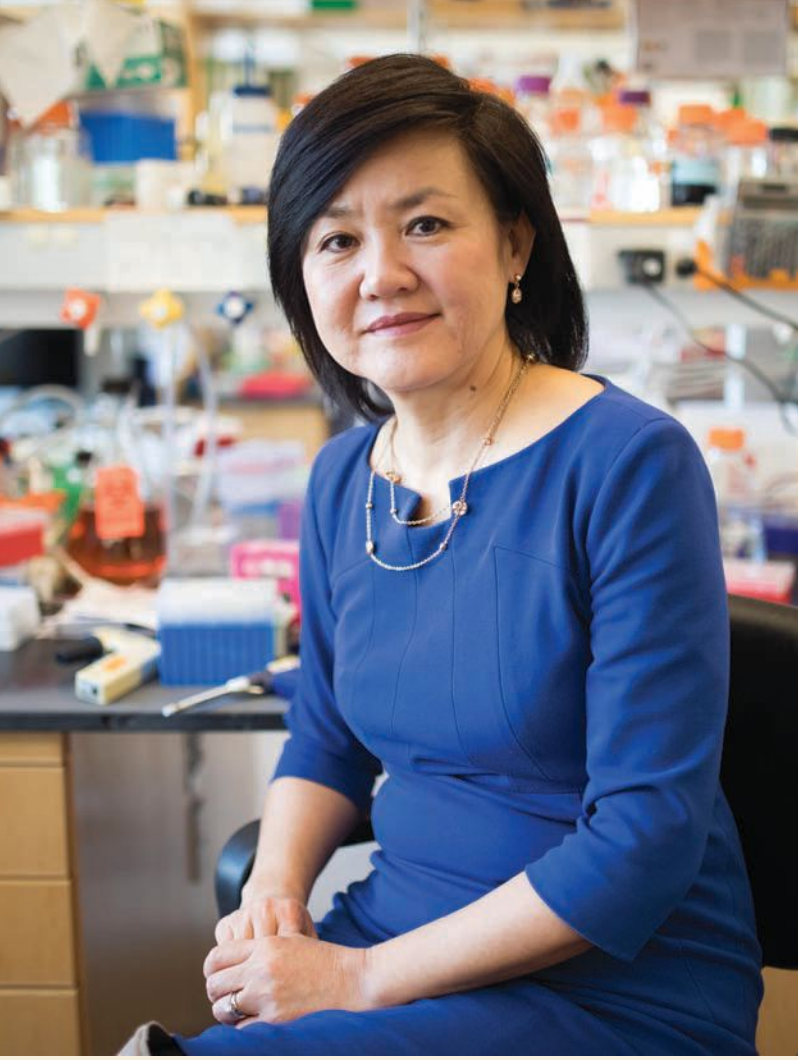
TSAI TACKLES

Jarring childhood memory. Tsai was born in Taipei, Taiwan, and raised by her maternal grandmother in Keelung, a small fishing village north of the capital, while her parents remained in Taipei working for the customs department. When she was five, her parents moved to Keelung. Tsai retains a vivid memory of her grandmother that has stayed with her for life: "When I was about three, we were walking home from our daily trip to the market, and there was a thunderstorm. We took shelter at a bus stop, and after the storm was over, I urged her to get us home. She looked at me and said, 'Home, where is home?' The startled look she gave me has never left my memory. She looked completely lost," Tsai recalls. "She was diagnosed with dementia around then, although we don't know if it was Alzheimer's or another form."

Drawn to science. Tsai's parents invested in her education and that of her younger brother and sister, filling their house with books. "I gravitated towards the science books—biology, astronomy, physics—and I assumed everyone had those interests," she says. "I was predetermined to be a scientist, even though I didn't know it yet."

Cold shock. Because of her love of biology and animals, Tsai decided to train as a veterinarian after high school. In Taiwan, a four-year college education was merged with professional school, and she entered a veterinary program at the National Chung Hsing University in Taichung in 1978. "I learned a lot of biology but no molecular biology, of course, and didn't know about the possibility of doing laboratory research." Toward the end of school, her classmates were choosing what type of veterinary

MIT



LI-HUEI TSAI

Picower Professor of Neuroscience, Department of Brain and Cognitive Sciences, MIT
Director of the Picower Institute for Learning and Memory, MIT
Senior Associate Member, Broad Institute of MIT and Harvard

Greatest Hits

- Identified mammalian Cdk5, a novel cyclin-dependent kinase that is only active in the adult and embryonic nervous system
- Found the mechanism by which p35, a regulatory subunit of Cdk5, controls Cdk5's neuronal tissue–specific activity
- Identified a mechanism by which p35 is converted to p25 in the brains of mammals, including humans; found that p25 accumulation in mice can result in learning and memory impairments and that it accumulates in the brains of Alzheimer's patients
- Showed in mice that loss of learning and memory behaviors and decreases in synaptic connections in the presence of elevated p25 levels can be reversed with an oral histone deacetylase inhibitor
- With Ed Boyden's lab at MIT, demonstrated in a mouse model that stimulating neurons to produce normal gamma waves using optogenetic or strobe lighting can reduce the severity of Alzheimer's disease–linked amyloid-β plaques

practice to join—zoo, farm, or pet-based practices. "All of a sudden it dawned on me that I didn't want to do any of that." Through friends at other universities, Tsai learned about graduate school opportunities abroad and applied to universities that had veterinary schools. She received a fellowship to do a master's program at the University of Wisconsin–Madison and arrived there in January 1984. "It was an interesting experience. I grew up on a subtropical island and had never before experienced snow. I stepped out of the airplane, and there was snow everywhere. It was so cold! There was nothing that I could have purchased in Taiwan that would have prepared me for a Wisconsin winter," Tsai says.

A turning point. At the University of Wisconsin, Tsai joined the lab of veterinary microbiologist Michael Collins. She studied a genus of bacteria, *Pasteurella*, that infects dairy cattle. "I realized that I loved laboratory research and took a molecular biology course that was eye-opening. I knew then that I wanted to do a PhD in molecular biology. It was when I found the direction of my life." After completing the two-year master's program, Tsai began her PhD studies at the University of Texas Southwestern Medical Center in Dallas. She joined Bradford Ozanne's tumor biology and virology laboratory. "I learned to think outside the box and not to be constrained by any dogma," says Tsai. Because the lab did not have much grant funding, obtaining resources for research was a struggle. Yet Tsai, who was studying *c-fos*, a proto-oncogene, published a paper characterizing its mRNA and protein expression in a leukemia-derived cell line.

TSAI TINKERS

Hub of productivity. After being told by her graduate committee that she could complete her PhD earlier than she expected, Tsai quickly decided she wanted to study tumor suppressor genes and applied to only one lab to do a postdoc. In 1990, she began her postdoc in Harlow's lab at Cold Spring Harbor Laboratory in New York. "I arrived and lived in student housing that was five minutes from the lab. The cafeteria was in the same building as the lab, and I realized that I could just work almost nonstop," she says. Within a few months, however, she and the rest of the lab packed up and moved to the Massachusetts General Hospital Cancer Center in Boston. "Almost right away I realized that with the proper resources and support, I could be incredibly productive, getting interpretable and beautiful experimental results," she says. Tsai published her first *Nature* paper only one year later, in 1991: she identified the human cyclin-dependent kinase 2 (cdk2)

gene, originally identified in *Saccharomyces cerevisiae,* then followed this work with the Cdk5-p35 story.

Self-educator. Once settled in her lab at Harvard, Tsai made it her first project to create a loss-of-function p35 transgenic mouse and evaluate the consequences for the developing fetal and adult murine nervous system. Using mouse cortical neurons in culture, the lab initially showed that the Cdk5/p35 kinase is essential for developing neurons to produce new projections, called neurite outgrowth. Then Tsai and her colleagues studied the function of the Cdk5/p35 complex in vivo using the p35 knockout mouse. The lab found that the deletion of p35 was not lethal, but did result in a postnatal phenotype of epilepsy, with severe seizures, and sporadic adult deaths. To study whether the histology of the brain is altered in the mutant animals, Tsai collaborated with a Harvard neuropathologist. "Everyone would send him their mouse tissue samples, and after looking at ours, he looked at me with a saddened expression and said, 'I know you want your mice to have a phenotype, but I'm sorry to tell you that they look normal.'"

In her bold, "I-need-to-see-for-myself" way, Tsai decided to have a second look. She bought a microscope for her office and spent days doing nothing but staring at brain sections. "And I found a difference between our mutant mice and wild-type ones. All of the brain tissues from the mutant mice had a phenotype in which the six cortex layers appeared inverted, with the deeper ones closer to the surface and vice versa. There was also a change in layer 5, which is normally composed of these huge neurons. In the mutants, these cells were more superficial, in a different location. I went back to the pathologist who studied the blinded samples, and he admitted that he had been wrong," says Tsai. Her lab's work characterizing mice that lacked p35—which proposed that the cortex in the animals without the protein is abnormal because p35 is needed for proper neuronal migration and proper differentiation into specific cortical neuronal subtypes—was published in 1997 in *Neuron*.

