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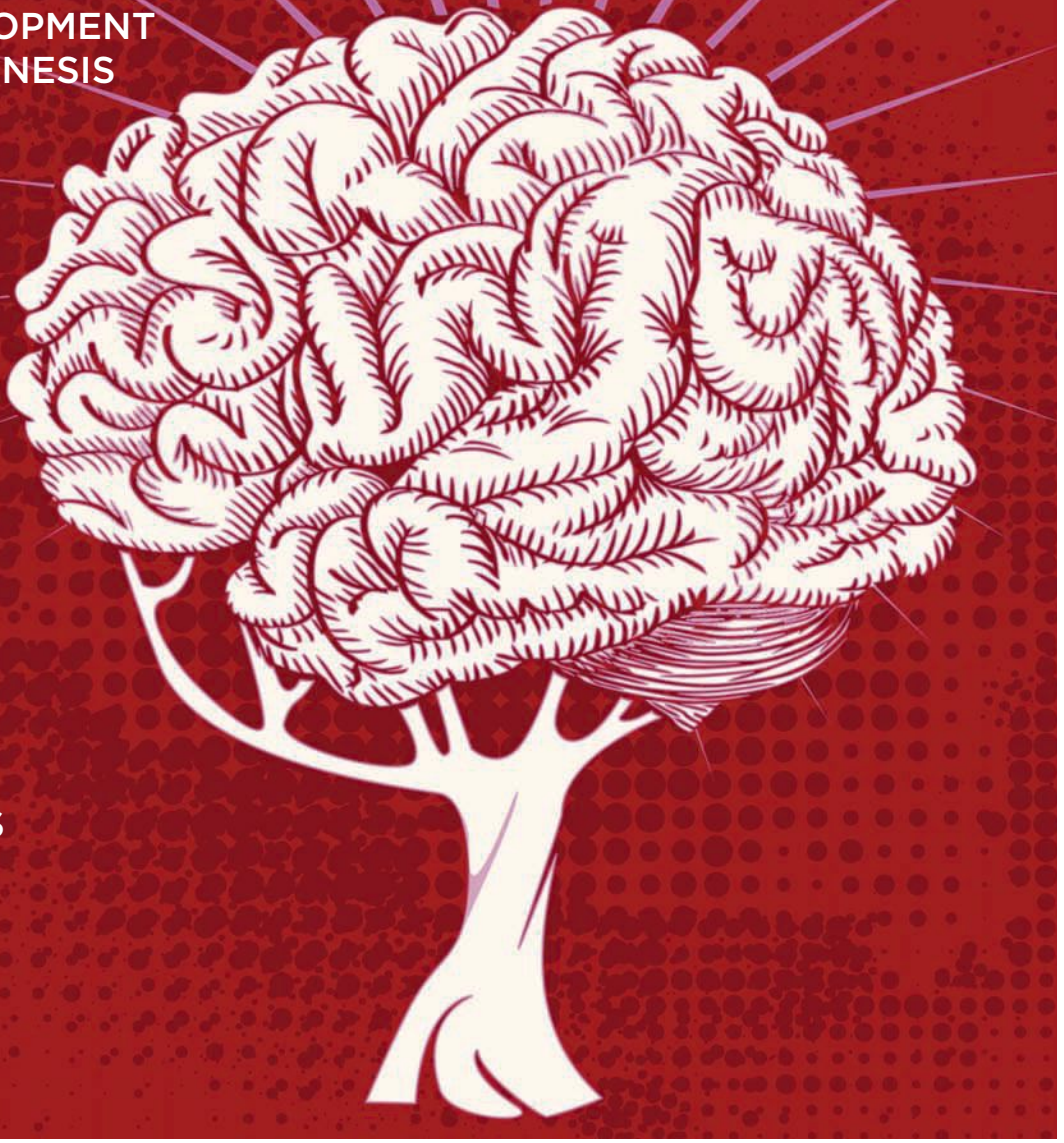
EXPLORING LIFE, INSPIRING INNOVATION

DYNAMIC BRAINS

FROM FETAL DEVELOPMENT TO ADULT NEUROGENESIS



SEX DIFFERENCES IN THE BRAIN

SPOTLIGHT ON GLIAL CELLS

MICROMANAGING NEURAL NETWORKS

PLUS

CULTURING ELUSIVE BACTERIA

Brain Gain

Young neurons in the adult human brain are likely critical to its function.

BY JEF AKST

At a lab meeting of Fred "Rusty" Gage's group at the Salk Institute for Biological Studies in the mid-1990s, the neuroscientist told his team that he wanted to determine whether new neurons are produced in the brains of adult humans. At the time, adult neurogenesis was well established in rodents, and there had been hints that primate brains also spawned new neurons later in life. But reports of neurogenesis in the adult human brain were sparse and had not been replicated. Moreover, the experiments had relied primarily on autoradiography, which revealed images of cell division but did not follow the fate of new cells, so researchers couldn't be sure if they really became mature neurons.

Gage's group, which included clinicians, was familiar with the use of bromodeoxyuridine (BrdU) to monitor the progression of certain cancers. BrdU is an artificial nucleoside that can stand in for thymidine (T) during DNA replication. As cells duplicate their genomes just before they divide, they incorporate BrdU into their DNA. To assess tumor growth, physicians inject the nucleoside substitute into a patient's bloodstream, then biopsy the tumor and use an antibody to stain for BrdU. The number of BrdU-labeled cells relative to the total number of cells provides an estimate of how quickly the cancer is growing. "If that nucleotide is labeled in such a way that [we] can identify it, you can birthdate individual cells," Gage says.

Because BrdU goes everywhere in the body, Gage and his colleagues figured that in addition to labeling the patients' tumors, the artificial base would also label the cells of the brain. If the researchers could get their hands on brain specimens from patients who'd been injected with BrdU, perhaps it would be possible to see new brain cells that had been generated in adults. With a second antibody, they could then screen for cell-type markers to determine if the new cells were mature neurons. "If you can . . . [use] a second antibody to identify the fate of a cell, then that's pretty definitive," Gage says.

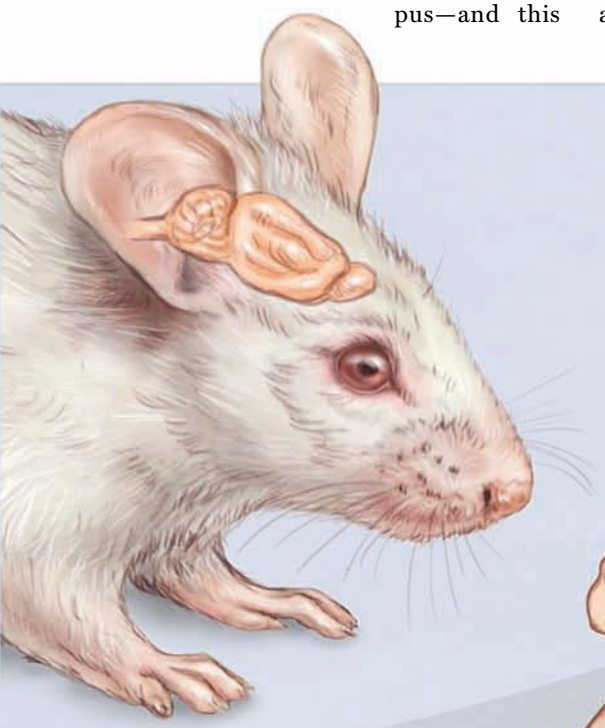
As Gage's fellows and postdocs left the lab and got involved in clinical trials involving BrdU injections, they began to keep an eye out for postmortem brain samples that Gage could examine. In 1996, one of them came through. Neurologist Peter Eriksson, who at the time was working at the Sahlgrenska University Hospital in Gothenburg, Sweden, began sending Gage samples from the brains of deceased patients. Every few months, a new sample arrived. And while waiting for the next delivery, Gage and his team were "getting fresh tissue from the coroner's office to practice staining fresh tissue," he says, "so that when we got these valuable brains we could see [what] they were doing."

Soon enough, a clear picture emerged: the human hippocampus, a brain area critical to learning and memory and often the first region damaged in Alzheimer's patients, showed evidence of adult neurogenesis. Gage's collaborators in Sweden were getting the same results. Wanting to be absolutely positive, Gage even sent slides to other labs to analyze. In November 1998, the group published its findings, which were featured on the cover of *Nature Medicine*.[1]

"When it came out, it caught the fancy of the public as well as the scientific community," Gage says. "It had a big impact, because it really confirmed [neurogenesis occurs] in humans."

Fifteen years later, in 2013, the field got its second (and only other) documentation of new neurons being born in the adult human hippocampus—and this time learned that neurogenesis may continue for most of one's life.[2] Neuroscientist Jonas Frisén of the Karolinksa Institute in Stockholm and his colleagues took advantage of the aboveground nuclear bomb tests carried out by US, UK, and Soviet forces during the Cold War. Atmospheric levels of ^{14}C have been declining at a known rate since such testing was banned in 1963, and Frisén's group was able to date the birth of neurons in the brains of deceased patients by measuring the amount of ^{14}C in the cells' DNA.

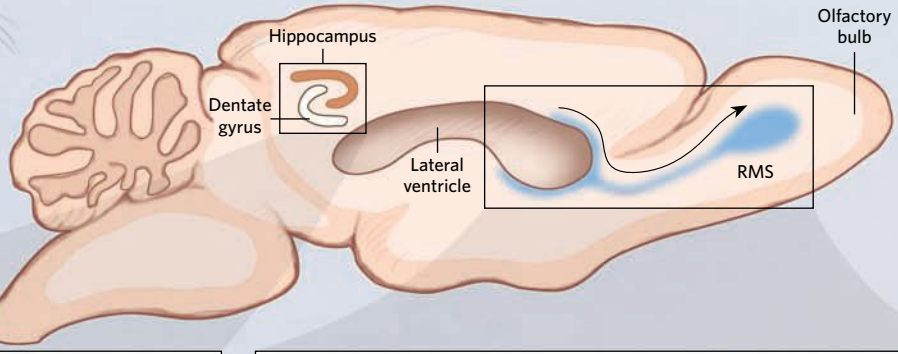
"What we found was that there was surprisingly much neurogenesis in adult humans," Frisén says—a level comparable to that of a middle-aged mouse, the species in which the vast majority of adult neurogenesis research is done. "There is hippocampal neurogenesis throughout life in humans."

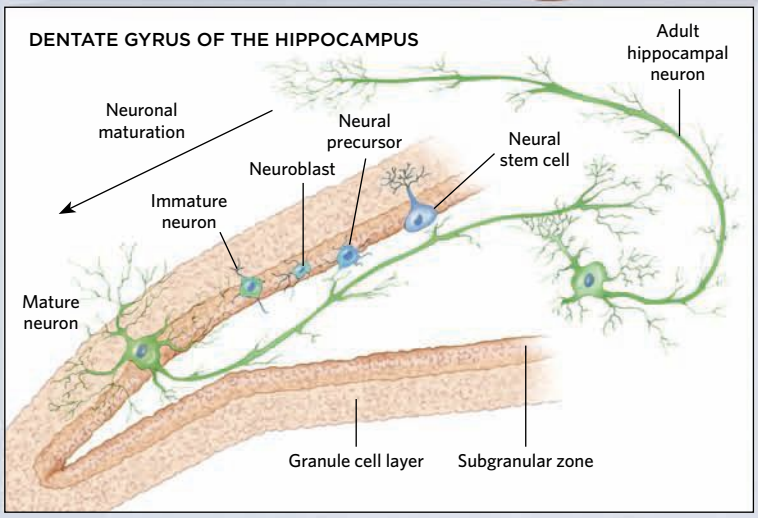


NEUROGENESIS IN THE ADULT MAMMALIAN BRAIN

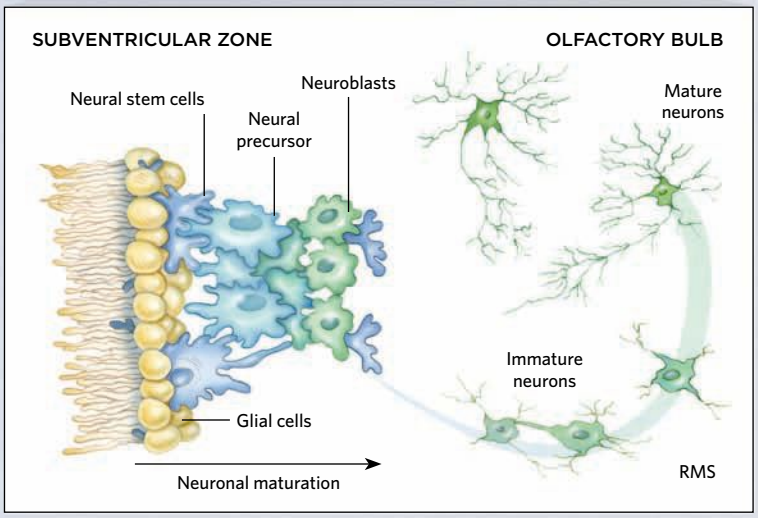
RODENTS

In rodents, there are two populations of neural stem cells in the adult brain. The majority of new neurons are born in the subventricular zone along the lateral ventricle wall and migrate through the rostral migratory stream (RMS) to the olfactory bulb. About one-tenth as many new neurons are produced in the subgranular zone of the dentate gyrus (white) of the hippocampus.





In the dentate gyrus, neural stem cells differentiate into neuroblasts before maturing and integrating with hippocampal circuits important in learning and memory.



In the subventricular zone, neural stem cells differentiate into neuroblasts, which make their way to the olfactory bulb, where they complete their development.

But many details remain unclear. How do newly generated neurons in adults influence brain function? Do disruptions to hippocampal neurogenesis play roles in cognitive dysfunction, mood disorders, or even psychosis? Are there ways to increase levels of neurogenesis in humans, and might doing so be therapeutic? Researchers are now seeking to answer these and other questions, while documenting the extent and function of adult neurogenesis in mammals.

Breaking the mold

In the early 1960s, MIT neurobiologist Joseph Altman used a hypodermic needle to induce lesions in rat brains, while simultaneously injecting tritiated thymidine, a radioactive form of the nucleoside commonly used for tracking DNA synthesis and cell proliferation. He found evidence of new brain cells that had been born at the time of injection, including some neurons and their neuroblast precursors.[3]

Researchers were immediately skeptical of the results. Long-standing theory held that neurons in the brain that had been damaged or lost could not be replaced; Altman was suggesting the opposite. "They were really good, solid indications, but it was such a strong dogma that neurons couldn't be generated in the adult brain," says Frisén. "It wasn't really until the '90s, when new techniques came along, [that researchers] showed, yes, indeed, new neurons are added in the rodent brain."

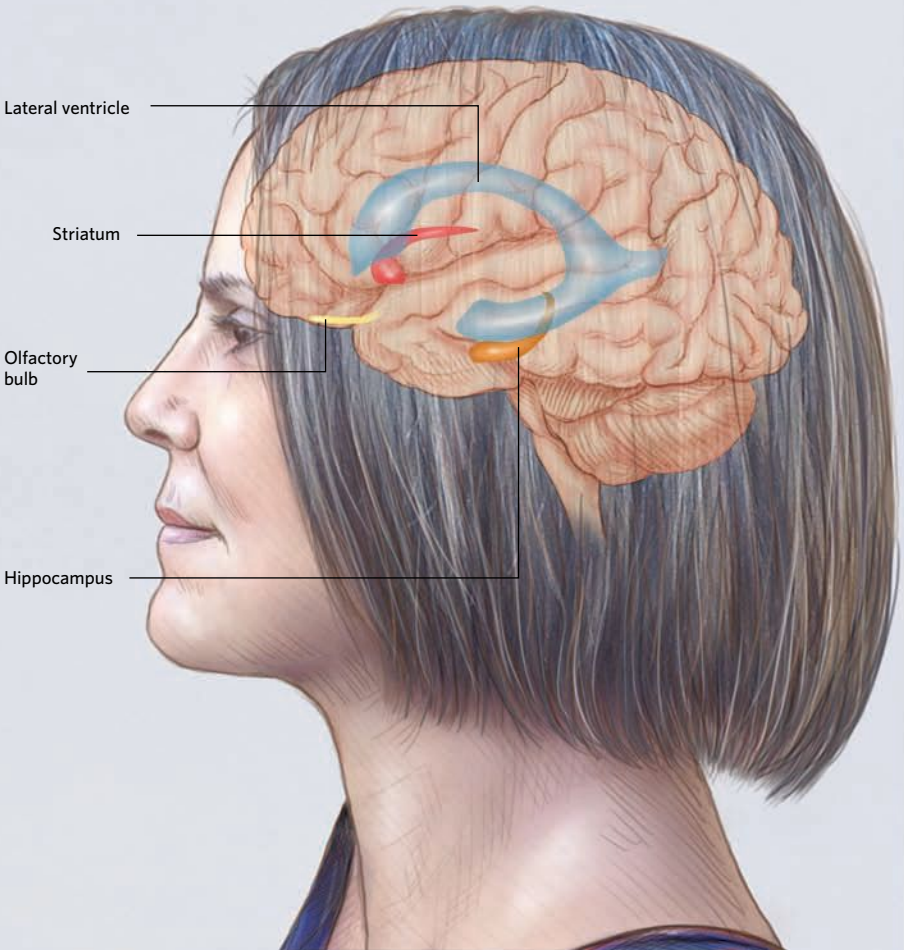
Those new techniques included BrdU, as well as neuron-specific protein markers and confocal imaging, which together enabled researchers to identify the newly generated cells. Multiple studies subsequently confirmed that neurogenesis occurs in limited regions of the rodent brain, specifically in the olfactory bulb and the dentate gyrus region of the hippocampus. (See illustration on opposite page.) Research also revealed that the rate of neurogenesis decreases with stress, depression, and anxiety, but increases with exercise and enrichment.