Ties to human disease. Tsai's lab found that p35 could be converted to a smaller, 25-kilodalton protein, p25, under conditions of neuronal stress such as oxidative stress or addition of amyloid-β peptides. The team also found that p25 was expressed in postmortem brain samples from Alzheimer's disease (AD) patients. Accumulation of p25 results in extended Cdk5 activation and mislocalization, resulting ultimately in primary neuron apoptosis. One year later, in 2000, the lab identified the mechanism by which neurotoxicity converts p35 to p25, suggesting its role in the pathogenesis of AD. Influx of calcium into the cell, Tsai's lab found, activates a calcium-dependent protease, calpain, which cleaves p35.

TSAI TRANSCENDS

Pill-popping mice. When postdoc Andre Fischer joined Tsai's lab in 2002, he began to investigate the behavioral and memory impairments of p25 transgenic mice, finding that transient expression of p25 actually facilitates memory and synapse formation in mice, but that prolonged p25 expression impairs long-term memory retention and retrieval. The researchers then discovered, to their surprise, that some of these memory and learning defects could be ameliorated when these mice were placed in what Tsai calls a "Mouse Disneyland," a stimulating environment with other mice and a constant stream of new toys for play—even though p25 was still present in the brains of the animals. Even more surprising to Tsai, the same amelioration could also be achieved with an oral pill, an inhibitor of histone deacetylases, which resulted in new dendrite sprouting, an increase in synapse number, and better learning and long-term memory retrieval. "We argued in that paper that the memory is not really lost, but just unable to be retrieved," says Tsai.

Life-changing results. In December of 2016, Tsai's lab published a finding that astounded the scientific community: exposing mouse models of Alzheimer's disease to flashes of light at the frequency of "gamma waves"—a pattern of neural oscillation in mammals between 30 and 80 Hz, detected by electroencephalography (EEG)—could reverse some of the characteristics of neurodegeneration in the animals' brains. The lab initially targeted interneurons in the brains of mice using optogenetics, and found that the exposure induced gamma oscillations of these cells—an activity linked to higher-order cognitive abilities.

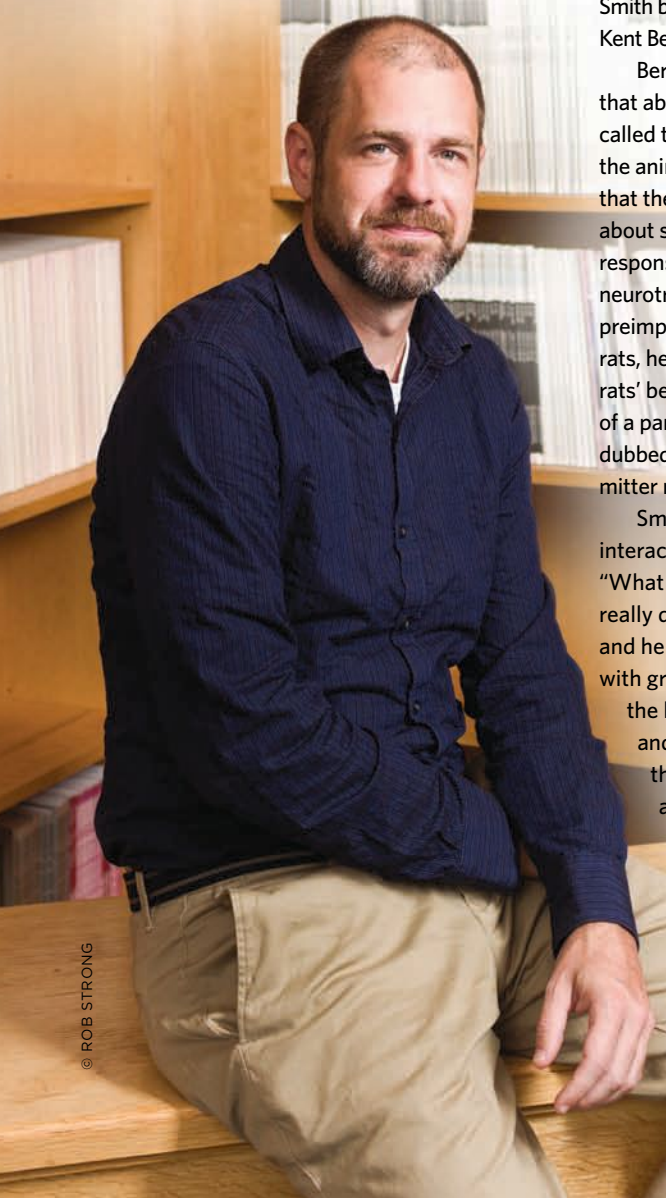
Then, a graduate student in Tsai's lab, Hannah Iaccarino, wanted to test whether optogenetic stimulation at gamma frequency would be beneficial for mouse models of AD. "After she did the experiment in the amyloid-β mouse model, she ran into my office saying that the amyloid levels in the brains were drastically reduced following one hour of gamma oscillation," says Tsai. Tsai only began to believe the results when they were repeated; when her team showed that no other frequency than gamma worked; and when the researchers saw major changes in the gene expression and morphology of microglia, the brain's immune cells. "The microglia just went crazy, their shapes were completely transformed to be much larger with more-elaborate processes," says Tsai.

The team also saw that the microglia could now more efficiently phagocytose the amyloid-β protein. Tsai's colleagues at MIT suggested that strobe lighting may be a way to noninvasively stimulate the same gamma waves. The researchers exposed the mice to 40 flashes per second for an hour and found that soon after, their levels of amyloid-β were about half those prior to the strobe-light exposure, along with the other effects seen with the optogenetic approach in a young Alzheimer's mouse model. Now, the lab is working on studying whether the behaviors of the mice are altered upon recurring strobe exposure and whether entraining the gamma frequency noninvasively through other sensory modalities could work similarly. ■

Kyle Smith: Habitually Creative

Assistant Professor, Department of Psychological and Brain Sciences, Dartmouth College. Age: 38

BY SHAWNA WILLIAMS



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When Kyle Smith was a kid, he didn't like science. "I didn't do very well" in the subject, he says. As an undergraduate at Indiana University, he initially saw himself going into film or television production, but he says the jump to psychology with a neuroscience bent wasn't really such a big one. With film, "basically you start out with nothing, come up with an idea, figure out how to get it done, be creative, make it interesting to people . . . push boundaries, [which] is exactly the same kind of thing I've found in science," Smith says.

Smith was drawn to psychology partly by the problem of drug addiction. "Watching people go through that, it just hijacks the person in a sad but really fascinating way," he says. As an undergraduate he studied at the University of Oxford, focusing on "the neuroscience side of psychology," which further hooked him, so Smith became a graduate student in the lab of Kent Berridge at the University of Michigan.

Berridge's group had previously found that ablating a region of the rodent brain called the ventral pallidum (VP) wiped out the animals' reward response so completely that they stopped eating. To learn more about specific areas involved in the reward response, Smith used tiny syringes to inject neurotransmitter-mimicking chemicals into preimplanted tubes in the brains of awake rats, he says. The resulting changes in the rats' behavior helped lead to the discovery of a particular area within the posterior VP—dubbed a "hot spot"—where the neurotransmitter mimics an enhanced reward response.[1]

Smith then delved into how that hot spot interacted with other areas of the brain. "What stood out about Kyle is that he was really dedicated to doing a scientific career, and he just threw himself into his projects with great energy and sort of swarmed over the literature, mastered the techniques, and then began to achieve results through a lot of hard work and talent—and did some beautiful, beautiful science," says Berridge.

When it came time to apply for postdoc positions, Smith was intrigued by the work of MIT's Ann Graybiel, who was using neural recording and other cutting-edge methods to decode habit formation in rodent brains. Working with her, Smith learned a new technique: optogenetics. "It developed into a very cool project where we were tracking changes in the brain as habits were formed," he says.[2]

In 2013, Smith started his own lab at Dartmouth College, where he's continued to study reward response, habit formation, and the VP. In one experiment, he and postdoc Steve Chang used optogenetics to disrupt VP function in rats that had been given a diuretic to make them salt-deficient. This VP impairment dampened the rats' ability to associate environmental cues with salt rewards—though they still ate the salt when it was presented to them.[3]

Like Berridge, David Bucci, who heads Dartmouth's Department of Psychological and Brain Sciences, says he has been impressed with Smith's command of the literature and embrace of new technologies. "He wasn't afraid to try new things and take a risk."

And his background in the visual arts still comes in handy, Smith says: "[I] go kind of overboard with the PowerPoints that I wind up doing." ■

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Lighting Up Monkey Brains

Optogenetic and chemogenetic tools illuminate brain and behavior connections in nonhuman primates.