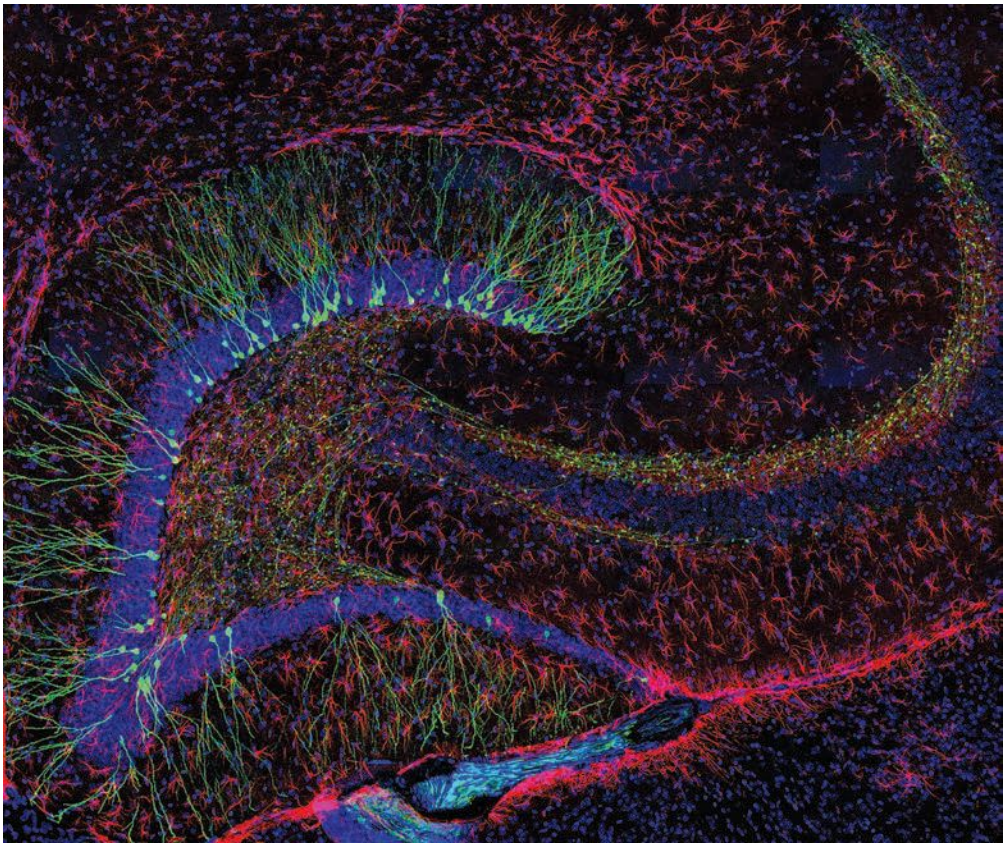
"The field grew enormously at this point," Gage says, and its focus began to shift from whether new neurons were being produced—they were—to whether those cells formed connections with existing networks to become functional—they do. Turns out, "these newly born cells have 5,000 synapses on their dendrites," Gage says—well within the range of other neurons in the brain.

But would those rodent results hold up in primates? All signs pointed to yes. In March 1998, Princeton University's Elizabeth Gould and colleagues found evidence of neurogenesis in the dentate gyrus of adult marmoset monkeys—and the researchers determined that the rate of cell proliferation was affected by stress, just as in rodents.[4] Six months later, Gage's group published its findings based on the clinical samples of human brain tissue. "It was a surprise to me, and I think to most

HUMANS

Researchers have also demonstrated that neurogenesis occurs in the adult human brain, though the locations and degree of cell proliferation appear to differ somewhat from rodents. Strong evidence now exists that new neurons are born in the dentate gyrus of the hippocampus, where they integrate into existing circuits. But so far, there is no definitive support for the migration of new neurons migrating from the subventricular zone (SVZ) of the lateral ventricle to the olfactory bulb, which is atrophied relative to the olfactory bulb of rodents and other mammals that rely more heavily on smell. However, one study did report signs of neurogenesis in an area next to the SVZ, the striatum, which is important for cognitive function and motor control.





They'll fire at anything, because they're young, they're uninhibited, and they're integrating into the circuit.

—Fred Gage,
Salk Institute for Biological Studies

people," Frisén says. And the point was hammered home with the Frisén group's analysis of ^{14}C in human brain samples.

"The human evidence now unequivocally suggests that the dentate gyrus in humans undergoes turnover in our lifetime," says Amar Sahay of Harvard University. "It really begs the question what the functions are of these adult-born neurons."

Young and excitable

The first step in understanding the function of the new neurons in the adult brain was to characterize the cells themselves. In the late 1990s and early 2000s, researchers delved into the cell biology of neurogenesis, characterizing the populations of stem cells that give rise to the new neurons and the factors that dictate the differentiation of the cells. They also documented significant differences in the behavior of young and old neurons in the rodent brain. Most notably, young neurons are a lot more active than the cells of established hippocampal networks, which are largely inhibited.[5,6]

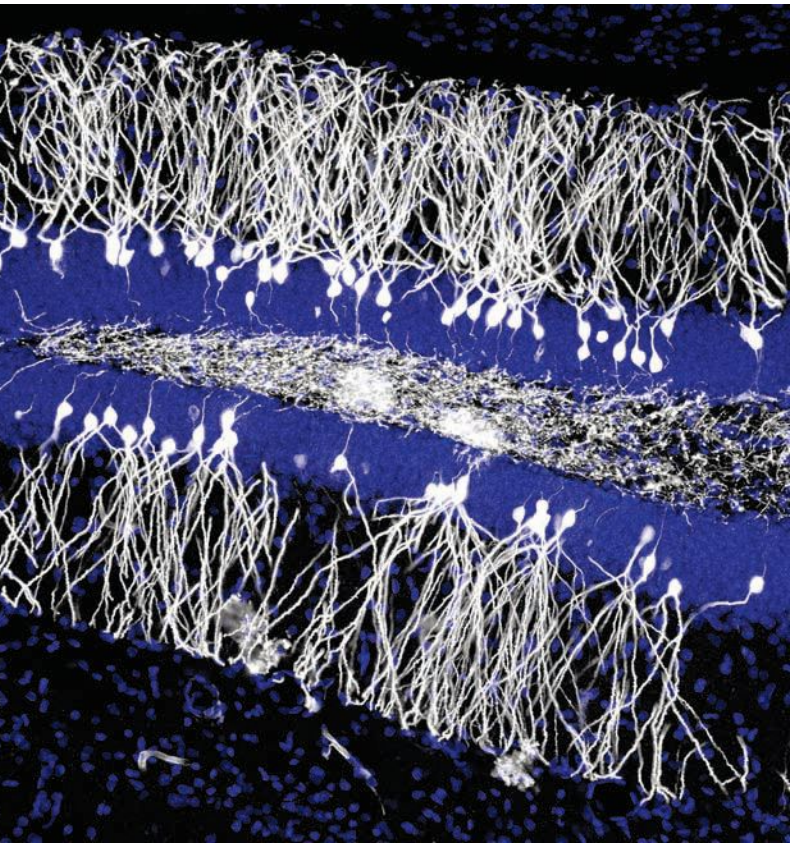
"For a period of about four or five weeks, while [the newborn neurons] are maturing, they're hyperexcitable," says Gage. "They'll fire at anything, because they're young, they're uninhibited, and they're integrating into the circuit."

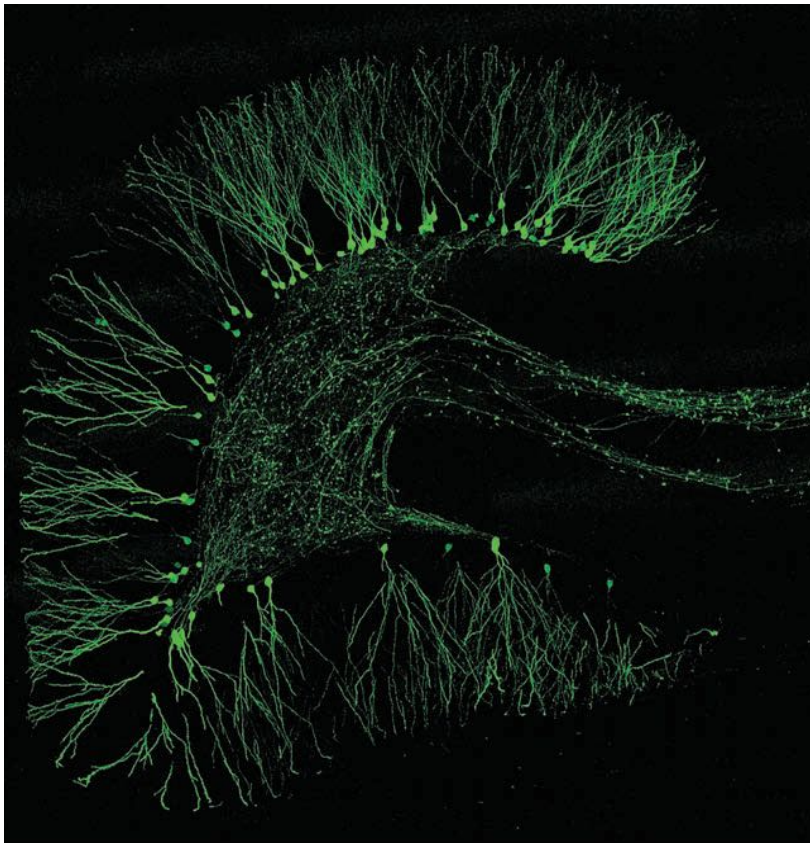
To determine the functional role of the new, hyperactive neurons, researchers began inhibiting or promoting adult neurogenesis in rodents by various means, then testing the animals' performance in various cognitive tasks. What they found was fairly consistent: the young neurons seemed to play a role in processing new stimuli and in distinguishing them from prior experiences. For example, if a mouse is placed in a new cage and given time to roam, then subjected to a mild shock, it will freeze for about 40 seconds the next time it is placed in that same environment, in anticipation of a shock. It has no such reaction to a second novel environment. But in an enclosure that has some features in common with the first, fear-inducing cage, the mouse freezes for 20 seconds before seemingly surmising that this is not the cage where it received the initial shock. Knock out the mouse's ability to produce new neurons, however, and it will freeze for the full 40 seconds. The brain is not able to easily distinguish between the enclosures.

This type of assessment is called pattern separation. While some researchers quibble over the term, which is borrowed from computational neuroscience, most who study hippocampal neurogenesis agree that this is a primary role of new neurons in the adult brain. "While probably five or six different labs have been doing this over the last four or five years, basically everybody's come to the same conclusion," Gage says.

The basic idea is that, because young neurons are hyperexcitable and are still establishing their connectivity, they are amenable to incorporating information about the environment. If a mouse is

COURTESY OF FRED H. GAGE





NEWBORN PHOTOS: Three different micrographs show new neurons (left and right, green; middle, white) generated in the adult mouse brain. Neurogenesis occurs in the dentate gyrus, a V-shape structure within the hippocampus. Bushy dendritic processes extend into a relatively cell-free region called the molecular layer (black in right photo) and axons project to the CA3 region of the hippocampus (green "stem" in right photo). Astrocytes are stained pink (left).

JASON SNYDER; COURTESY OF FRED H. GAGE

placed in a new cage when young neurons are still growing and making connections, they may link up with the networks that encode a memory of the environment. Just a few months ago, researchers in Germany and Argentina published a mouse study demonstrating how, during a critical period of cellular maturation, new neurons' connections with the entorhinal cortex, the main interface between the hippocampus and the cortex, and with the medial septum change in response to an enriched environment.[7]

"The rate at which [new neurons] incorporate is dependent upon experience," Gage says. "It's amazing. It means that the new neurons are encoding things when they're young and hyperexcitable that they can use as feature detectors when they're mature. It's like development is happening all the time in your brain."

Adding support to the new neurons' role in pattern separation, Sahay presented findings at the 2014 Society for Neuroscience conference that neurogenesis spurs circuit changes known as global remapping, in which overlap between the populations of neurons that encode two different inputs is minimized.[8] "We have evidence now that enhancing neurogenesis does enhance global remapping in the dentate gyrus," says Sahay. "It is important because it demonstrates that stimulating neurogenesis is sufficient to improve this very basic encoding mechanism that allows us to keep similar memories separate."

Pattern separation is likely not the only role of new neurons in the adult hippocampus. Experiments that have suppressed neurogenesis in adult rats have revealed impairments in learning in a variety of other tasks. More broadly, "we think it has to do with the flexibility of learning," says Gerd Kempermann of the Center for Regenerative Therapies at the Dresden University of Technology in Germany.

Last year, for example, neuroscientist Paul Frankland of the Hospital for Sick Children in Toronto and his colleagues found evidence that newly generated neurons play a role in forgetting, with increased neurogenesis resulting in greater forgetfulness among mice.[9] "If you think about what you've done today, you can probably remember in a great deal of detail," he says. "But if you go back a week or if you go back a month, unless something extraordinary happened, you probably won't remember those everyday details. So there's a constant sort of wiping

of the slate." New hippocampal neurons may serve as the "wiper," he says, "cleaning out old information that, with time, becomes less relevant."

Conversely, Frankland's team found, suppressing neurogenesis seems to reinforce memories, making them difficult to unlearn. "We think that neurogenesis provides a way, a mechanism of living in the moment, if you like," he says. "It clears out old memories and helps form new memories."

Neurogenesis in the clinic

While studying the function of hippocampal neurogenesis in adult humans is logistically much more difficult than studying young neurons in mice, there is reason to believe that much of the rodent work may also apply to people—namely, that adult neurogenesis plays some role in learning and memory, says Kempermann. "Given that [the dentate gyrus] is so highly conserved and that the mechanisms of its function are so similar between the species—and given that neurogenesis is there in humans—I would predict that the general principle is the same."

And if it's true that hippocampal neurogenesis does contribute to aspects of learning involved in the contextualization of new information—an ability that is often impaired among people with neurodegenerative diseases—it's natural to wonder whether promoting neurogenesis could affect the course of Alzheimer's disease or other human brain disorders. Epidemiological studies have shown that people who lead an active life—known from animal models to increase neurogenesis—are at a reduced risk of developing dementia, and several studies have found reduced hippocampal neurogenesis in mouse models of Alzheimer's. But researchers have yet to definitively prove whether neurogenesis, or lack thereof, plays a direct role in neurodegenerative disease progression. It may be that neurogenesis has "nothing to do with the pathology itself, but [with] the ability of our brain to cope with it," says Kempermann.

Either way, the research suggests that "the identification of pro-neurogenic compounds would have a therapeutic impact on cognitive dysfunction, specifically, pattern separation alterations in aging and early stages of Alzheimer's disease," notes Harvard's Sahay. "There's a growing list of genes that encode secreted factors or other molecules that stimulate neurogenesis. Identifying compounds that harness these pathways—that's the challenge."

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>
> —Amar Sahay, Harvard University

The birth of new neurons in the adult hippocampus may also influence the development and progression of mood disorders. Several studies have suggested that reduced neurogenesis may be involved in depression, for instance, and have revealed evidence that antidepressants act, in part, by promoting neurogenesis in the hippocampus. When Columbia University's René Hen and colleagues short-circuited neurogenesis in mice, the animals no longer responded to the antidepressant fluoxetine.[10] "It was a very big surprise," Hen says. "The hippocampus has really been always thought of as critical for learning and memory, and it is, but we still don't understand well the connection to mood."

Adult neurogenesis has also been linked to post-traumatic stress disorder (PTSD). While it is perhaps less obvious how young neurons might influence the expression of fear, Sahay says it makes complete sense, given the emerging importance of neurogenesis in distinguishing among similar experiences. "In a way, the hippocampus acts as a gate," he says, with connections to the amygdala, which is important for processing fear, and the hypothalamus, which triggers the production of stress hormones, among other brain regions. "It determines when [these] other parts of the brain should be brought online." If new neurons are not being formed in the hippocampus, a person suffering from PTSD may be less able to distinguish a new experience from the traumatic one that is at the root of his disorder, Sahay and his colleagues proposed earlier this year.[11] "We think neurogenesis affects the contextual processing, which then dictates the recruitment of stress and fear circuits."

Of course, the big question is whether researchers might one day be able to harness neurogenesis in a therapeutic capacity. Some scientists, such as Hongjun Song of Johns Hopkins School of Medicine, say yes. "I think the field is moving toward [that]," he says. "[Neurogenesis] is not something de novo that we don't have at all—that [would be] much harder. Here, we know it happens; we just need to enhance it." ■

References

1. P.S. Eriksson et al., "Neurogenesis in the adult human hippocampus," *Nat Med*, 4:1313-17, 1998.
2. K.L. Spalding et al., "Dynamics of hippocampal neurogenesis in adult humans," *Cell*, 153:1219-27, 2013.
3. J. Altman, "Are new neurons formed in the brains of adult mammals?" *Science*, 135:1127-28, 1962.
4. E. Gould et al., "Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress," *PNAS*, 95:3168 -71, 1998.
5. C. Schmidt-Hieber et al., "Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus," *Nature*, 429:184-87, 2004.
6. S. Ge et al., "A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain," *Neuron*, 54:559-66, 2007.
7. M. Bergami et al., "A critical period for experience-dependent remodeling of adult-born neuron connectivity," *Neuron*, 85:710-17, 2015.
8. K. McAvoy et al., "Rejuvenating the dentate gyrus with stage-specific expansion of adult-born neurons to enhance memory precision in adulthood and aging," Soc Neurosci, Abstract DP09.08/DP8, 2014.
9. K.G. Akers et al., "Hippocampal neurogenesis regulates forgetting during adulthood and infancy," *Science*, 344:598-602, 2014.
10. L. Santarelli et al., "Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants," *Science*, 301:805-09, 2003.
11. A. Besnard, A. Sahay, "Adult hippocampal neurogenesis, fear generalization, and stress," *Neuropsychopharmacology*, doi:10.1038/npp.2015.167, 2015.