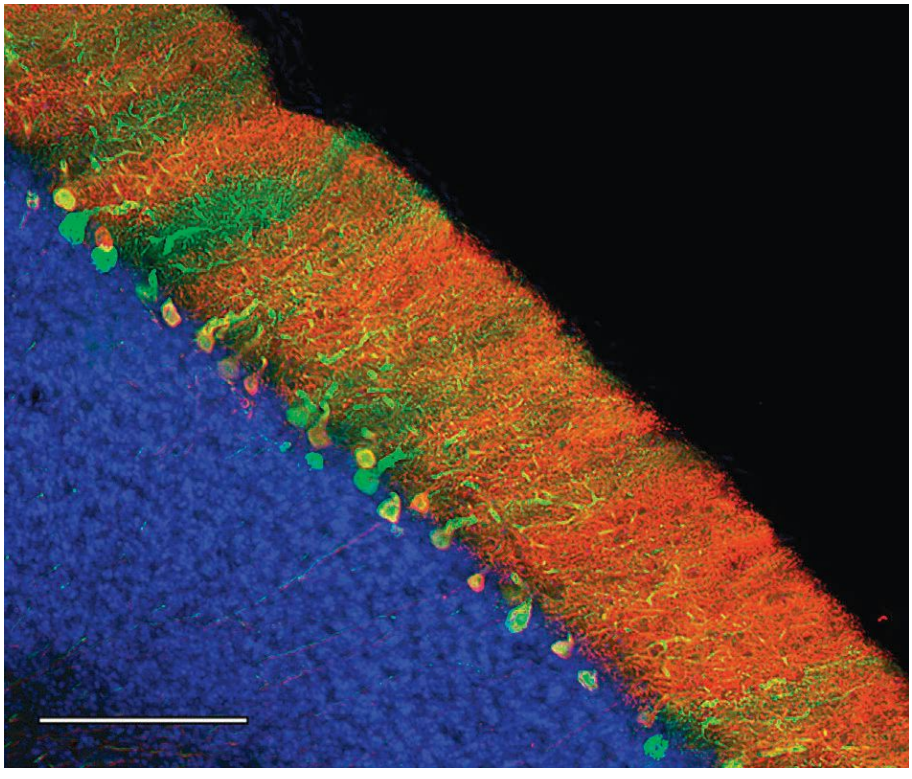
BY JYOTI MADHUSOODANAN

Since optogenetics burst onto the scene in the early 2000s, brain researchers have embraced the technique to study functions ranging from sleep and hunger to voluntary movements and sensory input. The vast majority of these studies have been conducted in rodents, and much has been learned, but extrapolating to humans from a species so different from us poses a challenge.

Brain research in nonhuman primates precedes optogenetics by decades. Attempts to understand the links between brain function and behavior have relied on techniques such as inserting an electrode into the brain to activate or interrupt neural signals, and creating lesions to disrupt pathways. But these approaches only reveal whether the altered brain regions are involved in the functions being studied, with little detail about the types of cells or networks involved.

Controlling neurons with light (optogenetics) or chemicals (chemogenetics) offers researchers a much more precise way to study brain function. Optogenetics utilizes a microbial protein known as channelrhodopsin (ChR), a light-activated ion channel. When inserted into animal cells under the control of a cell type–specific promoter, the protein is expressed in subsets of neurons, and a beam of light can be used to trigger its activity, spurring those neurons to action. Chemogenetics deploys chemicals rather than light. Cells are engineered to carry DREADD (designer receptors exclusively activated by designer drugs) proteins, which are then activated by a drug that doesn't otherwise affect animal metabolism.

Rodents are often genetically engineered to encode ChR, DREADDs, or other controlling elements. But so far, genetically modifying primates has proven more difficult and expensive, limiting researchers to using viral vectors for delivering genes for these proteins to the brain. These vectors are generally derived from adenoviruses, says Jessica Raper of the Yerkes National Primate Research Center. "Just like humans, nonhuman primates can have neutralizing antibodies for these viruses, so any method must prescreen for antibodies specific to the serotype being used," she explains.



TYPECASTING: Immunohistochemical staining shows selective labeling of Purkinje cells (green) and their axons (red) in the granular layer of the cerebellar cortex. (Scale bar = 200 microns)

The larger primate brain also requires larger amounts of vector to be injected directly into the brain, sometimes in multiple doses that may damage tissue. Furthermore, delivering light deep into the brain requires inserting an optical fiber, and chemicals designed to activate inserted genetic sequences must be able to cross the blood-brain barrier. (See "Into the Breach," page 32.) That means much more trial and error than in mouse studies. "There's no universal solution for primates as there is with the host of genetically modified rodents," says William Stauffer of the University of Pittsburgh.

Nonetheless, several recent studies have managed to probe the function of specific brain regions or cell types in rhesus monkeys, marmosets, and other primates using optogenetic and chemogenetic tools. Here, *The Scientist* profiles some of these recent efforts.

NEURON, 95:51-62, 2017

CELL TYPE BULL'S-EYE

INVESTIGATOR: Gregory Horwitz, University of Washington

PROJECT: Studying how primate brain cells control eye movements

PROBLEM: Before optogenetics, researchers experimentally manipulated groups of neurons by stimulating them with electrodes or altering their activity with chemical treatments. Cells can be classified into different groups based on their responses to such treatments, but it was difficult to know whether responsive cells within a given brain region differed by subtype, or whether they were all similar but acted differently because they were connected to different neuronal networks. "We couldn't dissociate the contributions of different cell types in a given brain region to behavior," Horwitz says. He considers this a major stumbling block in the field. "We need to be able to manipulate cells based on where they project or the genes they express."

APPROACH: Previously, Stauffer and his colleagues had targeted dopamine neurons in rhesus macaques using a two-vector strategy: one vector carried ChR in a form dependent on the enzyme Cre recombinase for activation, and the other carried the gene for the enzyme. Expression of Cre was controlled by a promoter specific to dopamine neurons, so ChR would only be activated in these cells. (*Cell*, 166:1564-71, 2016).

Horwitz and his colleagues built on this approach to design a single-vector system extendable to other cell types. They constructed an adeno-associated viral vector in which ChR was controlled by a promoter known as L7, which is only active in cerebellar Purkinje cells. "Our idea was to use a virus that would infect many types of cells, but use a promoter that would only affect a very specific set of cells," he says.

The team inserted an optical fiber near the injection site; activating cells with light produced strong neuronal activity in the Purkinje cells and altered a specific kind of quick eye movements within 15 milliseconds. "To study the kinematics of movement, you need a manipulation that will work fast, and it's gratifying to see that this one does," Horwitz says. To see precisely which neurons were being activated and causing the change, the team fused the opsin to a red fluorescent protein, and confirmed that the proteins had localized only to Purkinje cells in the cerebellar region being studied (*Neuron*, 95:51-62, 2017).

WHAT'S NEXT: Investigators will need to identify the best viral vectors and constructs for extending the method to other cell types. Few cell type–specific promoters have been characterized in monkeys; ones from mice or other species can offer some leads. Without well-characterized promoters, "it's a much harder road," Horwitz says.

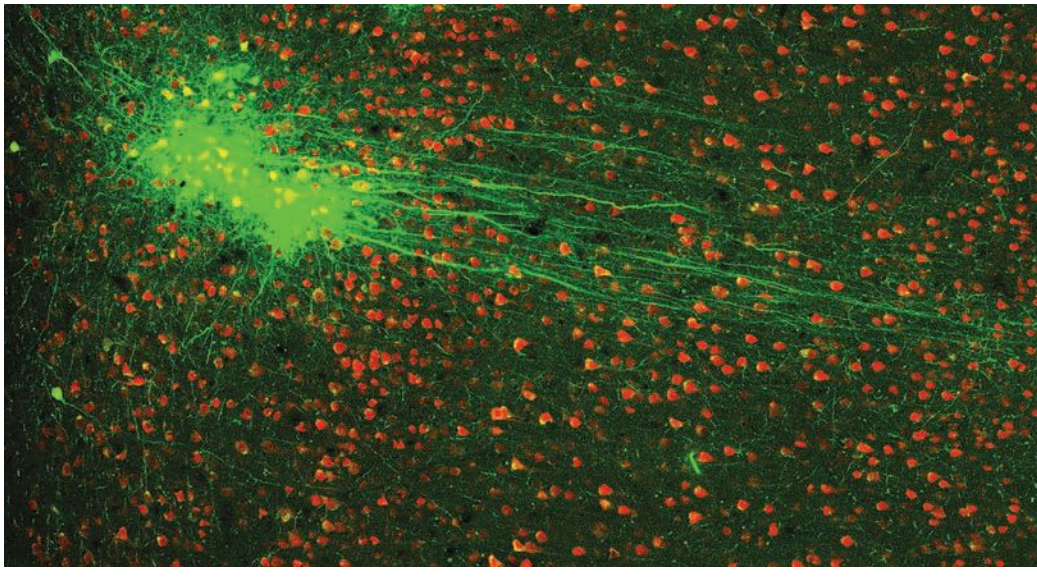
REVERSIBLE DISCONNECTION

INVESTIGATORS: Barry Richmond and Mark Eldridge, National Institute of Mental Health

PROJECT: Exploring reward processing in the visual system

PROBLEM: The orbitofrontal cortex in rhesus monkeys encodes information about properties of and preferences for rewards, while the rhinal cortex carries information about stimulus-reward connections. Previously, Richmond's team found that disrupting links between these two regions in monkeys led the animals to make errors when estimating the size of an expected reward after a task. But with the conventional method of creating lesions, the researchers could not reversibly disconnect the two regions to further test why these errors occurred.

APPROACH: The team created a lentivirus vector carrying a gene for a DREADD protein that silenced neurons when treated with a chemical called clozapine N-oxide (CNO). Expression of that DREADD protein is in turn controlled by a neuron-specific promoter. The researchers first removed the rhinal cortex from one side of the monkeys' brains, then trained them to associate a particular stimulus with a reward. The animals then received injections of the viral vector in the opposite orbitofrontal cortex and were tested on the task. When activity was silenced using CNO, the animals could not discriminate the size of expected rewards and made more errors in calculating reward size, suggesting that connections between these two brain regions help monkeys remember and gauge the relative value of different rewards (*Nat Neurosci*, 19:37-39, 2016).



COURTESY OF WALTER LERCHNER, NIMH

MONITORING REWARD BEHAVIOR: Neurons in the orbital prefrontal cortex expressing the chemogenetic DREADD receptor (visualized by GFP antibody)

The key to the technique is titrating the optimal amount of drug. The researchers also turned to positron emission tomography (PET) imaging to observe DREADD expression in vivo and see how much CNO was needed to induce silencing. For both optogenetic and chemogenetic methods, getting sufficient penetrance in the monkey brain, which is much larger than that of rodents, is a challenge. With chemogenetics, an additional issue is using drugs that cross the blood-brain barrier.

EXTENDING THE METHOD: To apply the method to other brain regions, PET imaging is useful for ensuring that DREADDs are expressed in the correct area or cell type, Eldridge says. But this is expensive, challenging, and requires chemists to synthesize the radio-ligands needed to image tissue. As an alternative, researchers could check DREADD production with histology, he adds.

Whether CNO works in primates and what dose to use needs more testing. Although Richmond's team found good results with intramuscular drug injections, recent rodent studies have found that CNO's activity is actually mediated by its metabolite clozapine, which can bind to other receptors. In primates, Raper and her colleagues reported that CNO does not cross the blood-brain barrier as effectively as clozapine does (*ACS Chem Neurosci,* 8:1570-76, 2017).

THALAMIC LIGHT-UP

INVESTIGATOR: Adriana Galvan, Emory University

PROJECT: Studying interconnected brain areas that control movement

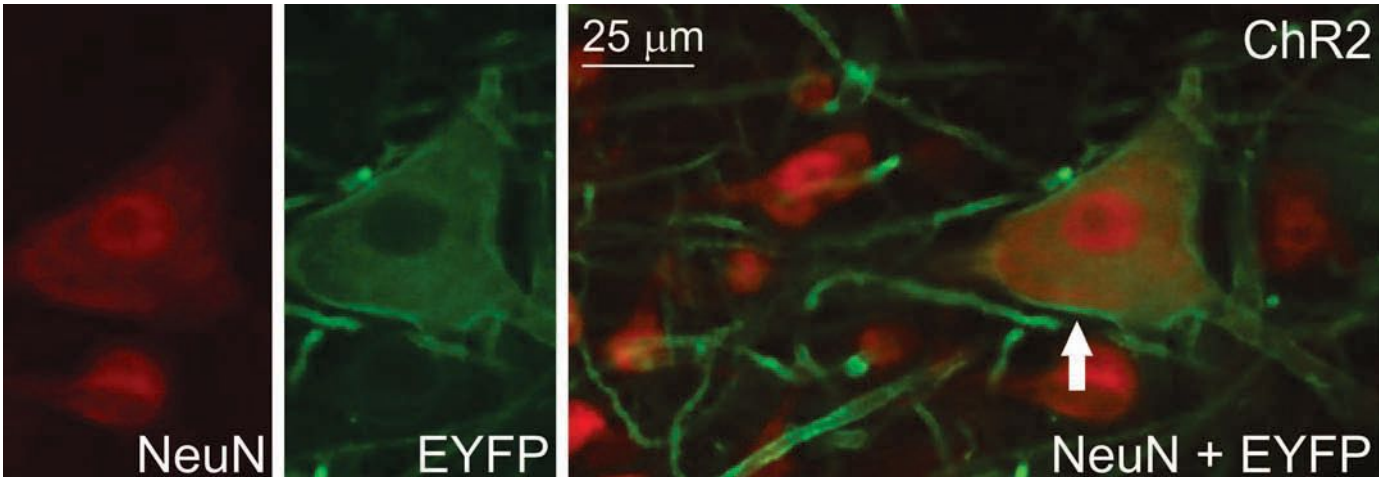
PROBLEM: Viral vectors injected into the brain aren't selective: they infect cells at random. Researchers can use unique promoter sequences to target specific cells, but not all neuronal subtypes are well characterized at the genetic level.

APPROACH: To selectively target one subtype of neuron and understand its activity, Galvan and her colleagues began by injecting adenoviral vectors carrying ChR into the motor cortex of rhesus monkey brains. The opsins were expressed in cortical neurons projecting into a variety of brain areas, but the team could selectively activate the pathway of choice by altering the placement of the optical fiber. Placing the light source at different points would thus activate different circuits. "So we activated selected brain regions, not specific cell types," Galvan says.

They chose to activate the pathway from the cortex to the thalamus. In addition to inserting the optical fiber close to neurons' cell bodies in the cortex, the researchers placed it millimeters away—closer to the axon terminals in the thalamus where the cells made connections to other neurons. Although light stimulation in the cortex activated excitatory cortical fibers as expected, when only the axon terminals in the thalamus were stimulated, the researchers found a decrease of activity in thalamic neurons, likely because illumination also stimulated inhibitory GABAergic neurons in the region. "In a sense that's an off-target effect, but it may also be what happens naturally," Galvan says. "Under normal conditions, this is probably a way for the cortex to exert inhibitory influence on the thalamus."

Teasing apart these distinct roles would have been difficult with an electrophysiological approach because the cortex and thalamus are reciprocally connected. "If we were to just electrically stimulate, we'd see activation of both pathways simultaneously, so it would be very difficult to see what's going on," Galvan says. Most previous studies have focused on sensory areas of the cortex and thalamus, but her study suggests that in the motor areas, these two regions interact in a distinctive way (*J Neurosci,* 36:3519-30, 2016).

EXPERT TIP: When relying on light to activate specific brain regions, make sure that illumination does not spread out of the brain region of interest and activate ChRs that may be expressed in other areas, Galvan says. ■



SELECTIVE STIMULATION: Immunofluorescence labeling shows neurons in the primary motor cortex (red) that also express channelrhodopsin (green).

J NEUROSCI, 36:3519-30, 2016

Caught in the Act

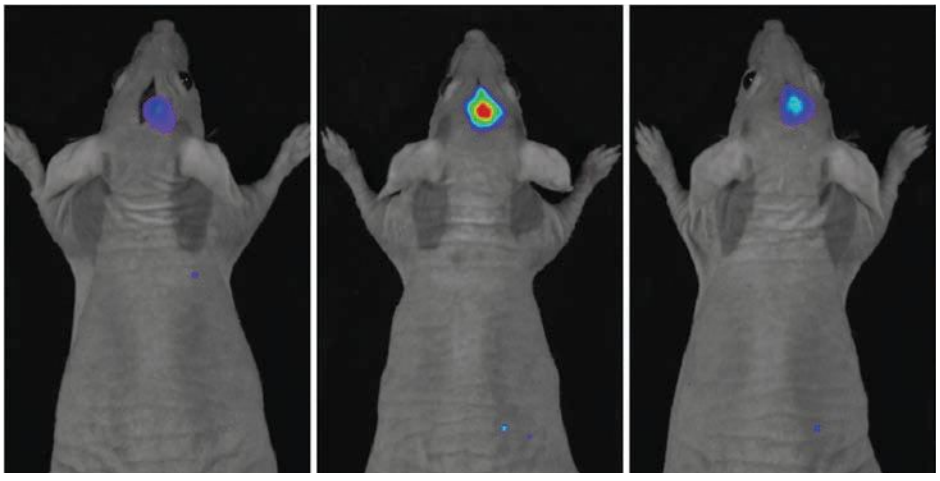
Molecular probes for imaging in live animals

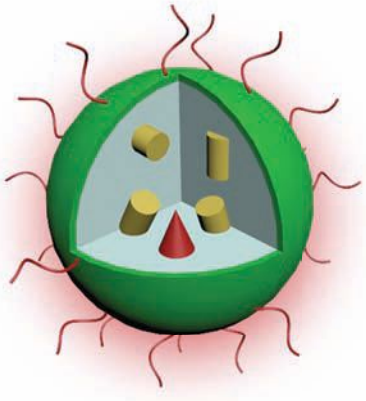
BY DEVIKA G. BANSAL

The ability to peer at molecular processes as they unfold in vivo can deliver invaluable insights to researchers. Molecules that shuttle through living cells are often vital biomarkers of disease conditions, and capturing tiny quantitative changes in their levels is an essential part of diagnosis in the era of precision medicine. What's more, dynamic monitoring of physiological changes can also help track and adjust drug treatments during preclinical studies.

In order to get a bead on key molecules that signal disruptions in regular cell functions, scientists need to cast a wide net. MRI, PET, and CT imaging are useful tools to visualize and measure cellular processes, but they are vastly more expensive than light-based imaging techniques.

A variety of fluorescent probes are available to detect specific molecules and monitor their activities. But optical imaging in living animals has mostly been limited to the study of skin, eyes, surface vessels, and epithelial tissues accessible to visible light. In recent years, however, investigators have been developing probes that work in the near-infrared range—longer wavelengths that allow visibility into deeper tissue layers. *The Scientist* spoke with some of these researchers about how they designed noninvasive probes to capture real-time, in situ changes in living animals.