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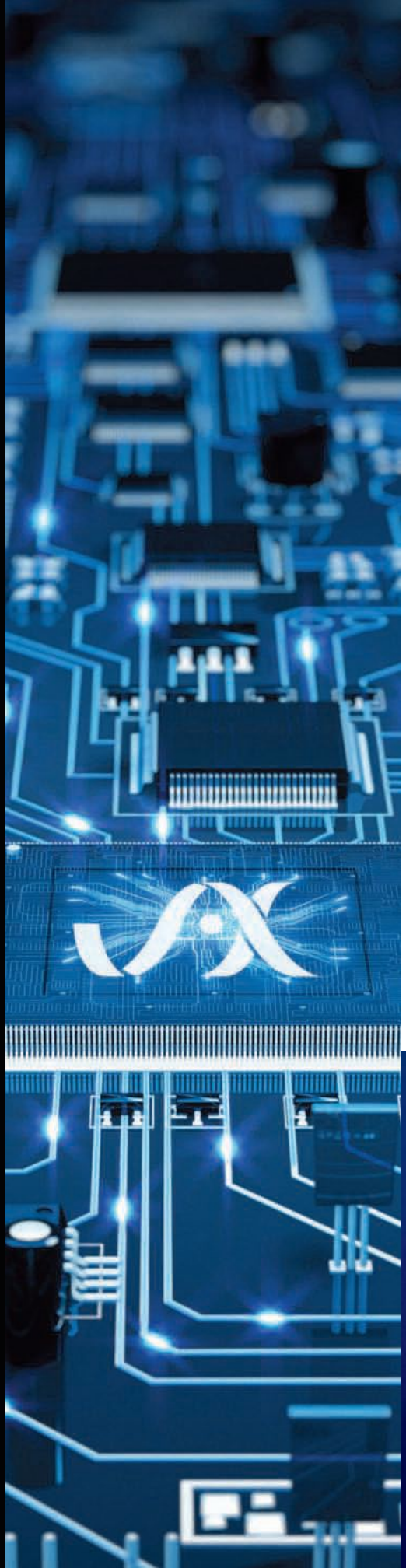

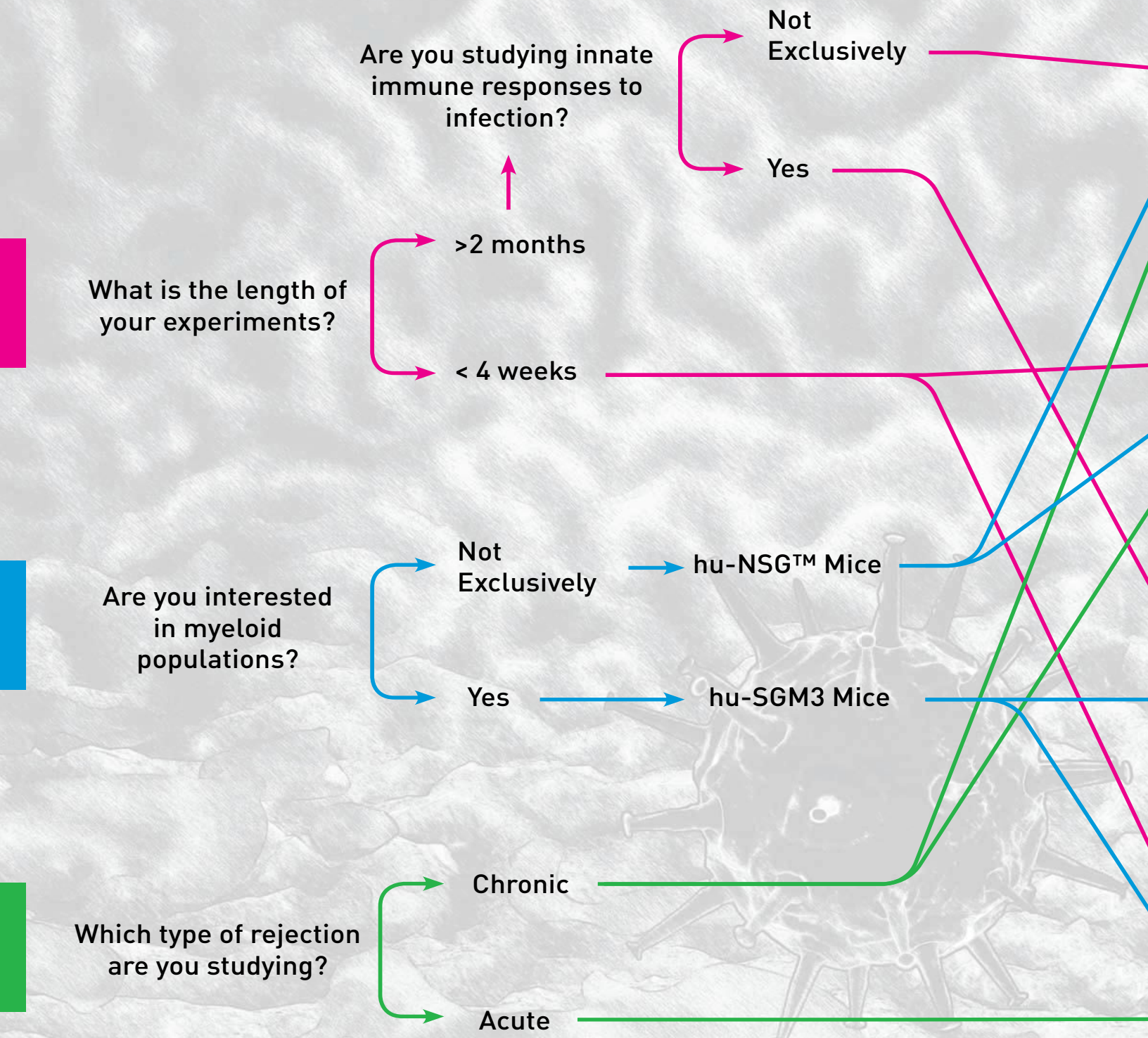
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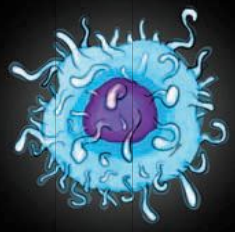
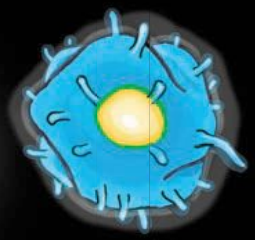
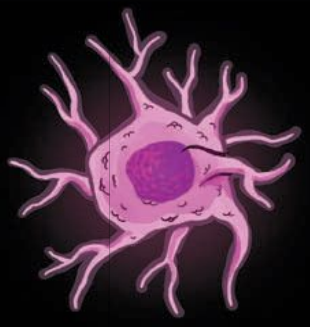


B and T cells (MHC-restricted CD4 and CD8)



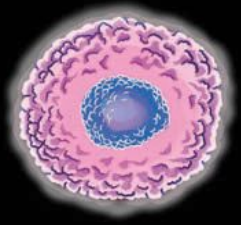
Monocytes

Macrophages

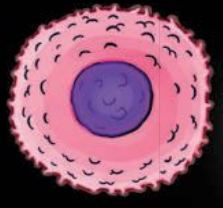


Dendritic cells

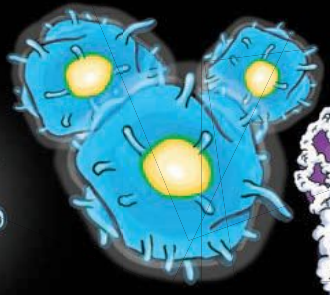
T cells (HLA-restricted CD4 and CD8)



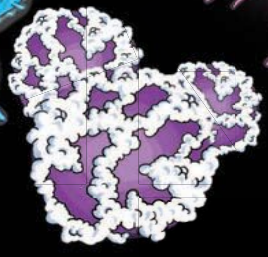
Natural Killer cells



B and T cells (MHC-restricted CD4 and CD8)



Monocytes



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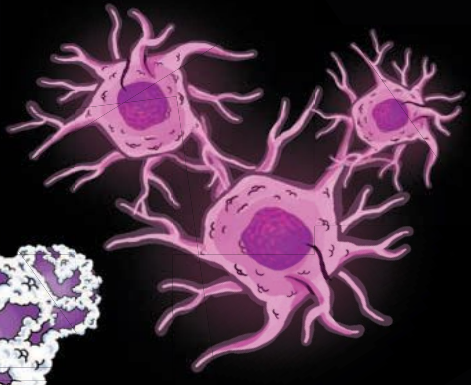

Dendritic cells

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HUMAN CYTOKINE EXPRESSION	N/A	N/A	IL3, GM-CSF, CSF	IL3, GM-CSF, CSF
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Lost Colonies

Next-generation sequencing has identified scores of new microorganisms, but getting even abundant bacterial species to grow in the lab has proven challenging.

BY ANNA AZVOLINSKY

TAMING THE BEAST: Colored scanning electron micrograph of a segmented filamentous bacterium (SFB, orange) reaching up from a bed of mouse intestinal cells (green). SFB was successfully cultured for the first time this year, a half a century after it was first discovered.

COURTESY OF PAMELA SCHNUPF, *NATURE*, 520:99-103, 2015

In 2001, Nicole Dubilier, a marine biologist at the Max Planck Institute for Marine Microbiology in Bremen, Germany, made a surprising discovery—two symbiotic bacterial species living inside a gutless marine worm, *Olavius algarvensis*. To better understand the unique relationship among the three species, Dubilier set out to culture the two symbionts. But nearly 15 years later, she has yet to successfully grow the bacteria in the lab.

Her best attempt kept the microbes alive for about 10 months, Dubilier says, but then the culture "just died on us. . . . It's a kamikaze project. How long can you have someone put in all their effort if it's constantly unsuccessful?"

Dubilier is hardly alone in her plight. A heaping teaspoon of soil or a shot of ocean water may contain as many as one million bacterial species. Many of them are potential gold mines of chemicals and metabolites with medicinal, engineering, and energy applications. But when researchers have tried to culture these microbes in the lab, only a minority of cells form colonies. Clearly, nutrients, a carbon source, and time are usually not enough to coax bacteria isolated from the wild to grow in a laboratory setting. So what's the missing ingredient?

"It's a significant intellectual teaser," says Slava Epstein, a microbial ecologist at Northeastern University in Boston, "why, after 150 years of the sweat and blood of smart, talented people, we can cultivate only a small proportion of microbes."

In 1873, Joseph Lister first introduced serial limiting dilution of bacteria in liquid medium to achieve a pure culture. Then in the 1880s, Nobel laureate Robert Koch invented methods for growing pure bacterial cultures on solid media and his laboratory assistant, Julius Petri, created the round, flat, stackable plate that is still a fixture in microbiology labs. For almost as long, scientists have struggled to cultivate newly identified soil or marine microbes. "I wanted to trace the [history] of this phenomenon," says Epstein. "But the search became difficult because soon I was tracking references from an era where references were done very differently, at a time when Pasteur was quoted as a contemporary. It's that old."

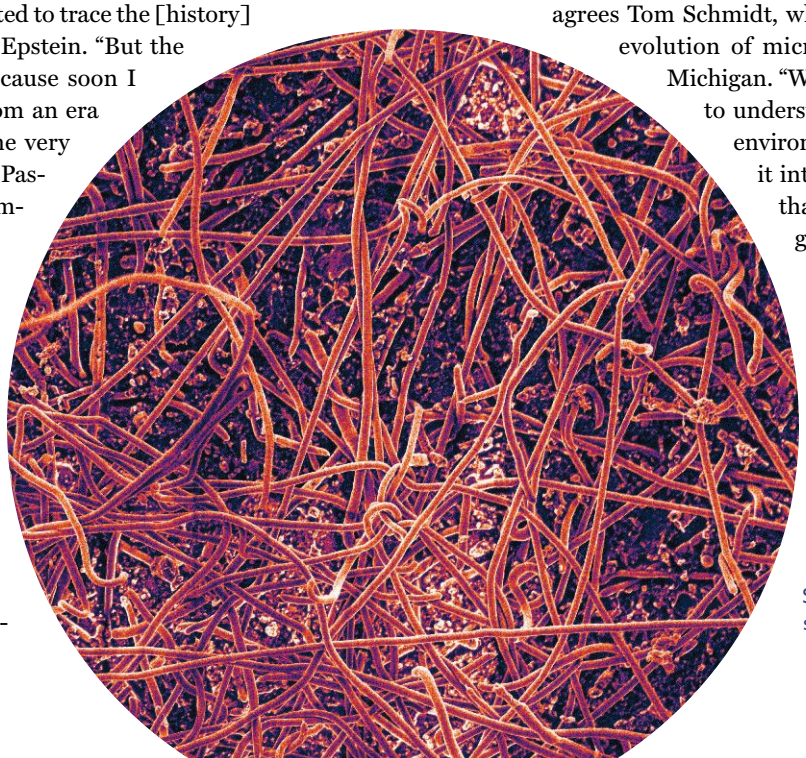
In 1985, microbiologists Allan Konopka of the Pacific Northwest National Laboratory and James Staley of the University of Washington dubbed this gap between the number of bacterial cells that could be directly counted in an environmental sample and those that could be reproducibly cultivated "The Great Plate Count Anomaly."[1] More recently, researchers have applied metagenomics approaches, extracting and sequencing all microbial DNA from an ecological sample, and have revealed tens of thousands of new species, causing the gap to widen exponentially. One oft-cited statistic posits that only 1 percent of bacterial species in an environmental sample can be cultured, although microbiologists say this is more a symbol of the inability to culture most bacterial species rather than a number rooted in rigorous science. (See sidebar on opposite page.) "I really hate the statement that 1 percent are culturable. The [actual proportion] depends on the environment," says Karsten Zengler, a microbiologist at the University of California, San Diego (UCSD). Either way, the true number of bacterial species is impossible to calculate without a better estimate of the total number of species around the globe.

If we can't grow them, we don't have the access to these organisms that rule our biosphere.

—Kim Lewis, Northeastern University

Researchers are working hard to improve their chances of culturing fussy bacteria and hope to continue mining the world's bacterial diversity for antibiotics and other therapeutic compounds, as well as for biotechnological advances. For example, the recently discovered CRISPR—an antiviral defense system found in many bacteria—has been widely adapted as a genetic-engineering tool. "If we can't grow them, we don't have the access to these organisms that rule our biosphere," says Northeastern University microbiologist Kim Lewis.

"The need to cultivate organisms is greater now than ever," agrees Tom Schmidt, who studies the ecology and evolution of microbes at the University of Michigan. "We need the whole organism to understand how it behaves in the environment and what organisms it interacts with; we can't know that from just the parts list the genome provides."



Scanning electron micrograph of segmented filamentous bacteria

COURTESY OF PAMELA SCHNUPF

No guts, no glory

One reason that only a fraction of microbial species has been successfully grown in the lab is simply that most researchers aren't willing to try. Microbiologists typically

stick to easily grown bacteria such as *E. coli*, simply inserting genes from other microbes into this genetically malleable model organism. "Cultivation is a bit of a dying art," says Zengler. "It can be a tedious, time-consuming, and often frustrating process."

Dubilier agrees. "[Cultivation] is not what young scientists are interested in—it's not sexy. They want to do the omics."

But a few stubborn researchers are taking up the challenge. And for some types of microbes that don't initially grow well using standard laboratory media, methodically offering the bacteria different culture conditions can pay off. David Fredricks, who studies how human microbiota affect health at the Fred Hutchinson Cancer Research Center in Seattle, was able to grow the majority of bacterial species found in human vaginal samples by plating them on a half dozen different media types kept in either aerobic or anaerobic conditions. Of course, patience, attention to detail, and a dissecting microscope were required, he notes, as some of the species formed only tiny colonies that were invisible to the naked eye.

Besides choosing the right growth medium, researchers also need to consider how bacteria are housed. What does not work well—at least for growing bacteria from vaginal fluid, says Fredricks—is growing colonies from single cells separated in wells, as is standard practice in clinical microbiology. That's because it's not just the nutrient and metabolite components of the medium that affect bacterial growth, but also signaling molecules and, potentially, physical signals that microbes get from their neighbors. McMaster University's Michael Surette, whose lab has cultivated many of the bacteria found in both healthy and diseased human respiratory tracts, credits his group's success to the use of unrestrictive culture conditions that allow interaction between species on standard media plates. "We treat each plate as a community rather than picking individual colonies."

But too much interaction can be a bad thing, adds Surette. A little physical separation is important to give slow-growing species a chance to replicate in a dish filled with faster-growing microbes. "If you separate the bugs even by a few millimeters, the slow ones can still grow, yet the interactions within and between colonies are preserved," Surette says. "It's a trivial thing, but it's powerful if you want to culture."

Some bacteria are particularly finicky, however, and no combination of culture conditions and cell housing seems to work. In such cases, researchers must get creative. A few years ago, as a postdoc in microbiologist Philippe Sansonetti's lab at the Pasteur Institute in Paris, Pamela Schnupf decided to culture segmented filamentous bacterium (SFB), a common gut bacterium in mammals. While SFB was well characterized in the 1970s, no successful attempts to culture the bacterium had been reported, and by the 1990s, the field had lost interest. Schnupf's motivation to try again stemmed from the recent discovery that the bacterium is a key modulator of host immunity.[2] "It acts as an educator of the immune system partly because it has this pathogenic pattern of attachment, and colonizes a niche different than other bacteria of the microbiota," she says.