VISUALIZING REACTIVE OXYGEN MOLECULES: To detect endogenous hydrogen peroxide in live animals, Kanyi Pu and colleagues constructed a chemiluminescent probe tucked inside a semiconducting polymer nanoparticle (SPN). The yellow cylinders denote the substrate, and the red pyramid denotes a dye that allows the probe to emit in the near-infrared range (left). The scientists tested the probe in a mouse model of neuroinflammation (above). From left to right, the images show mice treated with saline, lipopolysaccharide alone, which causes inflammation, or lipopolysaccharide with glutathione, which abates the injury. The probe lights up to mark peroxide levels in inflamed brain tissues.

RADICAL VISION

TARGET: Hydrogen peroxide

RESEARCHER: Kanyi Pu, Associate Professor, Nanyang Technological University, Singapore

PROBLEM: Reactive oxygen species act as essential signaling molecules at low concentrations, but free radicals wreak havoc when their levels shoot up in tumors and inflamed tissues. Several probes measure hydrogen peroxide (H_2O_2) levels in cell culture, but they are obscured by autofluorescence when used in vivo. Current probes designed for live imaging rely on small-molecule dyes that are unstable in the presence of hypochlorite and hydroxyl radicals, which are highly reactive. "Detecting peroxide in living animals is challenging, and you need to have a sensitive probe with the ability to pass through thick tissues," Pu says.

SOLUTION: Pu and colleagues developed a chemiluminescent probe that detects peroxide at levels as low as 5 nM (normal in vivo levels of H_2O_2 are closer to 100 nM). The probe relies on a reaction between H_2O_2 and peroxalate to chemically excite a luminescent reporter, eliminating the need for external light excitation, which in turn eliminates autofluorescence. "This design kind of minimizes the tissue penetration problem, and sensitivity is higher when you don't need any external light source," says Pu.

The probe sits inside semiconducting nanoparticles that are stable in the presence of free radicals, and it emits in the near-infrared range, which allows the emitted light to be detected even through the skull (*ACS Nano*, 10:6400-09, 2016).

ACS NANO, 10:6400-09, 2016

LIMITATIONS: The probe's current iteration can't reliably home in on the tissue of interest. The best way to ensure that the nanoparticles accumulate in a specific location is to inject them directly into the right spot.

FUTURE PLANS: Pu and colleagues are working on targeting strategies and are trying to reduce the probe size to ensure better accumulation and biodistribution.

EXPERT TIP: The probe is more stable than others out there, but for best performance, stick with fresh preparations, Pu says. "If you leave it out for one day, many substrates get consumed," he adds. Purge the buffer with nitrogen, dissolve the freeze-dried probe in it, and use immediately.

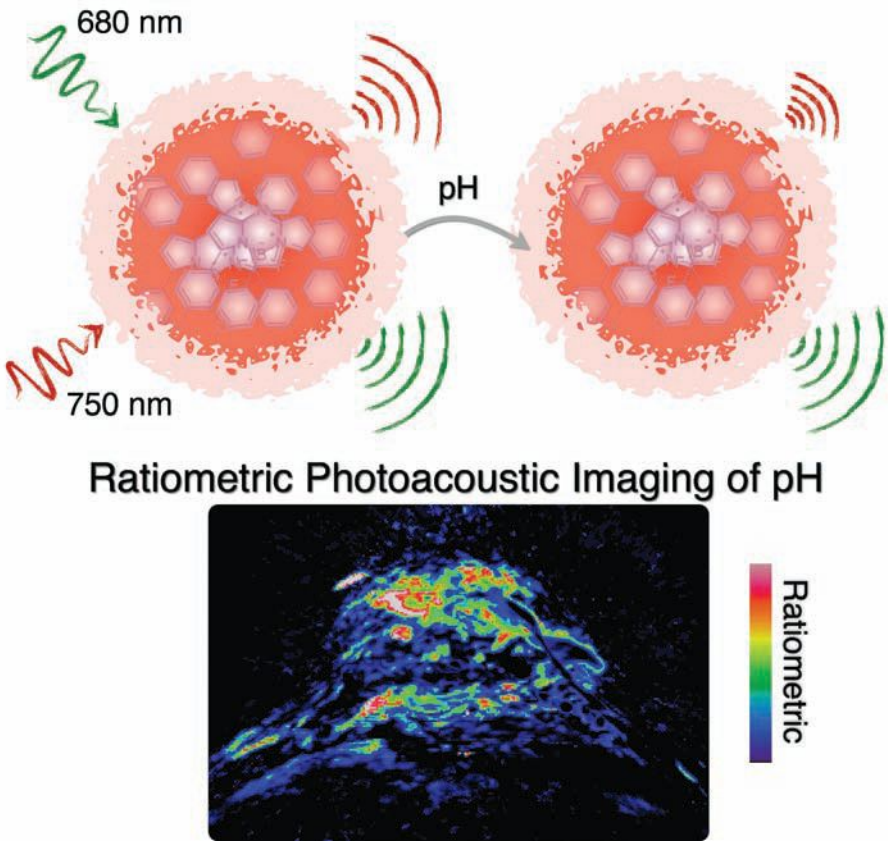
LITMUS TEST

TARGET: pH

RESEARCHER: Kanyi Pu, Associate Professor, Nanyang Technological University, Singapore

PROBLEM: pH is a physiological index that remains near neutral under normal conditions. In inflammation-related diseases, however, the tissue environment turns acidic. "If you can map the pH in disease, it can help to do better drug screening," Pu says, explaining that knowledge about the pH environment is useful for drug design. But most current probes designed to measure pH are based on fluorescence, which is often hampered by strong light scattering and tissue autofluorescence.

SOLUTION: Pu and colleagues developed a probe that detects pH in a ratiometric manner. The unique thing about this probe, Pu says, is that it is photoacoustic. "It's basically a light-in and sound-out process," he says. When researchers use a laser to target a tissue of interest, part of the energy gets converted into heat, leading to tissue expansion. The expanded tissue emits ultrasound waves that can be recorded. This imaging technique allows researchers to maximize signal-to-noise ratios and easily detect signals through tissues as thick as 6 cm. "In addition, it measures linearly," Pu says. "When the signal is high, the pH is high, and when the signal is low, the pH is low" (*Adv Mater*, 28:3662-68, 2016).



LIGHT IN, SOUND OUT: The schematic illustrates a mechanism for sensing pH changes related to inflammation. When a tissue of interest is targeted with two lasers of different wavelengths, part of the light energy gets converted into heat, leading to tissue expansion. The resulting ultrasound waves emitted by the tissue allow tissue probing at much deeper levels. When the specially designed probe detects a pH change from neutral to acidic, the signal goes green; as the pH increases, the signal turns red. This mouse tumor tissue (bottom) shows regions of varying pH.

LIMITATIONS: Despite the advantages and use in clinical applications, the photoacoustic probe is not that popular in basic research: researchers need ready access to a $1 million ultrasound transducer connected to a pulsed laser, equipment not always on hand.

FUTURE PLANS: The probe easily accumulates in tumor tissues because tumor blood vessels are leaky due to inflammation, Pu says. But it's not so easy for the probe to cross the blood-brain barrier, something his lab is tackling next.

EXPERT TIP: The probe is easy to use, Pu says: "It's stable, the size is small, you inject it intravenously, and you can do ratiometric imaging for four hours straight."

TRACING A TOXIN

TARGET: Formaldehyde

RESEARCHER: Weiying Lin, Professor, Institute of Fluorescent Probes for Biological Imaging, University of Jinan, China

PROBLEM: Exposure to formaldehyde (FA), a chemical used in plastics, cosmet-

COURTESY KANYI PU

ics, textile processing, wood processing, foods, and medicine, may cause memory loss, cancer, and spontaneous abortion. But recent work shows that FA is also an endogenously produced metabolite released during histone demethylation and DNA methylation. Functions of endogenous FA are hard to study due to a lack of robust molecular tools, Lin says, "which is why we decided to develop FA fluorescent probes."

SOLUTION: Lin and colleagues engineered a two-photon fluorescent FA probe based on the condensation of a hydrazine moiety with FA. This unique strategy endows the probe with a very large turn-on signal, a low detection limit, and very fast onset, Lin says. These critical attributes enable the tracking of endogenous FA in living tissues for the first time at levels as low as 0.7 μM (normal in vivo levels of FA are closer to 29 μM) (*Angew Chem Int Ed*, 55:3356-59, 2016).

LIMITATIONS: The probe exhibits good chemical and photostability. However, the maximum emission wavelength is only about 543 nm, which limits the probe's detection depth.

FUTURE PLANS: Lin hopes to continue developing more-stable and more-penetrant FA fluorescent probes for biomedical applications.

EXPERT TIP: The probe concentration for optimal sensing and imaging varies by applications, Lin says. For cell imaging experiments, use 5–10 μM; for tissue imaging, Lin recommends using the probe in a higher concentration range of 5–30 μM.