A NUMBERS GAME

There are about 12,400 cataloged bacteria species according to the List of Prokaryotic Names with Standing in Nomenclature, and most of these can be cultured to some extent. But these represent only a fraction of the presumed millions of species of microbes in the world, and only about half the known bacterial phyla have at least one cultured species.[12,13]

Although the exact numbers are unknown, it is often reported that only 1 percent of all known bacterial species have been successfully cultured in the lab. But no one has rigorously confirmed this estimate, says Slava Epstein, a microbial ecologist at Northeastern University in Boston. "It symbolizes a small number, no less but no more." Indeed, many microbiologists agree that the 1 percent statistic is misleading. "Often, about 1 percent of bacterial cells from the soil will form colonies in a given experiment," he says. "But that's not to say 1 percent of species do." It could be that most cells of a particular species will grow, while many other bacterial strains do not.

In marine environments, the percentage of bacterial cells that can be recovered and cultured is even smaller, about 0.01 to 0.1 percent. The easiest-to-grow species, it turns out, are those from the human gut. As many as one-half of the bacterial species identified in and on the human body will grow in the lab, says David Fredricks of the Fred Hutchinson Cancer Research Center in Seattle.

Other complications in calculating the proportion of bacterial species that can be cultured include defining a species at the genetic level and the fact that successful identification in metagenomic analyses depends on the sequence coverage. The greater the coverage, the greater the chance that more rarely represented sequences—from rarer bacterial species—will be detected, says Michael Surette of McMaster University and Farncombe Family Digestive Health Research Institute in Ontario, Canada. Conversely, low sequence coverage could underestimate the total number of species in a sample—and overestimate the percentage that has been successfully cultured.

Single-cell sequencing of a field sample may also fail to capture the fraction of cells that are in a state of dormancy, meaning even more species are missed. "There are lots of dormant cells recalcitrant to sequencing, and many surveys don't use the proper procedures for getting DNA out of spores," says Columbia University's Jonathan Dworkin. "Just because sequencing technology keeps getting better does not negate the need for being able to isolate these dormant cells."

AT HOME ON HUMANS: Researchers have had reasonable success culturing microbes found in and on the human body. Cultures shown here are derived from the human respiratory tract.

Schnupf and her colleagues initially poured over older SFB literature that cited failed cultivation attempts and undertook a brute-force approach, trying various conditions that might support SFB's growth. But when nothing proved successful, Schnupf turned to the bacterium's DNA for help.

"It was clear that SFB needed a rich environment because its genome was missing many biosynthesis pathway enzymes to make nucleotides and vitamins," she recalls. "It also likely needed iron because it had genes for several iron transport systems." Moreover, SFB's genes suggested that, despite being an anaerobe, the bacterium could withstand low levels of oxygen, to which it is probably exposed in the small intestine. Schnupf took all of this information into account as she created a laboratory version of SFB's in vivo niche: a human or murine cell line and tissue culture medium along with a rich array of nutrients regularly used for growing bacteria, spiked with extra iron, all cultured at a 1.5 percent oxygen concentration. Although it took several years to get the right cocktail, Schnupf was finally successful where others had failed for nearly half a century.[3]

"This was a pretty big breakthrough," says Jonathan Dworkin, a microbiologist at Columbia University. "Before, if you wanted to work with SFB, you had to purify it from mouse poop. It was doable, but not very good for making rapid progress."

Hiding out

Aside from the singular difficulty of getting SFB to thrive in the lab, microbiologists have generally had reasonably quick success culturing human-derived microbes, at least in comparison with species isolated from environmental samples. More than half the members of the human microbiome can now be cultured, while less than 1 percent of bacterial species found in the wild have been grown in the lab, according to some estimates.

Culturing environmental microbes is difficult because many bacteria in the soil exist in a dormant state—a survival mechanism employed when nutrients are scarce and conditions adverse. Transferring these bacteria to a rich medium does not necessarily entice them to grow, and researchers know little about how to coax dormant bacteria out of their slumber. "If nutrients are the factors that wake up dormant cells, we would have discovered these by now, but that is not the case," says Epstein.

Unlike hibernating bears or plants that respond in a predictable fashion to the changing environment, microbes may switch from dormancy to growth in a stochastic manner, he says. Epstein dubbed this idea "the scout model" in 2009.[4] If a bacterium awakes and conditions are poor, it dies; the "scout" is sacrificed for the sake of the population. If conditions are favorable, the scout forms a new population and may even relay signals for its dormant neighbors to wake up. Epstein's lab has provided evidence for this mechanism in laboratory isolates of *E. coli* and *Mycobacterium smegmatis*, as well as in a handful of species collected in the field.[5,6] Dworkin has also shown that the spore-forming *Bacillus subtilis* goes through a similar random waking process.[7]

But even the stochastic model does not explain why the majority of bacteria cannot be cultivated in petri dishes, says Epstein. "The implication of this model is that populations that are massively growing at the time of sampling should be easier to culture than rarer ones, but in fact the reverse is true," he says. "Typically, we can only cultivate a minor component of natural communities, and it's exactly the abundant communities that are missing from laboratory plates." A clade of marine bacteria called SAR11, for example, accounts for as much as one-quarter of the bacteria in seawater but has generally evaded cultivation.[8]

Epstein suspects that there may be a delay in bacterial growth as the microbes adjust to the alien conditions of the lab. The idea is that, while scout cells do randomly wake up to test the waters, they then adapt to their new environment by undergoing changes in gene expression, analogous to the process of differentiation in the cells of multicellular organisms. This delay in growth after a bacterial colony is transferred from one environment to another was noted by French biologist and Nobel laureate Jacques Monod in the early 1940s.[9] "Monod described that *E. coli* exhibit a lag when transitioning from growth on glucose to lactose," says Epstein. "But the distance between environmental and laboratory petri-dish conditions is huge, and adaptation may take a longer time, during which we pronounce the cells dead or uncultivable."

Smarter tools

To capture the efforts of Fredricks, Schnupf, Surette, and other microbiologists who've worked to culture fastidious bacterial species, Matthew Oberhardt, postdoctoral fellow in Eytan Ruppin's laboratory at the University of Maryland Institute for Advanced Computer Studies, has been building a large database of detailed

COURTESY OF MICHAEL SURETTE

strain and media combinations for growing cultivable microorganisms. He hopes the data will serve as a source of insight into culturing principles, which might then be turned around and used for prediction. "I saw this as an opportunity to find principles underlying the media, to improve culturing conditions, and bring the art of culturing bacteria to high-throughput data," he says.

Meanwhile, Epstein's laboratory uses a multiwell diffusion chamber that it created in 2002, hoping to strike a balance between allowing slower-growing species to compete against more dominant strains and ensuring open communication among the bacterial community. The device, called the iChip, physically separates bacterial cells by a breathable membrane but surrounds them in their natural environment—the soil or marine water—allowing some contact and diffusion of factors and metabolites without the need to identify each species' exact growth conditions.[10] The diffusion also allows the multitudes of bacteria-produced factors to be preserved, as these compounds likely help culture species that prove difficult to grow under standard lab conditions.

Another growth tool, developed by UCSD's Zengler around the same time Epstein's group introduced the iChip, traps a single bacterial cell inside a tiny droplet made of a permeable gel, then uses flow cytometry to separate out those droplets in which a microcolony has formed.[11] As with the iChip, the gel bead method isolates individual cells while preserving the interaction of the community, capturing both slow- and fast-growing bugs. The tool overcomes the sometimes painstaking microscopy needed to detect minute colonies that can get lost among the higher-density populations that grow like weeds on a petri dish. "Not every organism researchers are interested in grows to the density of *E. coli*," says Zengler. "This allows the capture of these less-dense colonies."

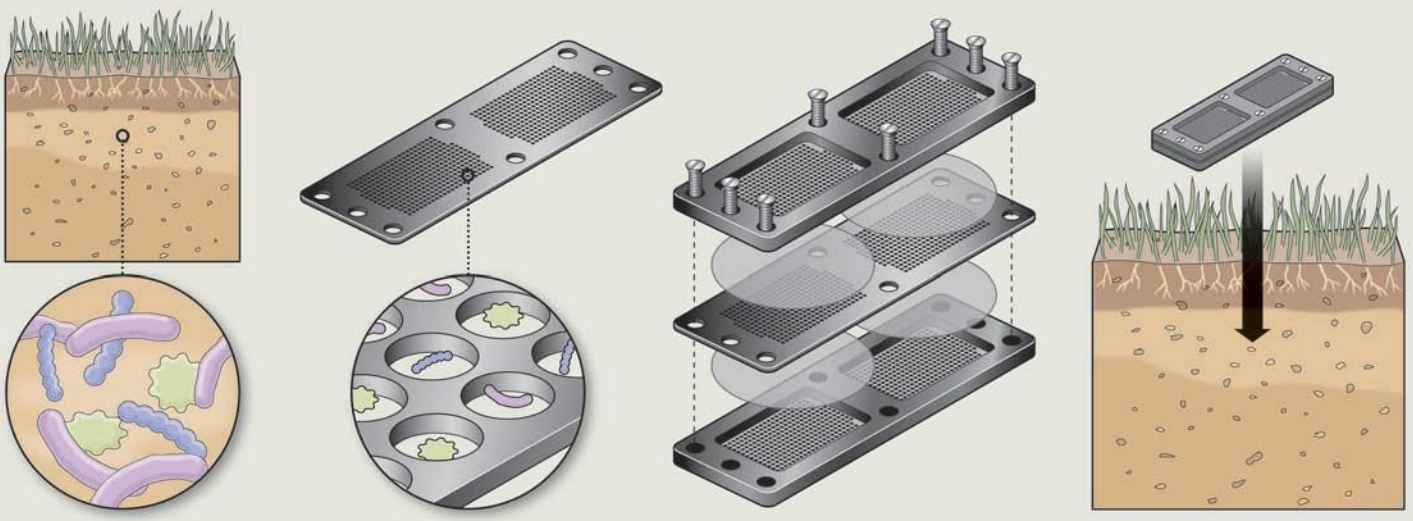
> **Cultivation is a bit of a dying art. It can be a tedious, time-consuming, and often frustrating process.**
>
> —Karsten Zengler, University of California, San Diego

More recently, Zengler cofounded a company called GALT to develop and market high-throughput microbiology cultivation and screening tools. These tools address the bottleneck of cultivation, he says, by eliminating the labor-intensive and mostly

CULTURAL RICHES

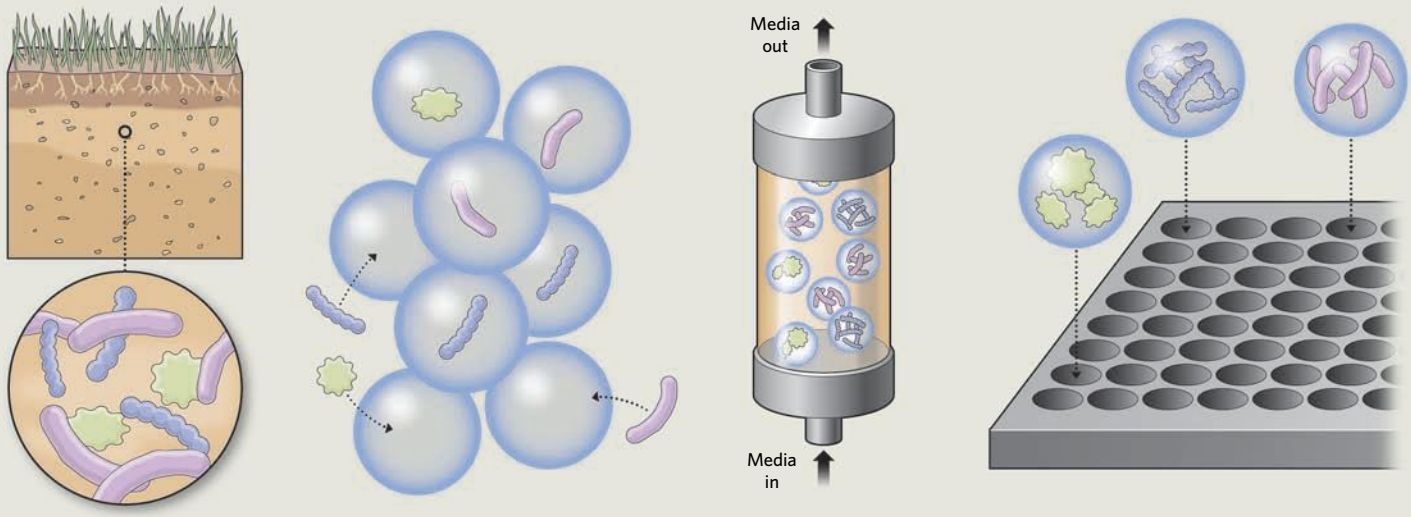
To facilitate growing bacteria collected from the environment, researchers have devised new techniques that allow different microbes to communicate, while keeping them separate in order to give slower-growing species a fighting chance.

iCHIP: A multiwell diffusion chamber separates individual bacterial cells in the wells of a 384-well plate. A breathable membrane surrounding the plate allows interaction with the natural environment, such as soil or ocean water, and sensing of the multitudes of molecular factors produced by neighboring bacteria.



1. Collect environmental sample.
2. Place iChip plate in a diluted environmental sample to capture one bacterium per well.
3. Enclose iChip plate in a breathable membrane that allows for bacterial communication.
4. Place iChip device back into the environmental sample, where small molecules and metabolites can promote bacterial growth.

MICRODROPLET-MICROCOLONY FORMATION: A device traps individual bacteria inside tiny, permeable gel droplets, which allow interactions among bacteria while keeping them separate. The droplets are bathed in a nutrient-rich media until a microcolony of 40–200 cells forms inside, then sorted and plated for further analysis.



❶ Collect environmental sample.

❷ Encapsulate individual cells in microdroplets.

❸ Nourish bacterial colonies until they are 40–200 cells strong.

❹ Separate the droplet-enclosed colonies using flow cytometry and transfer to a 96-well plate.

slow process of isolating and growing bacteria in a controlled environment. And the demand for such products is growing, says Zengler, partly driven by the increased interest in the human microbiome as well as in soil microbes that may boost agriculture yields. "We need cost-effective and high-throughput cultivation methods to study microbiome interactions and develop microbes as therapeutics and to promote crop growth and yield."

Access to microorganisms means access to their metabolites, says Gerry Wright, director of the Institute for Infectious Disease Research at McMaster University in Hamilton, Ontario. Wright has amassed a collection of 17,000 culturable species from previously under-sampled ocean and land environments and is now working to analyze the chemicals produced by these microorganisms. With a need for new antibiotics on the rise, researchers are hoping to discover antimicrobial molecules among the plethora of yet untapped bacterial biodiversity. In addition to antibiotics, there may be microbe-produced molecules that have immune modulatory, antiviral, or anticancer activities, he says.

The new tools allowing scientists to bring nature into the lab will perhaps convert some of the omics researchers into cultivation enthusiasts. The more bacterial species researchers are able to culture in the lab, the greater the chances of striking gold. "My view of the microbial world is as an iceberg," says Dworkin. "We are seeing some idea of how these bacteria come up with solutions [for growth and communication], but we study few types of bacteria. We're still missing a lot of interesting stuff." ■

References

1. J.T. Staley, A. Konopka, "Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats," *Annu Rev Microbiol,* 39:321-46, 1985.
2. I.I. Ivanov et al., "Induction of intestinal Th17 cells by segmented filamentous bacteria," *Cell,* 139:485-98, 2009.
3. P. Schnupf et al., "Growth and host interaction of mouse segmented filamentous bacteria in vitro," *Nature,* 520:99-103, 2015.
4. S.S. Epstein, "Microbial awakenings," *Nature*, 457:1083, 2009.
5. S. Buerger et al., "Microbial scout hypothesis and microbial discovery," *Appl Environ Microbiol,* 78:3229-33, 2012.
6. S. Buerger et al., "Microbial scout hypothesis, stochastic exit from dormancy, and the nature of slow growers," *Appl Environ Microbiol,* 78:3221-28, 2012.
7. A. Sturm, J. Dworkin, "Phenotypic diversity as a mechanism to exit cellular dormancy," *Current Biology,* 25:2272-77, 2015.
8. M.S. Rappé et al., " Cultivation of the ubiquitous SAR11 marine bacterioplankton clade," *Nature,* 418:630-33, 2002.
9. J. Monod, "Recherches sur la croissance des cultures bactériennes," Paris: Hermann et Cie, 1942.
10. T. Kaeberlein et al., "Isolating 'uncultivable' microorganisms in pure culture in a simulated natural environment," *Science,* 296:1127-29, 2002.
11. K. Zengler et al., "Cultivating the uncultured," *PNAS,* 99:15681-86, 2002.
12. N.R. Pace, "Mapping the tree of life: Progress and prospects," *Microbiol Mol Biol Rev,* 73:565-76, 2009.
13. M. Achtman, M. Wagner, "Microbial diversity and the genetic nature of microbial species," *Nat Rev Microbiol,* 6:431-40, 2008.

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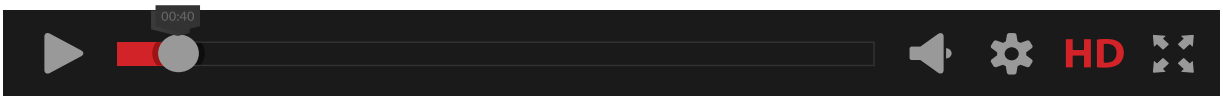

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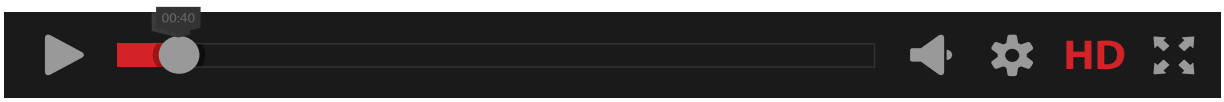

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C. Varin et al., "Glucose induces slow-wave sleep by exciting the sleep-promoting neurons in the ventrolateral preoptic nucleus: A new link between sleep and metabolism," *J Neurosci*, 35:9900-11, 2015.

That drowsy feeling after a big meal—the so-called food coma—may actually be the result of increased neuronal activity. New research in mice suggests that sugar excites neurons in a brain region, called the ventrolateral preoptic nucleus (VLPO) of the hypothalamus, that induces sleep.

"What [the researchers] show is that these ventrolateral preoptic neurons are glucose responsive, and they show that in times of high glucose, they fire more," says Harvard University neuroscientist Clifford Saper, who was not involved with the study. "If you've ever had a big meal, and you get sleepy afterward . . . this may be part of the reason why."

About 20 years ago, Saper discovered that sleep-promoting neurons within the VLPO usher the brain into the first stage of the sleep cycle, slow-wave sleep, by inhibiting the organ's arousal systems. When awake, these arousal systems inhibit VLPO sleep neurons. This two-way inhibition, says Saper, ensures that we are either awake or asleep, depending on which set of neurons has the upper hand, but that we spend little time—often just seconds—in between.

One such arousal system is a group of hypothalamic neurons that release neuropeptides called orexins (also known as hypocretins); loss of orexin neurons in humans leads to narcolepsy. Researchers have shown that glucose inhibits orexin neurons in vitro, inspiring Thierry Gallopin at ESPCI ParisTech in France to investigate how glucose affects the sleep-promoting neurons of the VLPO.

"If glucose is able to inhibit this arousal system, my hypothesis is that glucose could, in turn, excite sleep-promoting neurons," says Gallopin.

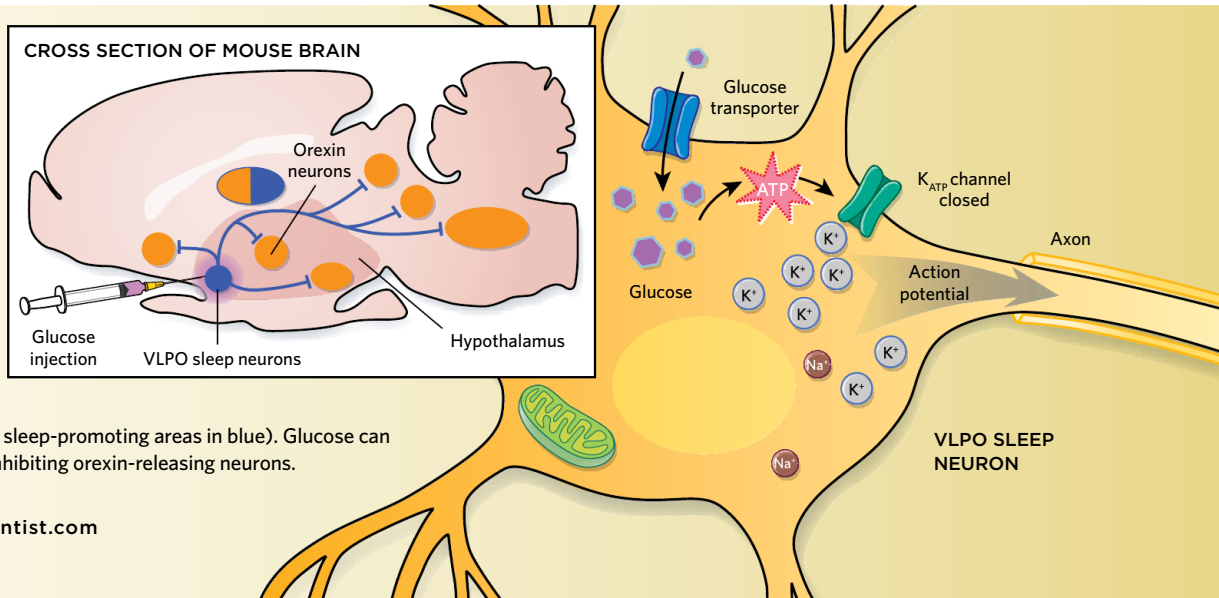
So Gallopin and his colleagues injected glucose—in concentrations at or beyond physiological limits—into the VLPOs of mice and monitored their sleep for two hours. The mice fell asleep faster and slept longer after an injection with glucose than they did after being given a glucose-free solution.

Brain-slice experiments revealed that VLPO sleep neurons fire more in the presence of glucose. More specifically, it's the energy molecule ATP, a product of glucose metabolism, that is responsible. These cells contain potassium channels that close in response to ATP, thereby making the neurons fire more readily. This leads to the inhibition of orexin neurons and other arousal regions of the brain.

With its arousal centers quieted, the brain goes to sleep. "The slow waves seem to be the natural state of the brain if it is not being stimulated," explains Saper in an email. Previous studies have shown that a slab of cortex disconnected from the rest of the brain will enter a slow-wave state spontaneously. This scenario isn't quite the same as sleep, Saper adds, given that the brain sample cannot wake up. "A slow-wave state that cannot be interrupted would more accurately be called a coma."

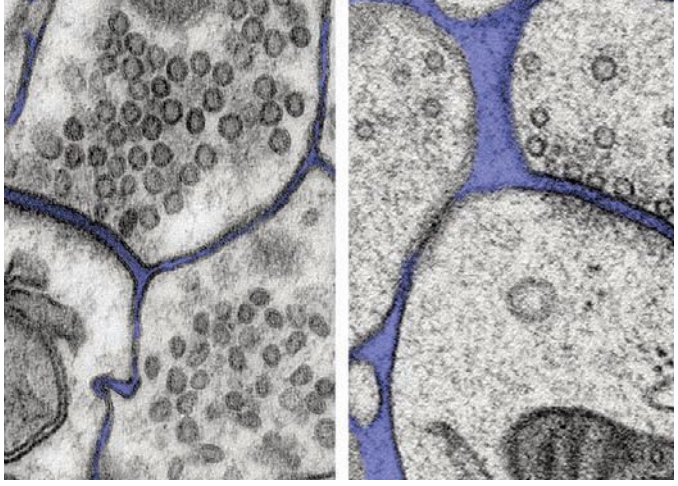
Thankfully, a "food coma" is less a catatonic state and more the result of a brain sent off to sleep by a molecular lullaby.

—Ashley P. Taylor



SUGAR COMA MODEL: An injection of glucose into the ventrolateral preoptic nucleus (VLPO) of the anterior hypothalamus fuels ATP production in sleep-promoting neurons located in the VLPO. This boost in cellular ATP closes potassium channels (K_{ATP}), leading to neuronal excitation and the onset of sleep. When the VLPO sleep-promoting neurons fire, they inhibit arousal-promoting areas of the brain (inset, orange; sleep-promoting areas in blue). Glucose can also reduce arousal by directly inhibiting orexin-releasing neurons.

GUIDING LIGHTS: Channelrhodopsins from algae such as *Guillardia theta* (center; false-color image) are endowing optogenetics with novel abilities.



TIGHT SQUEEZE: Chemical fixation compacts synapses in a mouse brain (left), compared to freezing, which maintains the extracellular space (blue; right).

NEUROSCIENCE

Negative Thinking

THE PAPER

E.G. Govorunova et al., "Natural light-gated anion channels: A family of microbial rhodopsins for advanced optogenetics," *Science*, doi:10.1126/science.aaa7484, 2015.

CHANNEL SURFING

Channelrhodopsins (ChRs) from green algae paved the way for optogenetics research in neuroscience and other fields. These membrane ion transporters are light sensitive, and importing them into neurons has given scientists unprecedented control over neuronal activity. The 50 or so known algal ChRs only transport positive ions, however, which trigger action potentials. Only one ChR from an Archean species and engineered algal ChRs can transport anions, which suppress neuronal firing by hyperpolarizing neurons, and neither is as fast or sensitive as the natural algal channels. "[ChRs] enabled fairly efficient activation of neurons, but neural inhibition was limited to much lower-efficiency tools," says molecular biologist John Spudich of the University of Texas Medical School at Houston.

AN ALTERNATIVE SOURCE

In their search for ChR diversity, Spudich and his colleagues looked to another type of alga called a cryptophyte. They examined the genome of *Guillardia theta* and cloned three genes that resembled those for known ChRs. After expressing two of the genes in human embryonic kidney cells, "the first thing we saw was an unusually large current," says Spudich.

THE NATURAL

The researchers found that these channels were natural anion transporters, and when expressed in rat neurons, they are 10,000 times more sensitive than engineered anion channels. "You have to search in nature to find these wonderful tools," says Wayne State University's Zhuo-Hua Pan, who is starting to use *G. theta*'s channels in experiments.

A BRIGHTER FUTURE

Spudich is not stopping at *G. theta*. He plans to look for more anion channels that will expand the utility of optogenetics. "There are many cryptophytes out there." —Amanda B. Keener

NEUROSCIENCE

Brain Freeze

THE PAPER

N. Korogod et al., "Ultrastructural analysis of adult mouse neocortex comparing aldehyde perfusion with cryo fixation," *eLife*, 4:e05793, 2015.

THE FIX

Soaking brain tissue with chemical fixatives has been the go-to method of preserving specimens for decades. Yet few neuroscientists take into account the physical distortion that these chemicals cause. And even among those who do pay attention, "we don't really know in quantitative terms how much really changes," says Graham Knott, a morphologist at the École Polytechnique Fédérale de Lausanne in Switzerland.

SHRINKAGE

Comparing fresh to fixed tissue, Knott and his colleagues found that chemical fixation shrank the tissue by 30 percent. "It raises the question of, 'What on earth is going on if it shrinks that much?'" says Knott. To find out, they turned to an alternative preservation approach, rapid freezing and low-temperature resin embedding, which was shown in the 1960s to better capture the natural state of the brain. Using a high-pressure version of this cryo-fixation technique, they observed neurons swimming in extracellular space and smaller astrocytes than are seen in chemically fixed samples.

REALITY

NIH investigator Kevin Briggman says Knott's technique offers a much more accurate snapshot of the brain. An added bonus is that the elbow room around neurons afforded by cryo fixation makes it easier for automated methods to count cells or analyze structures. The only problem, he adds, is that, in contrast to chemical fixation, "you can't freeze a whole mouse brain."

THE COMPROMISE

Briggman and Knott don't advocate doing away with fixatives. Rather, Knott says, scientists who use them should consider their effects when interpreting data. "We need to use models that pay very careful attention to how tissue has reacted to chemicals." —Kerry Grens

DR DAVID HILL; GRAHAM KNOTT

PROFILE

Circuit Dynamo

Eve Marder's quest to understand neurotransmitter signaling is more than 40 years old and still going strong.

BY ANNA AZVOLINSKY

Eve Marder has her junior-year college roommate to thank for her initial fascination with neuroscience. "She came back from the first day of an abnormal psychology course and said, 'Eve, you have to take this course! The professor has an English accent, wears a three-piece suit, and has a dueling scar,'" recalls Marder, a professor of biology at Brandeis University in Waltham, Massachusetts. "Of course, I agreed. What could be more romantic than that?" The course focused on schizophrenia, which at the time, in 1967, was thought to stem from a genetic predisposition coupled with competing sensory inputs or stressors that the brain couldn't turn off. "The professor, in passing, said that some people think there may be a [cellular] basis for schizophrenia, including deficient inhibition of electrochemical signals in the brain. I thought, 'What does that mean, *inhibition in the brain*?'" says Marder. To find out, she read everything she could about the role of inhibitory neurotransmitters—and, in the process, decided she would become a neuroscientist.

> "Every time we were really stuck—not trivially, but stuck on a deep intellectual level—that has driven us to rethink, go sideways, or turn the problem inside out and come up with something new."

Marder began her graduate studies in biology at the University of California, San Diego (UCSD) in 1969. "I was a molecular biologist at heart because I was intrigued by molecules and cells rather than the large systems many were studying," she says. That same year, a new assistant professor, Allen Selverston, joined the department. "He was the only real neurobiologist in the biology department, so I decided to work with him." In the summer of 1970, Selverston introduced Marder to the lobster stomatogastric ganglion (STG), a then-new and relatively simple model for studying neuronal connectivity that she has studied ever since.

Here, Marder explains why her choice to become a scientist during the 1960s counterculture was more conservative than what her friends and peers were up to, how her penchant for beautifully written neuropharmacology papers led her to a postdoc in Paris, and why we are not training too many PhDs.

MARDER'S MOMENTUM

A political slant. Growing up in the 1960s in Westchester County, New York, at the height of the civil rights movement, Marder was involved in a local youth civil rights group. In 1965, when she entered Brandeis University as a freshman, Marder thought she wanted to be a civil rights lawyer. But during her sophomore year, a post–World War II European history course that required memorization of every country's political parties, "all alphabet soup and boring, with an odious black textbook with double columns of text," changed her mind. After taking the final, Marder walked out of the lecture hall and pressed her mental delete button. "I forgot all of it on purpose and changed my major to biology," she says.

Perfect sense. "I remember in high school when we learned about respiration and photosynthesis and the other molecular machinery hiding inside things that on the surface looked solid. I realized all of these molecular dynamics were inside cells, and that is what really fascinated me—these mechanisms of biological systems. Biology just made complete sense to me."

The road less travelled. Before graduating and heading off to grad school, Marder presented an honors thesis on muscle biochemistry. At the time, most students who did an honors science thesis were men headed to medical school. "I remember having a funny conversation with one of them my senior year. He asked if I was also applying to medical school, and after I said no, said, 'But why not? You'd get in.' I said, 'But why would I want to go to medical school if I don't want to be a doctor?' And he just kept saying, 'But you would get in,' and 'Why wouldn't you want to be a doctor?'"

A new model. When Marder joined Selverston's lab as his first graduate student, he had just learned how to make in vitro preparations of the STG. "It was brand new and a simple example of a pattern-generating circuit," says Marder. The STG, just 30 neurons, controls the rhythmic movement of the lobster's stomach muscles during digestion, similar to the circuitry that controls breathing or walking. The STG cellular circuitry continues to generate motor patterns in vitro that resemble in vivo action potentials, but without the need for external stimuli. "This was something not possible then with vertebrate neuronal preparations."

Thinking outside the circuit. For her graduate thesis, Marder set out to identify the neurotransmitters of the STG. "It was already clear that there were a bunch of different molecules used as transmitters, but no one had any idea why there were so many. There were researchers studying GABA, dopamine, serotonin, and other neurotransmitters separately, but no one was asking why there were so

PHOTOGRAPH BY MIKE LOVETT/BRANDEIS UNIVERSITY

EVE MARDER

Professor, Department of Biology
Brandeis University

Greatest Hits

- Discovered that acetylcholine acts as a neurotransmitter in the crustacean stomatogastric ganglion (STG), where it functions as both an excitatory and an inhibitory signal
- Among the first to describe neuromodulators that acted differently than neurotransmitters, resulting in long-lasting effects on neuronal circuits
- Determined that neurons are robust, maintaining their electrical activity patterns despite the turnover of channels and other changes
- With colleagues, developed the "dynamic clamp," a neurophysiological method that can finely manipulate nerve cells and simulate neuronal and muscle systems using computer-adjusted parameters
- Showed that there are multiple sets of parameters in neurons and networks that can produce similar output patterns

many transmitters in the brain and what their functional organization was. I wanted to understand the whole circuitry," she says. Marder was the first to describe neurotransmitters in the STG. She discovered that acetylcholine functions as both an excitatory and inhibitory transmitter in some of the STG's neurons. "This turned into a lifelong chase into transmitters and modulators in functional circuits."

MARDER'S MERITS

A singular vision. Marder learned a lot in graduate school, but her desire to understand the molecular underpinnings of neuronal communication remained strong. "At the time, the field was interested in working out wiring diagrams, because the scientists, who mostly came from electrical engineering, thought the neuron circuits worked like electrical circuits. My advisor echoed what many said at the time. 'What you are doing is just pharmacology, it doesn't really matter.' In their minds, it only mattered whether the signal was inhibitory or excitatory; the actual signaling molecule wasn't going to matter."

A conservative choice. "Of the people I graduated college with, half were going to change the world, some were going to live on a commune, some guys ran away to Canada to avoid the draft, and others were going to a farm in Vermont to grow their own food. Long-term career plans were sort of weird for us—we were all counterculture. Graduate school at the time was a very conservative thing to be doing amongst my friends. When I finished my PhD, I just thought about the next step but didn't have long-term career goals." Marder spent a year as a postdoc in David Barker's laboratory at the University of Oregon and then, in December 1975, with a fellowship from the Helen Hay Whitney Foundation, set off for Paris to work in JacSue Kehoe's laboratory. "She had written, just head and shoulders, the most beautiful neuropharmacology papers. I had read every paper in the field and these were just so much better than what anyone else was doing." There, Marder collaborated with Danielle Paupardin-Tritsch, publishing three papers, including a study of the responses of the crab STG to three different neurotransmitters.

More than on/off switches. When she came back to the U.S. in 1978, Marder joined the faculty at Brandeis as an assistant professor in the biology department and has remained there ever since. Along with her first graduate student, Judith Eisen, whom Marder credits with early successes in her lab, Marder provided the initial evidence for the existence of neuromodulators that can prompt longer-term changes influencing how neurons respond to

fast-acting neurotransmitters. Eisen initially characterized the pharmacology of the synapses, figuring out which neurons had receptors for which signaling molecules.

Predicting behavior. In 1989, Marder met Larry Abbott, then a theoretical physicist at Brandeis. "It was clear that for a true mechanistic understanding of how the dynamics of the circuit arise from its components, we needed to be able to do modeling," she recalls. With Abbott, Marder's lab developed the "dynamic clamp," a method that uses a computer to introduce a conductance—the ease with which an electric current can pass through a circuit—into neurons to model their behavior. They also worked out the negative-feedback system within neurons that allows for changes in parameters while maintaining their normal function. "We were building models and they were fragile. We would change a parameter, and the model would crash. I kept saying that the cells don't crash all the time, so how do cells balance their number of ion channels? What we came up with is a very simple way of thinking about this."