MONITORING A MARKER

TARGET: β-galactosidase

RESEARCHER: Zhiqian Guo, Associate Professor, East China University of Science and Technology, Shanghai

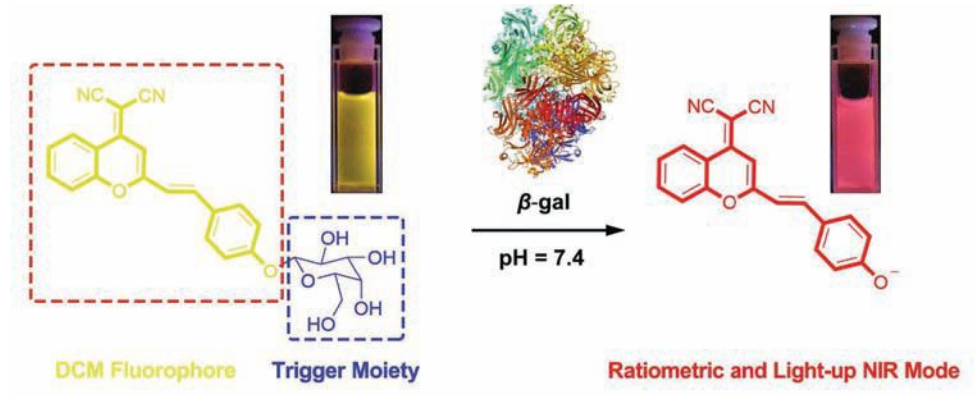
PROBLEM: β-galactosidase (β-gal) activity is a well-known biomarker for aging cells and primary ovarian cancers. Although a number of fluorescent probes can help visualize β-gal in cell lines, very few can detect real-time enzyme activity in living animals—a critical ability for cancer diagnoses. "Precise in vivo tracking of enzyme activity is still challenging due to its dynamic complexity and intrinsic background noise," says Guo.

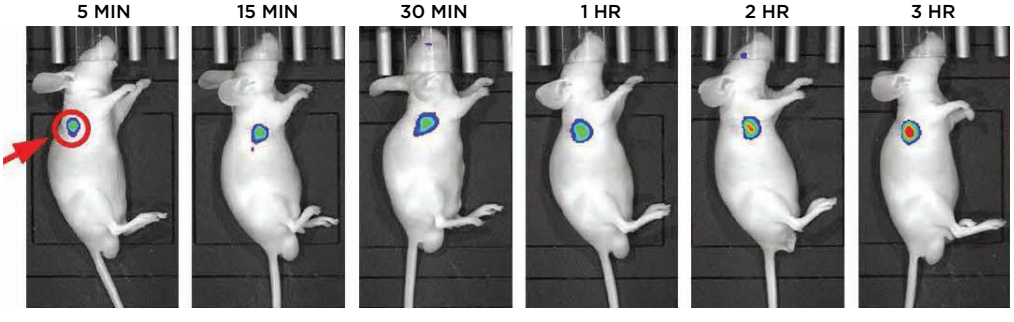
SOLUTION: To get around some of those issues, Guo and colleagues developed a new β-gal sensor that works in the near-infrared range and lights up only when enzyme activity triggers the probe. The conditional fluorescence and the longer wavelength dampens background noise and autofluorescence, thus improving penetration depth for imaging. When researchers inject the probe intravenously, target cells brighten up in as little as five minutes, and the signal reaches a maximum at three hours. "The rapid response to β-gal activity at the tumor site enables real-time, in vivo imaging at high resolution," Guo says (*J Am Chem Soc*, 138:5334-40, 2016).

LIMITATIONS: Even though the probe fluoresces only in cells with active β-gal, there is no way to target it to specific tissues, Guo adds. The lack of a localization mechanism can ultimately affect probe accuracy.

FUTURE PLANS: Guo and his team plan to directly address the drawback by developing a probe with better "active-targeting ability," he says.

EXPERT TIP: The probe is fairly straightforward to use, Guo says, but inject as close to the target site as possible for best results. ■





TRACKING TUMORS: When the fluorophore detects β-galactosidase in the tissue, the probe lights up in the near-infrared range (top). Zhu and colleagues show real-time β-gal activity by imaging tumor-bearing nude mice after injecting the mice with a form of β-gal that localizes to the tumor, reaching maximum signal three hours after injection of the fluorescent probe.

J AM CHEM SOC, 138:5334-40, 2016

Interleukins
IL-2, IL-3, IL-4, etc.

Chemokines
MCP-1, RANTES, MIP-1β , etc

Growth Factors
EGF, FGF-basic, PDGF-BB, etc

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Misconduct Under the Microscope

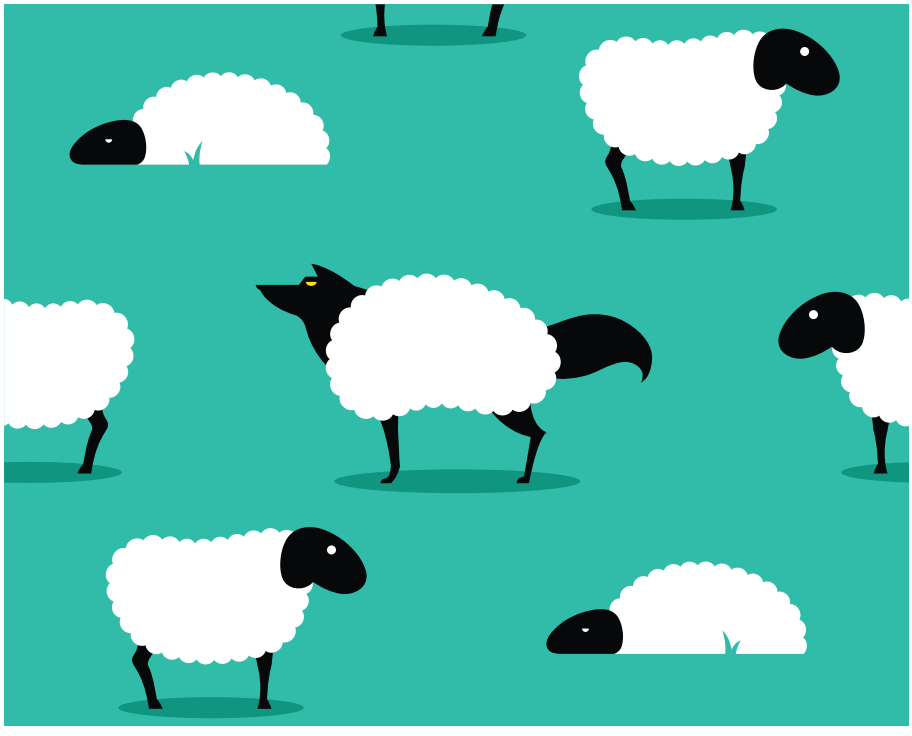
Are colleges and universities doing enough to protect their students and staff from professors who break the rules, or even the law?

BY ANNA AZVOLINSKY

In 2013, Dong-Pyou Han, then an assistant professor of biomedical sciences at Iowa State University, resigned after admitting to tampering with experiments. He had spiked animals' blood samples with human antibodies to make it appear as if an HIV vaccine he was helping to develop could successfully protect rabbits. After being charged by a federal prosecutor the following year, Han pled guilty to two felony charges of making false statements on a National Institutes of Health (NIH) grant application and follow-up progress report. He was sentenced to 57 months in prison and had to repay $7.2 million of the NIH grant that had been awarded to him for the research.

While other cases of fudging the data might result in a suspension of funds or mandatory supervision for some period of time, Han's represents one of the most severe punishments meted out for scholarly malfeasance. Federal criminal charges for scientific misconduct are rare, as are criminal proceedings at any level against professors. Over the past few years, however, increasing media scrutiny, particularly of cases of scientific misconduct and of sexual harassment, has shined a spotlight on how universities handle faculty transgressions. And, at times, the publicity has revealed less-than-ideal responses to cases of alleged sexual harassment and of unethical or illegal conduct by faculty.

For example, in 2016 at the University of Rochester in New York, graduate students and current and former professors within the department of brain and cognitive sciences had filed complaints to university administrators, accusing professor Florian Jaeger of sexual harassment and intimidation. But the university cleared him of violating the school's harassment and discrimination policy even after an appeal by several of the faculty members, and promoted him to full professor even while the internal investigation was ongoing. This September, the case was brought into the public eye by *Mother Jones* magazine after the accusers submitted formal complaints to the US Equal Employment Opportunity Commission (EEOC) at the end of August. Three weeks later, the university finally placed Jaeger on leave, and the Rochester president said he regretted promoting Jaeger.



This is but one example of cases of faculty wrongdoing that got swept under the rug by universities. In some instances, accusations are never properly explored. When complaints are investigated, the internal inquiries are typically conducted behind closed doors, where a committee of the accused's colleagues, not quite impartial, listens to the case and makes a judgment.

"Academia is one of the last bastions where power imbalances spill over into the governance system, including faculty disciplinary committees that are charged with objectively judging their peers," says a former university administrator who asked to remain anonymous because of involvement in an ongoing university gender discrimination case. As a result, justice is not always served.

How to fire a professor

When a formal complaint of harassment or another transgression is filed within a university, administrators decide whether it is substantial enough to warrant review by a faculty committee, which hears the evidence and rules on whether there was misconduct (and sometimes recommends specific sanctions). Following the committee's decision,

PUZZLE ON PAGE 14

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the case goes to the desk of a university provost or president for a final decision. For a tenured professor whose misconduct may be grounds for revoking tenure, there is often an additional hurdle to clear before disciplinary action can be taken.