The bigger picture. "The big themes that have come from the STG model, and partly from our lab, are that neuronal circuits are multiply modulated, that modulators reconfigure circuits, and that there have to be pretty simple global regulatory mechanisms that help neurons maintain stable electrical activity despite the fact that their ion channels—the proteins in the cell membranes that carry out electrical signaling—are being constantly replaced."

Heterogeneity. For the last 10 years, Marder's lab has been working to understand the extent of variability within individual nervous systems that still allows the systems to remain stable. "For quite a long time, people thought that all nervous systems had to be very tightly tuned," she says. "And what we are seeing is that, actually, they can be quite variable and change over time and still work well enough because there is a lot of degeneracy in the way circuits are constructed."

MARDER'S MIND

The new normal. Before 1968, many life-science PhD programs had informal quotas, restricting the number of women accepted, says Marder. But that year, the draft law changed, and graduate school enrollment was no longer a valid way to defer the draft. Life-science graduate programs suddenly switched to gender parity. Marder's class at UCSD was the first to have a large proportion of women—13 women out of a class of 30; in prior years there were only two or three women per class of 30. "There was a lot of hubbub about it. 'What are we going to do with all these women? Civilization as we know it will end!' was how the professors talked about it. But by May that year they had completely forgotten about it," Marder laughs.

The big picture. "I have always been good at figuring out how to use the same nervous system to ask a bunch of different questions. And I've always been pretty good at finding the general principles amongst the idiosyncrasies of a neuronal system. I think that is key if you work on model organisms."

The benefits of being stuck. "Everything important we did in the lab arose because something wasn't working as expected. In Judith's original work, we finally realized after getting different answers from every experiment that there were nine different possible outcomes connecting only three neurons, all consistent with our recordings. So we had to design a completely different experiment. The activity-dependent regulation work arose only because of my total frustration with having to tune conductance-based models. So every time we were really stuck—not trivially, but stuck on a deep intellectual level—that has driven us to rethink, go sideways, or turn the problem inside out and come up with something new."

A worrisome trend. "I am very concerned with science right now because I think the push to have high-impact papers is having a deleterious effect on the way people design experiments, and it definitely has a deleterious effect on the way researchers write their papers. The most common critique you see from reviewers is that authors are overselling their work, and it's because the researchers believe high-impact papers are necessary for their careers. So the science we are doing is being warped."

A scientist in the rough. Marder recently wrote a commentary against the push to decrease the number of students accepted into science PhD programs."We're very bad at spotting who is going to be a good scientist and who isn't [based on graduate-school applications]," she says. "While students are in college, they are fundamentally consumers of knowledge, and becoming a scientist means you have to learn to be a creator of new knowledge. But the tools that allow you to be a great consumer of knowledge don't set you up for the kind of frustrations and sidewise thinking and problems that you have to confront to be a knowledge creator. You can take the best undergrad students, and they may not be the best graduate students. And the ones who complete the best PhDs may not be the ones that stay in the field. I think it comes down to drive, persistence, willingness to confront failure, creativity, passion—all of these other attributes that we can't measure easily on an application. So to take just the top undergraduates, you're going to be missing many of the best ones. The real problem here is that the US government is not funding enough science."

Potential to fly. "It's crazy right now because biology in general, and neuroscience specifically, is at this extraordinary moment in time when there is so much really wonderful science that can now be done that was inconceivable even 10 years ago. We are at this incredibly exciting moment for discovery and at the same time the field is being destroyed by extraordinary anxiety about [funding] resources. If we didn't have this extent of resource anxiety we could be flying, but the funding situation is crippling, and we are crippling ourselves with this incredible collective anxiety." ■

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Jacob Hooker: Weaver of Brain Science

Director of Radiochemistry, Athinoula A. Martinos Center for Biomedical Imaging; Associate Professor, Harvard Medical School. Age: 35

BY BOB GRANT

As a kid growing up outside Asheville, North Carolina, Jacob Hooker spent a lot of time tinkering underneath cars with his mechanic father. But it was a guest speaker in his high school chemistry class who provided the spark that propelled him into research. Kent Hester, director of student and career services at North Carolina State University's College of Textiles, told students about opportunities for studying textile chemistry. Hooker applied to NC State and won a $5,000-per-year North Carolina Textile Foundation merit scholarship, graduating in 2002. "That was one of the largest college-based scholarships on NC State's campus at the time," Hester recalls.

Hooker published four papers while at NC State, but when the time came to apply for grad school, he says he was "geographically driven" to branch out from his home state and explore the western half of the country. On a visit to the University of California, Berkeley, he met Matt Francis, a young biochemist who had just set up his lab the year before.

"From the very second I met him, I could just tell he was a very special individual," says Francis, who develops techniques for the chemical modification of proteins. "He's got a certain intensity and scientific sophistication to him that supersedes any kind of training he had." While in Francis's lab, Hooker published a technique for chemically modifying the interior surface of a viral capsid that would pave the way for viral drug-delivery methods.[1]

Near the end of his PhD research, Hooker learned about radioactive labeling and radioisotopes from Jim O'Neil at Lawrence Berkeley National Lab. Then, at a nuclear medicine meeting in the winter of 2006, he attended a presentation given by Brookhaven National Lab organic chemist Joanna Fowler about using specially synthesized radioactive molecules and positron emission tomography (PET) technology to study the neurobiology of addiction. "It was incredibly exciting that a chemist could label a small molecule and observe something fundamental about the brain using that molecule they made," Hooker says.

He immediately wrote to Fowler, asking if she had an open postdoc position in her lab. "She said she didn't, but she'd be willing to help me look for money to make one," Hooker says. He submitted an NIH training proposal as he was finishing his PhD dissertation and got the grant. Hooker moved to Fowler's lab in 2007. "Getting him was like winning the lottery," says Fowler, now a professor emeritus at Brookhaven.

Fowler introduced Hooker to PET, which is used in concert with radionucleotide tracers to image molecules in the active brain. Hooker and colleagues built new radiotracers that target novel molecules to gain insights into their in vivo function. He also helped develop a new method for quickly and efficiently labeling the carbamate functional groups with carbon-11 (^{11}C), one of the most important isotopes for PET research.[2]

Hooker started at the Athinoula A. Martinos Center for Biomedical Imaging at Massachusetts General Hospital in 2009, overseeing the construction of the center's new MRI/PET facility while setting up his own lab. Earlier this year, Hooker and Mass General neuroscientist Marco Loggia devised a PET imaging strategy to record the activation of glial cells in the brains of chronic-pain patients.[3] With other colleagues Hooker developed a novel histone deacetylase–binding radiotracer, [^{11}C]Martinostat, which could open an unprecedented window into DNA expression in the brain.[4]

"He's the best I've ever seen," Fowler says. "He's going to ask questions we haven't thought of before." ■

REFERENCES

1. J.M. Hooker et al., "Interior surface modification of bacteriophage MS2," *J Am Chem Soc,* 126:3718-19, 2004. (Cited 212 times)
2. J.M. Hooker et al., "One-Pot, direct incorporation of [^{11}C]-CO_2 into carbamates," *Angew Chem Int Ed,* 48:3482-85, 2009. (Cited 68 times)
3. M.L. Loggia et al., "Evidence for brain glial activation in chronic pain patients," *Brain,* 138:604-15, 2015. (Cited 8 times)
4. C. Wey et al., "In vivo imaging of histone deacetylases (HDACs) in the central nervous system and major peripheral organs," *J Med Chem,* 57:7999-8009, 2014. (Cited 6 times)

Into the Limelight

Glial cells were once considered neurons' supporting actors, but new methods and model organisms are revealing their true importance in brain function.

BY KATE YANDELL

By 1899, nearly 50 years after glia were discovered, Spanish neuroscientist Santiago Ramón y Cajal recognized that research on these cells was lagging behind studies on their flashier neuronal cousins. "What functional significance may we attribute to the neuroglia?" he wrote in his multivolume book *Texture of the Nervous System of Man and the Vertebrates*. "Unfortunately, in the present state of science it is not possible to answer this important question except through more or less rational conjectures. In the face of this problem, the physiologist is totally disarmed for lack of methods."

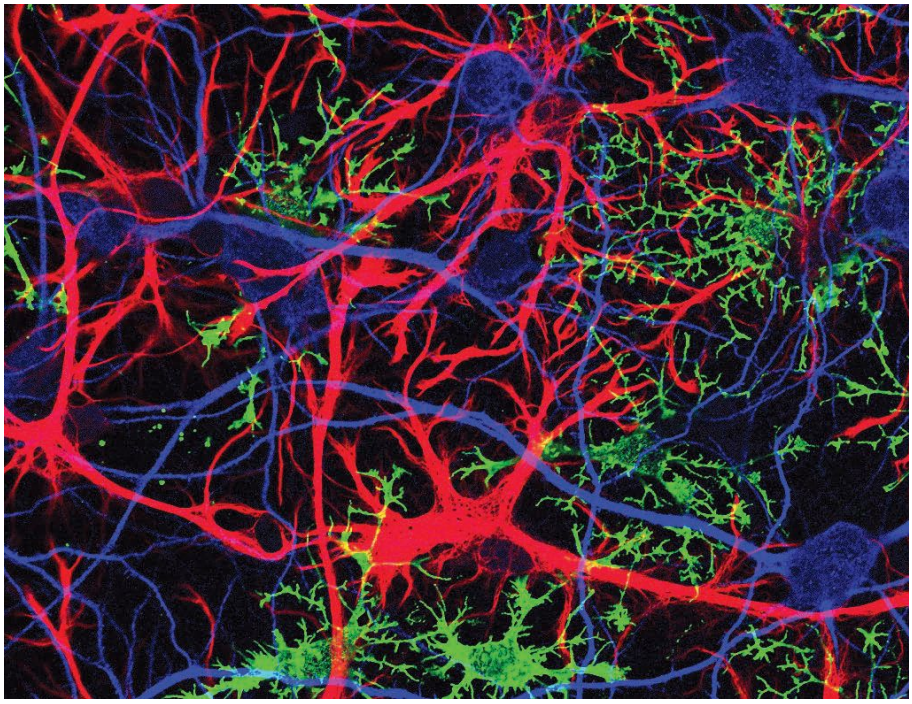
"People are saying neurons are overstudied, and glia are understudied."

—Ben Barres, Stanford University

Despite making up 50 to 80 percent of the cells of the human brain (estimates vary), glial cells were thought to simply provide structural integrity to the brain and to nourish and mop up after neurons. And it would be the better part of a century before Ramón y Cajal's call for more methods to study glia was answered.

"Glia were not a major point of interest in neuroscience," says Marc Freeman, an investigator with the Howard Hughes Medical Institute (HHMI) who studies fruit fly glia at the University of Massachusetts Medical School. "Neurons were the ones that fired signals. . . . Glial cells, when you plug them in, don't do a whole lot."

But attitudes towards glia are finally changing. In recent years, researchers in the field have shown that glia, including astrocytes, oligodendrocytes, and microglia, help form and prune synapses and otherwise actively influence brain structure and function. There is also increasing evidence that glial dysfunction can contribute both to neurodegenerative diseases such as Alzheimer's and to neurodevelopmental diseases including autism.



GLIAL CELL JUNGLE: Star-like astrocytes (red) and oligodendrocytes (green) intertwine with neurons (blue) in culture.

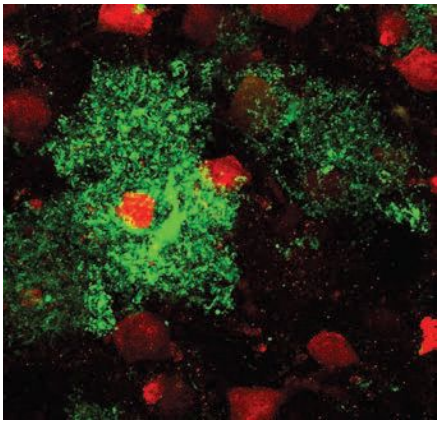
JONATHAN COHEN, NATIONAL INSTITUTES OF HEALTH; COURTESY: NATIONAL SCIENCE FOUNDATION

"All of a sudden, students are sensing that glia are interesting," says Stanford gliobiologist Ben Barres. "People are saying neurons are overstudied, and glia are understudied."

Here, *The Scientist* profiles an assortment of methods—both adapted from neuronal work and developed specially for glia—that are propelling glial research forward.

Panning for glia

One challenge in studying glia is that the cells have proven difficult to purify and grow in culture. More than two decades ago, Barres began to extract neurons and glia from rodent tissues using a method called immunopanning, in which cells are progressively depleted or harvested from solutions as they bind to selected antibodies stuck to the bottom of petri dishes. Over the years, Barres developed immunopanning antibodies to capture a variety of cells, such as myelin-forming oligodendrocytes, which coat neuronal processes with fatty insulation, as well as astrocytes from the rodent optic nerve. But he and others were unable to purify intact, mature brain astrocytes and maintain them outside the body. Researchers had figured out how to isolate astrocyte precursors from the brains of newborn animals, but the cells lacked normal gene-expression patterns and were flat, not star-shape.



STAR STRUCK: The many branches of an astrocyte (green) wrap around neuronal cell bodies (red) and ensheathe synapses (not shown) in the cortex of a mouse. Two-way communication between neurons and astrocytes is necessary for normal brain development and function.

In 2011, Barres and his colleagues finally devised an immunopanning method to extract mature brain astrocytes and developed a protocol to grow the cells in a medium that lacked serum, which contains proteins that are filtered out of blood before it enters the brain. Under these conditions, the astrocytes thrived, retaining their 3-D structure and exhibiting gene-expression patterns characteristic of the cell type in vivo (*Neuron*, 71:799-811).

Soon after developing this new immunopanning method, Barres's group demonstrated a new role for murine astrocytes: eating synapses. Astrocytes isolated from mice engulf nerve terminals in culture, and cells missing the phagocytosis-related genes *Megf10* and *Mertk* gobble up fewer neuronal synapses, indicating the genes' importance in synaptic pruning (*Nature*, 504:394-400, 2013). "It's been really like taking candy from a baby," says Barres. "As soon as you can purify the cells and culture them, you can start to gene profile them and start to understand what genes are unique, and that gives you clues immediately about their function."

RNA readouts

Techniques for purifying glia, along with increasingly advanced RNA-sequencing technologies, have aided researchers in ambitious projects to record the transcriptomes of glial cells. Last year, Barres and his colleagues isolated neurons, astrocytes, three types of oligodendrocytes and oligodendrocyte precursors, microglia, and two types of blood vessel–forming cells from mouse brains and published a database detailing gene expression in the purified cells (*J Neurosci*, 34:11929-47, 2014). Barres is working to publish a similar database of gene expression in human brain cells. "We figured out how to purify human astrocytes from any age: from fetal all the way to 70-year-old human brains," Barres says. "There's just some extraordinary information there."

Glial transcriptomics should help researchers elucidate the roles of different glial subtypes, design better conditional knockout mice, and identify genes that are conserved among mammals, fish, and invertebrate model organisms. And it may not even be necessary to isolate and culture the cells first. Jim Eberwine, a molecular neurobiologist and genomicist at the University of Pennsylvania's Perelman School of Medicine, has developed a new method to sequence RNA from single cells still lodged in their tissue microenvironments (*Nat Methods*, 11:190-96, 2014).

Transcriptome in vivo analysis (TIVA) relies on molecular tags that researchers introduce into live brain tissue. The group can selectively activate the tag in certain cells or regions using light. Once activated, the TIVA tags change conformation, exposing a section that binds the tails of messenger RNA. The researchers then purify and sequence the bound mRNA. (See "Molecular Multitasker," *The Scientist*, April 2014.)

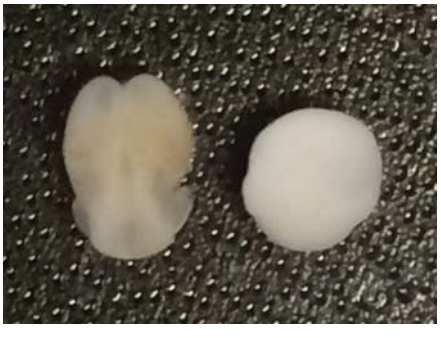
Investigators interested in applying the technique to gliobiology can use microscopy to view how individual astrocyte processes interact with neurons, for instance. Then, using TIVA tags, they can collect RNA from specific astrocyte populations, specific astrocytes, or even from specific astrocyte appendages. "When you homogenize all the cells, they lose their shapes, they lose their interactions," says Eberwine.

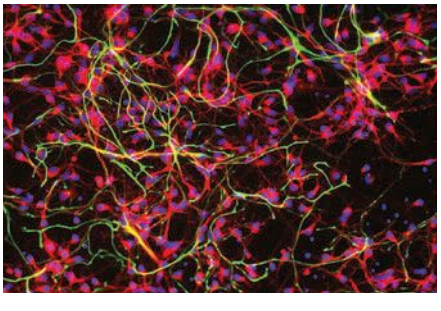
Since publishing their original paper, the researchers have gathered transcriptomic data from mouse and human astrocytes.

Grow your own glia

Some researchers have dispensed with complex methods for isolating glia in their natural context. Instead, they are generating their own glial cells for study. Last May, a Stanford University team led by neuroscientist and stem cell biologist Sergiu Pasca demonstrated a method for creating spheres of astrocytes and cortical neurons from human induced pluripotent stem cells. The neurons in the balls, which reach diameters of 4 to 5 mm, show gene expression patterns that recapitulate neural maturation during human fetal development. "We noticed and were very happy to see that the astrocytes were not flat," says Pasca. "They were these beautiful star-like cells."