"A tenured professor has to go through academic review boards to be ousted. Regardless of what the allegations are, the university has obligations to go through that process before pulling someone's tenure," says Sharon Vinick, a plaintiffs' employment lawyer in California who has represented many women who have brought claims of sexual harassment or discrimination within colleges and universities. "There is a heightened scrutiny compared to your typical private employer." W. Scott Lewis, a partner at the Pennsylvania-based National Center for Higher Education Risk Management (NCHERM) group, agrees: "The additional layer for tenured faculty to be removed can be a difficult and arduous process." Sometimes, the layers of bureaucracy can stand in the way of efficient disciplinary action. But there are also more egregious cases of universities choosing to go easy on tenured faculty.

Geoffrey Marcy, a former University of California (UC), Berkeley, astronomy professor, was found to have sexually harassed multiple students over the span of a decade. Although the university's own internal proceedings took place in 2015, Marcy was not penalized until the records were published by *BuzzFeed* later that year. At that point, Marcy received only a mild reprimand by the vice provost of Berkeley's faculty, involving an agreement that held Marcy to "clear expectations" regarding his future interactions with students; otherwise he could get suspended or fired. It was only after subsequent public outrage—including a letter to *The New York Times* penned by 278 of Marcy's peers, who expressed concern that Marcy had been portrayed in too positive a light—that Marcy resigned. In another instance, the University of Southern California (USC) reportedly sat on complaints of Keck School of Medicine Dean Carmen Puliafito's illegal drug use and mistreatment of colleagues for at least a year before news reports about the accusations forced his resignation.

Part of the reason why universities are not eager to remove high-ranking professors has to do with maintaining the ability of their faculty to secure grants. "The first thought is always to protect the brand," says Arthur Caplan, a professor of ethics and bioethics at the New York University's Langone Medical Center. "As federal and state dollars dry up and grant money slows, schools need to and strive to protect and enhance their reputations, making the management of misbehaving and exploitative faculty members sensitive to the financial woes of institutions."

William Kidder, a research associate at the Civil Rights Project and himself a university administrator, emphasizes that tenure has a valuable role in protecting academic freedom. But there should be no distinctions between how tenured versus nontenured faculty are treated when someone complains about their behavior, he says. "The same standards should apply whether it's to a rock star professor or a first-year assistant professor." Indeed, most state laws, university faculty contracts, and university or college faculty handbooks contain a clause that lists fraud, misrepresentation, plagiarism, and moral turpitude as justifiable reasons for termination, regardless of tenure status.

"The bottom line," says Caplan, "is that even if professors are serving noble goals like fighting cancer, we still have to be tough" when it comes to disciplining misconduct.

Jumping ship

Even if universities choose to dismiss a faculty member following some transgression, all too often the allegations and proceedings remain confidential. For example, the Title IX law passed in 1972 requires that each institution keep a private record of all gender-bias allegations and cases, but only the statistics from each university are publicly available, while individual cases are typically not shared. This raises the risk that the inappropriate actions will continue at another institution.

"I've been involved in instances of professors sleeping with undergraduate students, throwing their names on academic publications that they didn't write, and had illegiti-

mate personal budget expenses, and nothing happened to them," Caplan tells *The Scientist*. "They got passed along, hired by other institutions and repeated the behavior."

This phenomenon of faculty sexual harassers moving from one college campus to another is what Kidder calls "pass the harasser." "In the current environment of expensive litigation and very long time periods to complete a full faculty disciplinary process, an individual campus may accurately conclude that a confidential separation agreement with the professor is in the best interest of the college," says Kidder. Yet that decision may not be aligned with the collective good of the academic community, he adds, especially if the faculty member gets a job at another campus that does not know about the prior misconduct.

Failing to keep records of allegations, or keeping them locked up and secret, is wrong, agrees Vinick. "Schools cannot take a position on the case if it is unresolved, but the administrators could disclose the facts of the complaint, and they could certainly disclose when there have been multiple complaints against a faculty member," she says. "By not doing that, institutions allow these professors to move from one institution to another and their careers are no worse for it."

And even if there is disclosure that a prospective faculty member has been found to have violated a university policy, a new school may also be "willing to gamble to get a high-visibility faculty member despite being warned about his or her irresponsible behavior," says Caplan. One prominent example is that of Thomas Pogge, professor of philosophy and ethics at Yale, who has had several complaints of sexual harassment against him both at Yale and at his prior institution, Columbia University. "I think investigators with lots of grants or who bring fame and visibility can slither their way through the current system," Caplan says.

Addressing the problem

To avoid bringing on faculty with a track record of wrongdoing, universities have begun to more thoroughly screen prospective hires. Although formal background checks of criminal and public records are still relatively uncommon for all but the higher echelons of schools' administrative positions, more schools are beginning to require them for all levels, says Terry Leap, a professor in the department of management at the University of Tennessee who studies white-collar crime.

The chance that a prospective faculty member is found to have a criminal record is minuscule, however. "Doing a formal background check of public records basically shows that the school made a good effort that could later negate the chances that someone files a hiring suit against the university. But the background check itself is not likely to uncover anything," says Leap. In fact, since 2004, the American Association of University Professors has recommended that criminal background checks not be performed for all new hires, arguing that such intrusive measures are "out of proportion to the actual problems facing the academy."

More telling of a professor's character may be the letters of recommendation from colleagues, though this requires reading between the lines, says Caplan. There is a practice of not writing anything negative, he explains, but letters that are only lukewarm hint at hidden issues. Going forward, this is a practice Caplan would like to see change. "I think this culture of only orally communicating negative comments but not in letters is wrong, and frankly, unprofessional, because the letters are confidential and we want individuals who are well-vetted."

When it comes to sexual harassment, schools have also stepped up their game, with many adopting a zero-tolerance policy. "In recent years, sexual harassment complaints are a hot-button item, which institutions of higher learning are acting swiftly and decisively to eliminate," says Leap. "The adverse media publicity and potential monetary liability pose too great a risk to simply sweep the matter under the rug."

Still, Leap acknowledges that the term "zero tolerance" is usually poorly defined. "It can't be a 'no questions asked'—you're expelled if accused—because that is unfair and too harsh," he says. "What it actually means in most cases is that the university takes acts of sexual harassment seriously, and the institution will take immediate and decisive action to investigate, adjudicate, and punish offenders." In addition, faculty found guilty of sexual harassment are often required to undergo training on how to interact with students, says Michael Olivas of the University of Houston Law Center.

I think investigators with lots of grants or who bring fame and visibility can slither their way through the current system.

—Arthur Caplan, New York University

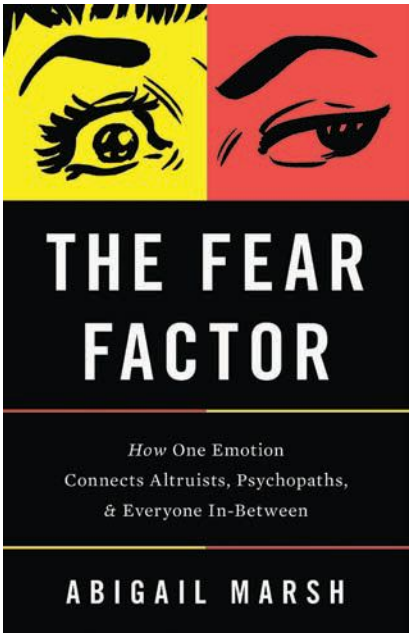
Some institutions are taking strides to prevent such incidents in the first place. At the University of California, Berkeley, for example, new university-wide procedures outline how to handle alleged sexual misconduct by faculty and staff. Harassment seminars are another option, and Lewis says his trainings are now frequently requested by faculty. "To me, that is a canary in the coal mine of how faculty and campuses are feeling about these issues," he says. "This is not the administrators thrusting this training upon their faculty—this is the faculty asking for the training—which shows the faculty are interested, and that is a changing tide."

Meanwhile, to curb scientific misconduct, earlier this year the National Academies of Science, Engineering, and Math committee on research integrity recommended the formation of an independent, nonprofit advisory board to evaluate potential foul play in the lab, so that universities aren't left to internally handle accusations against faculty. But there's a long way to go on this front, says Caplan. "[The scientific research community] has not yet found effective programs to teach research integrity and how to avoid misconduct despite the realization that these are growing threats to the integrity of medicine, science, the social sciences, and the humanities." ■

The Benefits of Trepidation

While wiping fear from our brains may seem attractive, the emotion is an essential part of our behavioral repertoire.

BY ABIGAIL MARSH



Basic Books, October 2017

Wouldn't a life without fear be lovely?

It might seem that way. Intense fear not only feels exceedingly unpleasant, it can, in its extreme forms, disrupt life. The one in five American adults who are affected by anxiety disorders such as phobias and posttraumatic stress disorder might feel that ridding themselves of fear entirely would be a blessing.

But there is something much worse than too much fear—too little of it. For fear is an emotion with deep and vital benefits, not only for the people experiencing it, but for those around them.

Fear's most obvious advantage is that it motivates escape in the face of danger—or the avoidance of danger in the first place. Without fear, basic urges for self-preservation evaporate.