The approach allows Pasca and his team to generate such cortical spheroids using cells isolated from people with autism or schizophrenia, which could help researchers study abnormalities in the for-





GROW YOUR OWN GLIA: (Top) A 61-day-old human cortical spheroid sits to the right of an intact embryonic mouse brain. At this stage of the spheroid's development, astrocytes are beginning to appear and neurons are forming connections. (Bottom) Glial cells (astrocytes, green) and neurons (red) from a human cortical spheroid that has been enzymatically dissociated and cultured.

BEN BARRES AND LU SUN; PASCA LAB, STANFORD UNIVERSITY

mation of neuronal connections that are characteristic of these disorders. "There is something unique about the 3-D interaction between the cells. They're very close to each other, and they're interacting much more like they are doing in the brain," says Pasca, who is currently maintaining more than a thousand cortical spheroids from multiple patients in his lab.

In vivo visions

Researchers are also trying to assess the role of astrocyte signaling in living mice. In 1990, Stanford neuroscientist Stephen Smith demonstrated that in response to glutamate, astrocytes release calcium in spikes that propagate as waves through networks of astrocytes (*Science*, 247:470-73). "This was a landmark discovery that kind of shook the foundations of neuroscience," says Dwight Bergles, Smith's former student, now a neuroscientist at Johns Hopkins University. "People thought that excitability and long-range signaling was the sole province of neurons."

> "There's a lot of things that fly glia do that mouse glia do."
>
> —Marc Freeman, University of Massachusetts Medical School

Since then, scientists have discovered that calcium released by astrocytes can regulate cerebral blood flow, metabolic rates, and structural changes in astrocytes. It can even trigger release of signaling molecules from astrocytes. What's still unclear is when and why these various glial functions kick in. For this, genetic calcium indicators such as GCaMPs have proven a perfect tool. While GCaMPs were designed to serve as an indirect measure of neuronal firing, they are a direct measure of the calcium activity within cells. "The genetically encoded calcium indicators are just absolutely ideally suited for understanding astrocyte biology," Bergles says.

But because calcium indicators were designed for use in neurons, researchers have had to optimize their use for imaging glial activity. For instance, users found that it was difficult to image signaling in astrocytes' narrow processes, as the GCaMP molecules gathered primarily in the cell body. So Bergles and his colleagues generated mice whose astrocytes tether GCaMP molecules to their cell membranes, making it easier to see when calcium floods the membrane-rich processes.

And, of course, gliobiologists are hard at work generating transgenic mice whose glial cells can be specifically tracked and manipulated. To do this, researchers must identify genes that are uniquely expressed in the subset of glial cells they hope to target, then put a gene for Cre recombinase under the control of the promoters of these glia-specific genes. When this enzyme is expressed, it deletes or inserts genes at sites in the genome that researchers have marked with short identifying sequences.

Researchers have created Cre lines that can be altered specifically in astrocytes, but the lines are not yet perfect. Various progenitor cells are often edited along with astrocytes, Bergles warns. And it is not yet possible to selectively manipulate astrocyte subtypes.

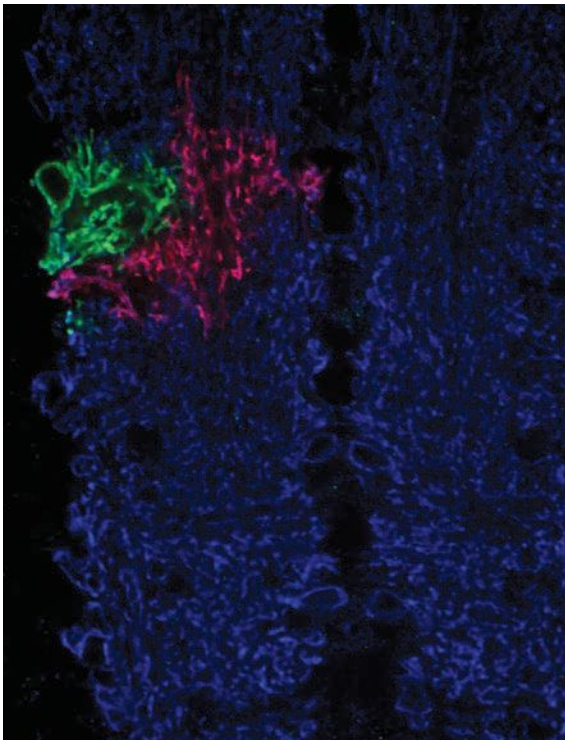
"I think down the line it would be really marvelous for people to develop tools to study heterogeneity among astrocytes [and] heterogeneity among oligodendrocyte progenitor cells," says the University of Virginia's Hui Zong, a biologist currently working on developing new tools for overexpressing genes in glial cells. "That will be another big wave of research."

Exploring other models

Fly biologist Marc Freeman thinks the way to settle some of the basics of gliobiology is to use simpler model organisms. "For whatever reason, the glial field has not embraced invertebrate model organisms like *C. elegans* and *Drosophila* anywhere near to the extent that the people who work on neurons have," he says.

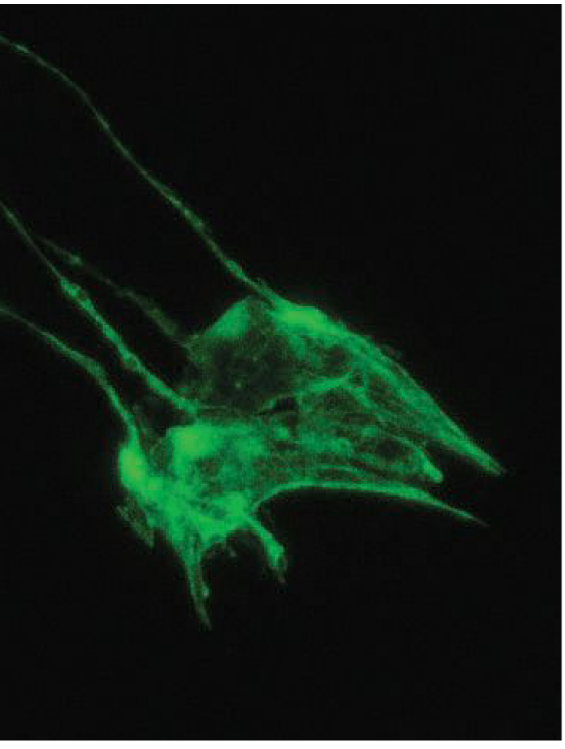
In the 1990s, some researchers decided that fly glia were too dissimilar from mammalian glia to serve as useful analogs. But after 10 years characterizing subsets of glia, "we are now finally at a point where we have a cell type in the fly that we call an astrocyte," Freeman says—and these cells are proving to be remarkably similar to mammalian astrocytes. For example, Freeman's group showed that fly astrocytes engulf synapses, in a publication paralleling Barres's study showing the same in mice (*Genes Dev*, 28:20-33, 2014). "There's going to be some things that mouse glia do that fly glia don't," says Freeman. "But there's a lot of things that fly glia do that mouse glia do, and I think those things are going to keep us busy for years and years."

Another organism that might prove beneficial in the study of glial cells is the zebrafish. These animals do not have classical star-shape astrocytes, but their transparent embryos have proven an excellent system for studying how oligo-



PRETTY FLY: Two differentially labeled sibling cells (red and green) in a group of *Drosophila* astrocytes (blue) in the brain of a third instar larva. Such labeling shows the size, morphology, and unique placement of each astrocyte in the developing fly brain.

TOBIAS STORK/MARC FREEMAN; GEORGIA RAPTI (OPPOSITE PAGE)



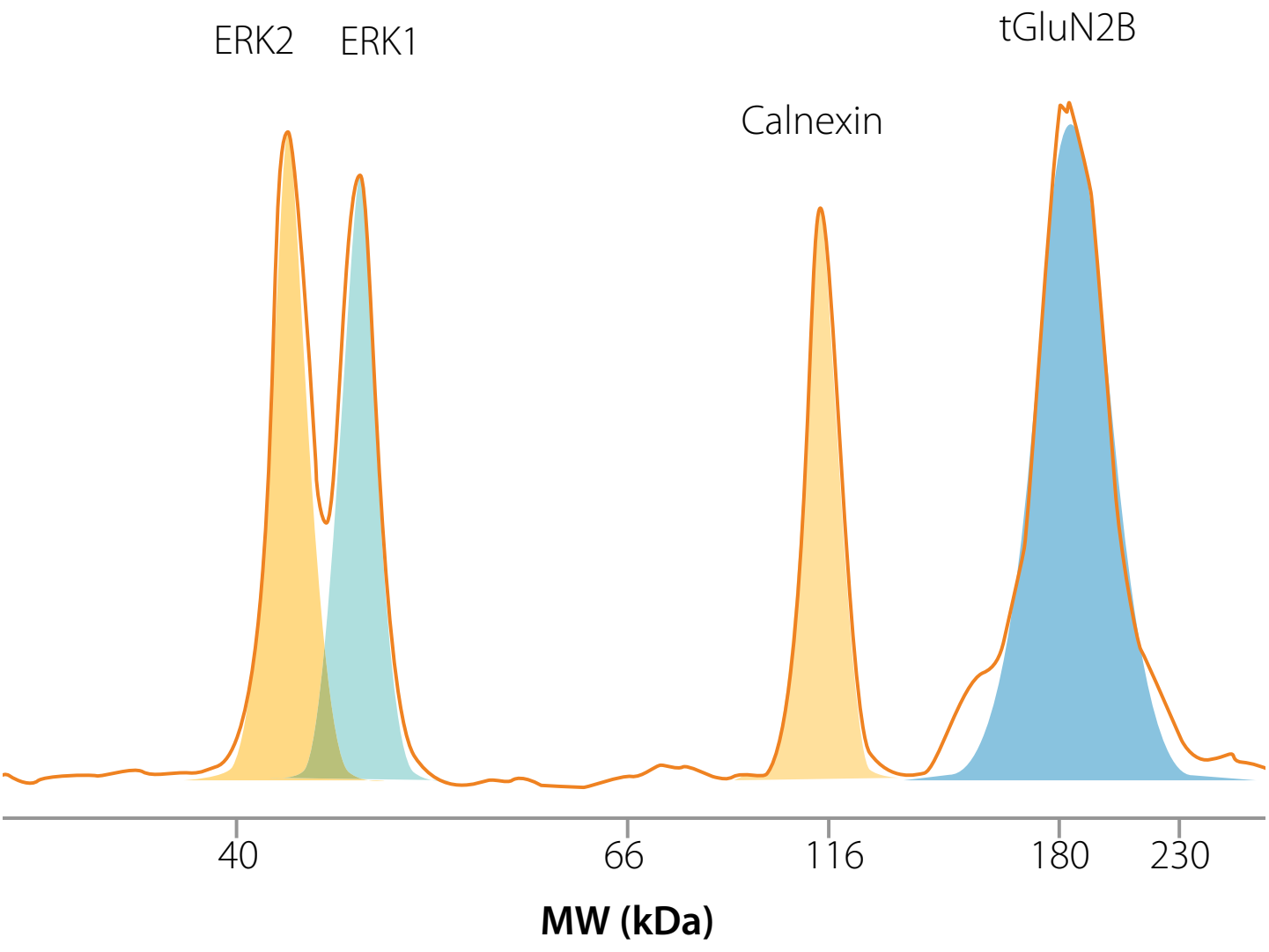
WORM POWER: The nematode *C. elegans* is a simple creature with just 50 glia, two of which are shown here in green. These glia have multiple processes reminiscent of those of vertebrate astrocytes and are key to the worms' development.

dendrocytes myelinate neurons. Developmental neurobiologist Bruce Appel of the University of Colorado, Denver, for example, made a line of transgenic zebrafish with fluorescently labeled glial cells. "It marked those cells very beautifully," he says. Earlier this year, Appel and an independent group at the University of Edinburgh used the fish model to show that neuronal signals influence how oligodendrocytes myelinate axons (*Nat Neurosci*, 18:683-89, 2015; *Nat Neurosci*, 18:628-30, 2015).

A third model system that could shed light on glial function is *C. elegans*. The nematode's glia are less morphologically complex than those of mammals, zebrafish, or flies, but these worms have a unique advantage: researchers can ablate specific glia in the worms without damaging associated neurons. Moreover, the simple nervous system of *C. elegans*—all 302 neurons and 50 glial cells of it—has been comprehensively mapped. Working in *C. elegans*, "we've shown there are many levels to the glial influences," says Rockefeller University's Shai Shaham. "Glia affect the shapes of neuronal receptive endings [and] the ability to transmit electrical stimuli."

"People who are very forward-thinking, they see that worms and flies helped project the field of neuron biology forward by decades, probably, in terms of gene identification and functional characterization," says Freeman. "If there's a chance that fly glia could do that [for gliobiology], you have to try, right? And I think it's going to happen." ■

Multiplexed by Wes.



ERK1/2, Calnexin and tGluN2B detected in rat brain tissue by Wes for Sarah, a researcher at the Neuroscience Institute, Medical University of South Carolina.

Decon Recon

Published genomes are chock-full of contamination. But as awareness of the problem grows, so do methods to help combat it.

BY SARAH C.P. WILLIAMS

When Supratim Mukherjee noticed the same bacteriophage sequence popping up again and again in hundreds of microbial genomes from a database he was analyzing, he got excited. Mukherjee, a bioinformatician at Lawrence Berkeley National Laboratory, was comparing the metabolic pathways of the microbes, and he began to wonder what the nearly ubiquitous sequence was. "I thought we must have discovered something novel," he recalls. "This entire bacteriophage genome was intact in all these diverse microbes."

But when Mukherjee looked up the bacteriophage sequence, he learned that it was the sequence of PhiX, a bacteriophage sold by Illumina as part of the company's sequencing kits to run alongside a genome of interest. Ironically, PhiX is intended as a quality-control measure, to track error rates on any given sequencing run. But in many hundreds of cases, Mukherjee found, researchers had failed to remove the PhiX sequences from their published genomes.

Mukherjee's experience isn't an isolated one. A recent slew of reports has shown that the contamination of published genomes is more widespread than ever. (See "The Great Big Clean-Up," *The Scientist*, September 2015.) How does this happen? And what can you do to ensure that your sequences don't become part of the problem?

The Scientist asked a handful of researchers to share their experiences with contamination and their best tips to detect or prevent these rogue sequences. Here's what they said.

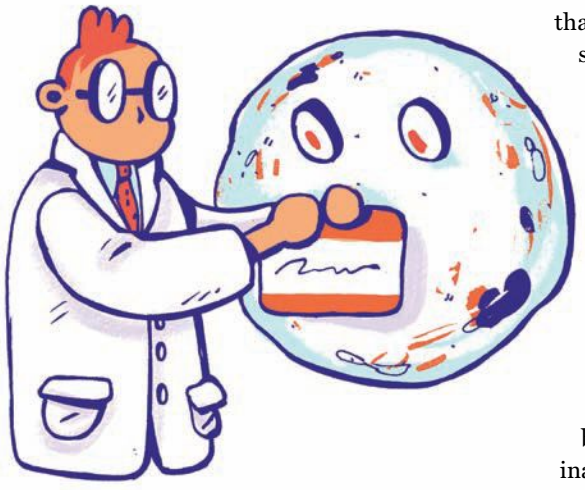
Widespread genome contamination

When Mukherjee's team realized that PhiX contamination was rife in published microbial genomes, the group decided to quantify just how often this happened. Among the 18,000 bacterial and archaeal genomes published in the public Integrated Microbial Genomes database, more than 1,000 are contaminated with PhiX sequences, the researchers reported earlier this year (*Standards in Genomic Sciences,* 10:18, 2015). Around 10 percent of the contaminated sequences had also been published in peer-reviewed publications.

PhiX contamination is just the tip of the iceberg—and the problem is growing exponentially, says David Lipman, director of the National Center for Biotechnology Information (NCBI), which has been screening sequences submitted to its GenBank database for the past five years. "In 2012, we were detecting con-

ILLUSTRATIONS BY JACKIE FERRENTINO

tamination in 2 to 3 percent of bacteria and archaea submissions," Lipman says. "But then it started climbing steadily, and by 2014 it was close to 10 percent. This year, so far, it's on the order of 23 percent." The rise, Lipman hypothesizes, is tied to the increase in the number of labs—including many that don't specialize in genomics—that are now sequencing genomes.



Microbial sequences aren't the only ones riddled with contamination. Last year, computer scientist William Langdon of University College London discovered that at least 7 percent of the human genomes included in the 1000 Genomes Project were contaminated with mycoplasma genetic material (*BioData Mining*, 7:3, 2014). (See "Out, Damned Mycoplasma," *The Scientist*, December 2013.) So it's safe to say that if you're struggling with a contaminated genome, you're not alone.

Sources of contamination

There are a few ways that contamination happens, says bioinformatician Rob Edwards of San Diego State University. "The first is that someone in the lab can confuse two samples and accidentally mislabel a specimen or a file," he says. "That's something you can easily mitigate by having a good lab management system and improving your record keeping." (See "Lab 2.0," *The Scientist*, December 2012.)

Alternatively, contamination can stem from extraneous genetic material that's been introduced into a sample. Or, if you're culturing bacteria you collected from the environment, Edwards says, it's not uncommon for multiple species to appear in a sequencing run, even if you think you only streaked a single culture. Likewise, if you're sequencing microbes from the human gut, your sample will naturally have human cells. Even the genes in a mitochondrion or chloroplast could be considered contamination if you only want an organism's nuclear genes. These contaminants can't be completely avoided, but there are measures that can be taken both to clean up your sample before you start sequencing and to purge your sequencing results of any residual contamination.