Neuroscientists have learned this from studying people in whom injury or disease has damaged the amygdala, a critical hub of the brain's fear network. These patients experience lives peppered with danger and trauma, much of it avoidable but not avoided. In one such patient, known only as SM, a congenital condition completely destroyed her amygdala and, with it, her capacity for fear. Although her intellect and reason are intact, she cannot detect or learn to avoid dangerous environments, and she has repeatedly found herself in life-threatening situations. SM has been held up at gunpoint and knifepoint, the latter by an obviously threatening stranger she approached, and has been the victim of multiple assaults. She has had to be stopped from touching poisonous snakes and spiders by the researchers who study her. Her son reports that when he was young, SM spotted a massive snake spanning the road near their house. Her response? To race *toward* the snake, pick it up, then set it loose in her yard.

Fear carries less obvious but no less significant social benefits as well, such as the ability to empathize with others' fear. This is because interpreting others' emotions involves the same machinery used to experience those emotions. The sight of another person's fear—the wide eyes and raised and contorted brows of a fearful facial expression, for example—normally sparks activation in the amygdala and other brain structures that together may enable the viewer to internally simulate that state. As my lab's research has demonstrated, the ability to empathize with others' fear may motivate altruism, including extraordinary acts such as donating a kidney to a stranger. By contrast, not only are SM and others like her unable to experience fear normally, they also have difficulty understanding others' fear. Without functioning amygdalas, they are reliably stumped when asked to interpret the meaning of even a clearly frightened-looking face. In this sense, they can be said to lack a fundamental form of empathy.

A similar problem seems to lie at the heart of a psychological disorder characterized by catastrophic empathy deficits: psychopathy. People with psychopathic traits are set apart even from other aggressive or antisocial people by their lack of remorse, compassion, and empathy. A critical clue to the cause of these traits has emerged from behavioral research: psychopaths—just like SM—have significant and specific impairments in recognizing fear in others, which brain imaging studies reveal are underpinned by amygdala dysfunction. Findings from my laboratory show that the weak amygdala response to others' fear consistently observed in psychopathic adolescents and adults may serve as a biomarker of sorts for the kinds of goal-directed, cold-hearted aggression for which psychopaths are notorious. These findings suggest that amygdala dysfunction leaves such people struggling to recognize when others are afraid—and keeps them from really even understanding what being afraid feels like. This impairment leaves them unmoved by the prospect of threatening or hurting people, or engaging in other behaviors that cause fear.

It seems that Franklin Delano Roosevelt was wrong, then, when he said, "The only thing we have to fear is fear itself." Deployed appropriately, fear can be a vital tool and guide in both the physical world and the social one.

What we need really to fear is those who lack this guide entirely. ■

Abigail Marsh is an associate professor of psychology and neuroscience at Georgetown University. She directs its prize-winning Laboratory on Social and Affective Neuroscience. Read an excerpt of The Fear Factor: How One Emotion Connects Altruists, Psychopaths, and Everyone In-Between *at the-scientist.com.*

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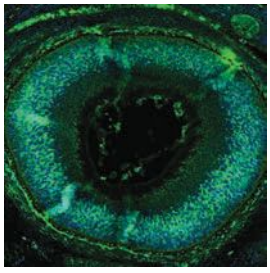

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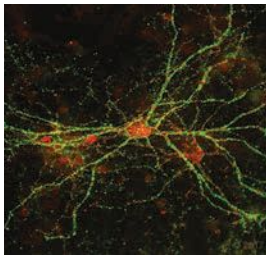

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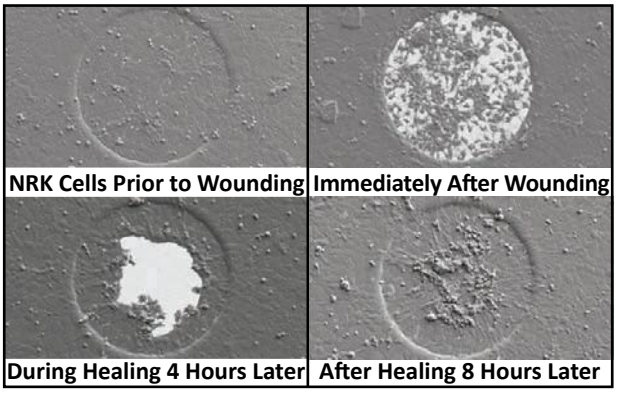

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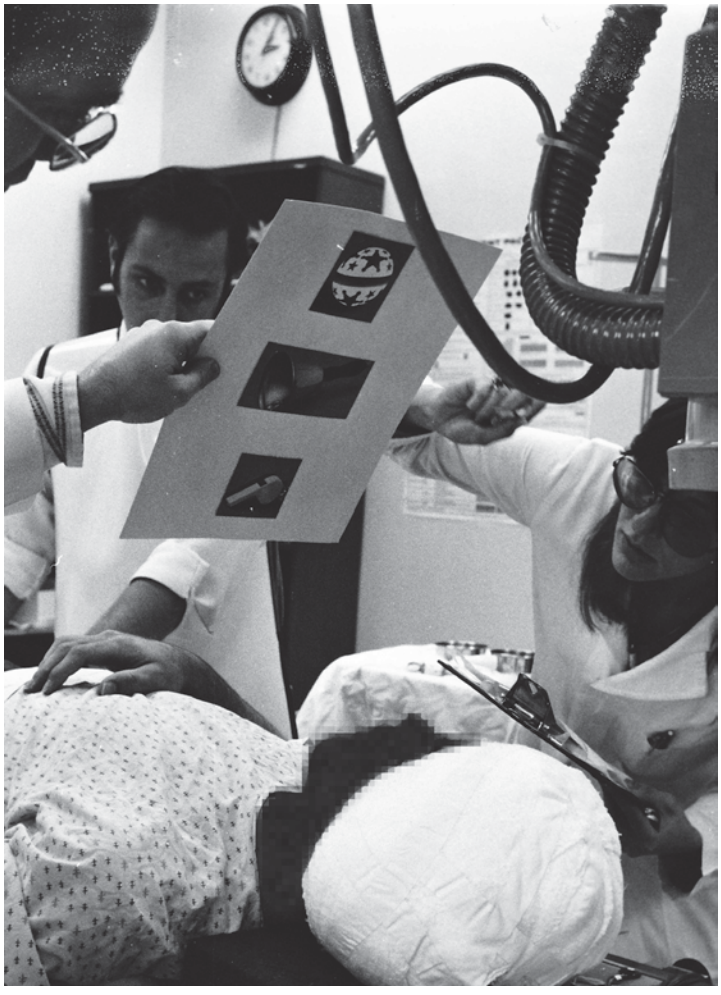
The Wada Test, 1948

BY PHILIP JAEKL

As a medical student on the Japanese island of Hokkaido in the 1940s, Juhn Wada developed an interest in neurology, before "neurology" was formally a word and before dedicated departments existed anywhere in Japan. After completing his degree, Wada began researching electroconvulsive ("shock") therapy, which works by inducing seizures—abnormal electrical activity in the brain. At that time, the procedure was becoming common for patients with severe depression or schizophrenia. The shocks, however, also caused language and memory impairments. Based on existing evidence showing that linguistic and, to a lesser extent, memory functioning predominantly take place in a single brain hemisphere, Wada proposed anesthetizing that hemisphere to avoid causing the impairments.



LANGUAGE AND MEMORY ASSESSMENT: Rebecca Rausch (far right), now a senior neurologist at the University of California, Los Angeles, is pictured here performing a modified version of the Wada test in the early 1970s. The patient is a candidate for epilepsy surgery to remove tissue from the brain hemisphere causing seizures. The test is required to determine if brain areas that underlie language and memory also reside in the same side of the brain. During the test, the patient names and recalls objects shown to him while an anesthetic—usually sodium amobarbital—is introduced to each hemisphere in turn. If linguistic deficits coincide with the anesthetization of the seizure-causing area, surgery will not be performed.

His proposed approach was highly criticized until 1948, when Wada learned of a patient who had developed persistent, uncontrollable seizures—a young cook at an American base camp who had been shot in the head by a drunken soldier attempting to shoot off his hat. As a last resort, the cook agreed to let Wada anesthetize his brain, one hemisphere at a time. Upon the injection of an anesthetic barbiturate into an artery known to deliver blood only to the left hemisphere, the man's seizures were successfully controlled. But the side effects of the barbiturate treatment were startling and immediately apparent. According to his written account, chills ran up and down Wada's spine as the patient temporarily lost all language ability and motor function on the right side of his body.

Wada eventually moved to McGill University in Montreal, Canada, where he further developed the procedure into what is now commonly known as the Wada test. After he published the details of the technique in 1960, it became widely used to localize linguistic and memory function in epilepsy patients. Such testing is critical because not all patients use the left brain hemisphere for these functions, as the cook did. Surgery to treat seizures is to be avoided if the region causing seizures is in the same hemisphere that controls language and memory. Remarkably, this remains the routine method of language and memory lateralization assessment in presurgical epilepsy patients to this day.

"Over half a century, thousands upon thousands of patients have benefited from invaluable information that had been only obtainable from the Wada test," Rebecca Rausch, a senior neurologist at the University of California, Los Angeles, tells *The Scientist* in an email.

Noninvasive alternatives are now coming to the fore, however. Just this year, the first set of detailed fMRI guidelines for language and memory assessment in epilepsy patients was published in *Neurology* (88:395-402). Although the Wada test remains the gold standard for the localization of language and memory, this publication likely initiates a switchover to using noninvasive fMRI methods for epilepsy patients scheduled for surgery. ■

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