Edwards, whose team focuses on metagenome sequencing from environmental samples, says his group often uses size filtering to separate organisms upon starting with an especially mixed bag of viruses and bacteria, as in a test tube of seawater. Or, if the researchers suspect contamination with human DNA, they hybridize their gene samples with human genetic material to remove it, leaving only the microbes.

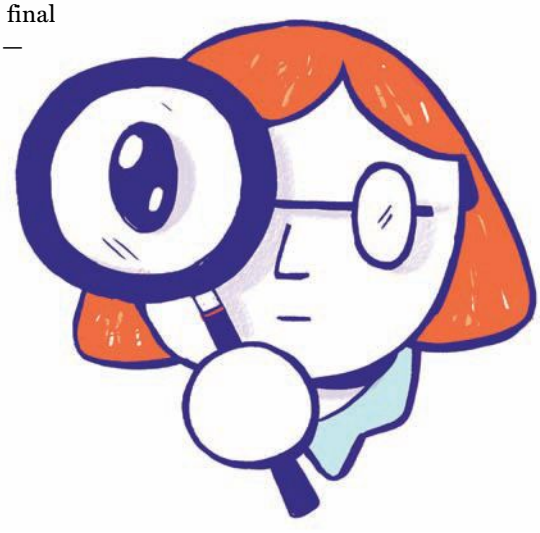
Similar clean-up approaches can be used when you're dealing with contamination that comes with the protocols you use, such as sequences from PhiX controls, from primers and adapters added to samples to amplify and sequence genes of interest, and from cloning vectors—the genetic vehicles that allow foreign DNA to be copied by host cells.

Yet another source of contamination is dirty machines that bleed through between experiments, letting genes from the prior sequencing run appear in the next one. Just being aware that this kind of contamination might exist in your experiments can help you choose methods to remove it after sequencing, Edwards says. Or, if it shows up repeatedly, you can try changing protocols or troubleshooting your machine.

Detective work

There's no question that getting rid of contaminants as early as possible in your process is ideal—for all sorts of reasons. "Contaminants are bad because they increase the direct costs," says Dominik Laetsch of the University of Edinburgh. "You literally get fewer nucleotides per dollar" when you spend time processing and analyzing unwanted sequences. But here's the good news: even if your sequences are filled with PhiX, primers, vectors, and genes from species you didn't intend, you can get rid of all the evidence before anyone else sees your final genomes.

Laetsch is among those developing tools to help clean up sequence data before analysis. Blobtools-light, the newest version of his software, compares your DNA contigs—the overlapping bits of sequenced DNA that are eventually assembled into a final sequence—with known sequences from NCBI's databases. Then, it provides an easy-to-interpret visualization of this com-



ILLUSTRATIONS BY JACKIE FERRENTINO

parison, lumping together sequences thought to be from similar organisms. "We use this as an initial screening tool," says Laetsch, who studies the bacteria that inhabit pathogenic nematodes, and so is frequently faced with data sets from multiple species. "We can see right away when there are low coverage contigs that aren't needed."

A similar program, called ProDeGe (Protocol for fully automated Decontamination of Genomes), was also described this year (*ISME,* doi:10.1038/ismej.2015.100, 2015). Like Blobtools, the protocol uses public databases to detect contamination in a genome assembly, then groups contigs into "Clean" or "Contaminant" groups. While Blobtools-light provides a visual charting of sequence groups, ProDeGe spits out lists that users can read through to identify contaminants and determine what they might be. "You don't have to know a lot about it to use it," says Mukherjee, who has used the ProDeGe software. "So for biologists who are scared of these kinds of tools because they don't know how to do bioinformatics, it's a great solution."

More-specific tools also exist. NCBI, for instance, offers VecScreen, which quickly identifies contaminating vectors in your sequence. And tools that are even more advanced will be available on the NCBI site later this year, Lipman says.

All the tools available to detect contamination must balance specificity and sensitivity—identifying sequences that are definitely contaminants without removing sequences that are part of the target genome—so it's important to understand the results in the context of your data set, says Edwards. If you're analyzing a genome from a novel group of species, for instance, programs may flag high levels of contamination simply because existing databases don't already contain homologues for the organism's genes—so take the results with a grain of salt.

Or, if you're expecting to see high levels of bacterial genome contamination, you can immediately home in on those sequences among the full list of contaminants. Because you know more about the source of your initial sequence and how it has been handled than any automated program, you can make these kinds of judgment calls, Edwards says. "I definitely recommend running your assembly through several different tools and comparing the results."

Decontamination strategies

Once you've pinpointed the contamination—and, hopefully, where it came from—you can move toward cleaning up your data. There are tools to do that, too, including DeconSeq, developed by Edwards's group. Unlike the more-automated contamination screen programs, DeconSeq requires the user to input contaminant species. Then it automatically removes sequences belonging to those species from the genome assembly.

If you skip the step of contamination removal before you finalize a sequence, someone else might catch it. At NCBI, for instance, Lipman's group runs a foreign contamination screen on every sequence submitted to GenBank. He hopes that when the screen flags a sequence as contaminated, scientists use it as an opportunity to learn more about their data set and its weaknesses, and change their methods to avoid the problem in the future. "If you just say, 'OK, I had a little problem in my submission and now I fixed it,' then this problem is going to keep happening," Lipman says.

If a genome is already published in the literature or a database by the time you detect contamination—when you're running more experiments, for instance—then own up and fix the mistake as soon as possible, before it ricochets into the work of other researchers who might be relying on your data for their own experiments. In some cases, this might mean contacting a journal to find out whether a correction or a full retraction is warranted.

"People need to take ownership of their data," says Mukherjee. "If you find a problem, retract it, clean it up, and then give it back."

Will the contamination problem get better?

It's tempting to speculate that as sequencing technologies advance, many of the sources of contamination will simply disappear. There's some truth to this, says Laetsch. "As the assembly process gets easier, and read lengths start to increase, it will definitely become even easier to pinpoint contamination," he says. But researchers shouldn't take that as a signal to stop screening for it, he adds. "Sequencing machines are only as good as the samples you put in them."

And as databases of genomes become larger, it becomes increasingly difficult to go back and sort through them all to ensure clean sequences. It's up to individual researchers to do their best with each genome, says Mukherjee. "I think the scientific community in general acknowledges that contamination is a big problem, but there has to be a bigger concerted effort to do something about it."

Even as contamination rates in GenBank skyrocket, Lipman agrees that there is growing awareness of the problem. Part of what's leading to the increasing contamination, he points out, "is the positive story that more and more labs are able to do sequencing, which is a wonderful thing." ■

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Ready, Willing, and Able

Researchers with disabilities are making their fields more accessible.

BY AMANDA B. KEENER

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A few years ago, the University of Delaware's Karl Booksh realized he was the only tenured chemist that he knew of at a top-tier research university who had gone through all of his scientific training, from college onward, with a disability—in his case, paraplegia in his legs and limited use of his hands and arms. This made him wonder: Where were all the scientists with disabilities?

A statistics-oriented person, Booksh scoured data going back to the mid-1980s on the numbers of PhD-level scientists in the U.S. who were living with disabilities. According to one report from the National Science Foundation (NSF), in 2013 about one in nine scientists and engineers under the age of 75 identified as having a disability, not far from the national rate of about 12 percent. But for more than half of those scientists, the onset of disability occurred after the age of 40, once their careers had already started rolling. The number of students who have disabilities when they enter science, Booksh says, has been lagging for decades. He realized that if he wanted something done about the bottleneck for would-be scientists with disabilities, he was going to have to do it himself. "There are not that many people in a position to and willing to pick up the flag and start waving it," he says. "This is my first charge at the castle."

Booksh has teamed up with University of Delaware biochemist Sharon Rozovsky to run an NSF-funded Research Experiences for Undergraduates (REU) program specifically for students with disabilities. Once a week for the past three summers, students with diverse disabilities, from mental health conditions and learning disabilities—such as autism spectrum disorder and severe obsessive-compulsive disorder—to physical disabilities, such as deafness and blindness, have gathered to devise strategies for success. They discussed when to disclose disabilities to potential employers, how to identify specific needs and request accommodations, and ways to overcome preconceived ideas about what someone with a disability can or can't do.

Twenty-five years after the passage of the Americans with Disabilities Act, there are still hurdles for scientists with disabilities, but there are also resources available to these researchers to adapt their labs and offices and to improve accessibility for future generations. *The Scientist* spoke with a handful of these individuals.

Strategic choices

For Oregon Health and Science University (OHSU) biologist John Brigande, who has suffered from progressive hearing loss since age nine, the key to success in science was finding the right lab. After about a year as a postdoc at the University of Texas at Austin, Brigande says his hearing loss had gotten so bad that he was forced to take it into consideration when making career decisions. "If I was going to be able to realize this dream of a career in science, I needed to find a training environment that could support my hearing loss most effectively."

Brigande accepted a postdoc position in the Purdue University lab of Donna Fekete, who studies inner ear develop-

ment. Fekete had been a member of Brigande's doctoral thesis committee at Boston University, so he knew she was sensitive to the needs of students with disabilities. He also found Purdue to be "incredibly proactive," he says. "From the moment I stepped into the lab, I had assistive listening technology that helped me communicate with lab members."

Brigande says even his choice of field was strategic. "If any scientific meeting on the planet might have captioning during the presentations so that I could read what was being said . . . it should be the Association for Research in Otolaryngology."

Richard Mankin, an entomologist with the US Department of Agriculture and current president of the Foundation for Science and Disability, was also strategic in choosing a research path that would suit his physical abilities. Mankin was born with arthrogryposis, a condition that causes the muscles to shorten and limits joint flexibility. "I didn't pick a field where I had to go mountain climbing," he says. His research does involve some fieldwork, but, "I'm mostly just walking around in citrus groves or other things that I can do . . . without a lot of accommodation," he says.

To disclose or not

Many researchers with disabilities acquired their limitations after their careers were already underway. Booksh was a freshman in college when a neck injury he sustained during a flag football game caused him to lose the use of his legs and some use of his arms and hands. Fortuitously, he was already doing chemistry research by that time, and he credits that line of study with keeping in him school after his injury. Although he could no longer do bench work efficiently, he could still design experiments and analyze data, so he focused on data-driven projects for the rest of college and through graduate school.

When writing letters and things, don't disclose your disability on page one. Page one should be about what you've accomplished.

—Karl Booksh, University of Delaware

Things got harder for Booksh as he began applying for jobs after earning his PhD. Potential employers would ask him, "How can someone in a wheelchair do chemistry?" he recalls. "I had to keep pointing out that the most successful faculty didn't know where the pipettes were in the lab."

Booksh says he often felt he needed to prove he could be as successful as any other scientist without asking for extra or resources. "I had to go about things by completely denying that I had a disability and by doing as much as I could, asking for no accommodation." For example, he delayed adopting voice recognition software to help him write faster, and instead took to working extra long hours. As a mentor he now realizes "that attitude is not healthy [long-term]."

Instead, he encourages his students to be up front with potential employers about their particular needs. "I've told the students, whatever they do, don't do what I did." But, he adds, "when writing letters and things, don't disclose on page one. Page one should be about what you've accomplished. [That] puts the person out there primarily as a darn good scientist who happens to have done all of this while having a disability."

It's also important to disclose your limitations in the lab, Brigande says. Some people will go out of their way to accommodate you once they know; others will simply avoid you, he says. "I respect both approaches."

Bill Jacobs, a microbiologist at Albert Einstein College of Medicine in New York with progressive vision loss, says he was never afraid to ask for help from his colleagues. Three years ago, he asked his institution to pay for a personal assistant to help him read, edit, email, and more. It makes Jacobs's job a whole lot easier, and the unique position benefits the assistants as well, by giving them a taste of life as a principal investigator. So far, all have gone on to earn higher degrees, Jacobs notes.

Not everyone is anxious to broadcast their limitations, however. "It's very difficult for a person with a disability of any kind to come into a challenging training environment and confidentially seek the sort of resources they need," Brigande says.

Stephanie Kerschbaum, an English professor at the University of Delaware who studies disability among faculty, says that advisors should be careful not to make students or trainees feel compelled to disclose disabilities. "There are [equal opportunity]

EARLY START: In 2013, the University of Delaware's Karl Booksh (middle) launched a Research Experiences for Undergraduates (REU) program designed to help students with disabilities prepare for a career in science.

EVAN KRAPE, UNIVERSITY OF DELAWARE

offices aimed at negotiating and ensuring the provision of accommodations," she wrote to *The Scientist*.

Making it work

Savannah Stark, one of the REU students who trained at Delaware this summer, says that one of the most valuable aspects of the program is simply learning what kinds of accommodations she needs to ask for. While doing experiments in the lab full-time, Stark, who was diagnosed with ADHD in fourth grade, has worked with her advisor to come up with helpful strategies, such as writing out the details of their discussion after each meeting. "I've definitely found some ways to adapt," Stark says.

Other researchers with disabilities have also found ways to adapt the lab to them. In addition to assistive listening technologies, Brigande uses captioning software to make calls from his office. And his former employee, who was also hearing impaired, brought a full-time sign language interpreter to the lab each day.

For Albert Einstein's Jacobs, adapting to his progressive blindness has been an ongoing process. At first, only his peripheral vision was poor; now, he essentially sees through a tunnel and can only read one word at a time. So rather than read, Jacobs often listens to research articles using a program called Dragon Medical, which he says has vocabulary for most of the science papers in his field. And when he's working on papers with his students, he has them read the text aloud to him to expedite the editing process. "I find it to be very effective," he says.

Maxine Linial, a virologist at the Fred Hutchinson Cancer Research Center in Seattle who lost her sight five years ago after being hit by a car as she crossed the street, also relies on technology to help navigate the logistics of life as a scientist. Specifically, she uses voice recognition software called JAWS to read and write emails and simple documents. But the program can't pronounce scientific terms very well, she says. Early in her days back in the lab after the accident, she says, "I kept getting emails from 'Oz.' . . . It took me a few weeks to realize it was AAAS. I thought the wizard was emailing me."

AUBREY MOORE OF THE UNIVERSITY OF GUAM

STRATEGIC FIELDWORK: Richard Mankin, an entomologist with the US Department of Agriculture, chose to study insect smell and communication—a line of research that was compatible with his limited mobility as a result of arthrogryposis.

For more involved work, such as reading scientific literature, analyzing data, and reviewing papers, Linial has found that a full-time, scientifically trained assistant is the better option. She works closely with biochemist Karen Craig, who also does literature searches and collaborates with Linial to draft manuscripts and grant applications. "She's a science sister," Linial says.

Improving access

In addition to changing their own lives and labs to work with their disabilities, many scientists are also working to improve accessibility within their institutions and fields. (See "Handicapable," *The Scientist*, September 2015.) Brigande has worked with other hearing-impaired researchers to expand captioning at the Association for Research in Otolaryngology's annual meeting to make it possible for them to participate without having to ask for help. "[Currently], we expect a hearing-impaired person to come to us and say, 'I need assistive listening technology for this presentation.' That's the culture," he says.

Margaret Price, an English professor at Spelman College in Atlanta who has written extensively on disability in academia, says that being proactive is key. "There's a huge difference between, 'Let us know if there's anything you need,' and 'We're already doing x, y, and z, and we hope that people will join in a conversation about what more can be done,'" she wrote to *The Scientist*.

Price began collaborating with Delaware's Kerschbaum on improving the accessibility of academic meetings when Kerschbaum, who is deaf, found there was no interpreter at a disability committee meeting at the Conference on College Composition and Communication, forcing her to rely on Price's notes. Since then, the committee has put together a website called composingaccess.net, which is full of resources to make academic presentations more accessible. "It is not only for deaf people, but also benefits people who may have auditory processing difficulties, who don't see images well, [or] who may retain information better when they read [it] than when they hear [it]," says Kerschbaum. "The list goes on."

To make meetings and seminars work for her, Linial says she often finds seminar speakers before their presentations and tells them, "If you give the kind of seminar I can understand, I will ask you a good question." She has also learned to give talks without dependence on visual aids, a skill that she's started to teach students in her department. "I used to be the 50 minutes, 45 slides kind of [speaker]," she says. "Now I know you have to get up and talk about your work." ■

Brain New World

The melding of mind and machine uncovers mysteries harbored in the brain.

BY MALCOLM GAY

This summer, Duke University researchers announced with much fanfare that they had successfully created a monkey "brainet," a new frontier in brain-machine interfaces whereby scientists used computers to link the brains of multiple animals. Pooling their combined mental activity, the monkeys cooperated to gain control of a virtual arm, moving it to various targets on a computer screen.

The invasive study, led by Miguel Nicolelis, was undoubtedly a breakthrough: a collaborative cyborg network. But such accomplishments are par for the course in a field where, for more than 15 years, researchers have routinely announced advances using electrodes and computers to "read" the intentions of behaving research subjects. Translating the neural code thus deduced, scientists in this relatively narrow field have granted animals and humans neural control over everything from computer cursors and wheelchairs to video games and flying drones. In the case of Jan Scheuermann, one of the brave paraplegics I feature in my new book, *The Brain Electric*, researchers successfully connected her brain to a flight simulator for an F-35 fighter jet.

Resplendent with sci-fi sizzle, brain-machine interfaces, or BMIs, are indisputably having a moment. Companies such as Emotiv and Muse are selling off-the-shelf EEG headsets aimed squarely at the consumer market. Auto manufacturers are experimenting with neurally integrated cars. A new breed of "neuro-marketers" has entered the advertising realm, and a slew of smaller enterprises now promise that BMIs will deliver myriad benefits, including enhanced relaxation and heightened mental clarity.

Fueled by pop-cultural offerings, including James Cameron's *Avatar* or the 2009 Bruce Willis vehicle *Surrogates*, it's easy to imagine that our brain-tapped future is already upon us—that Google, once confined to our desktops or handsets, will soon be in our brains.

Still, in many ways the hard science that underlies this imagined future of BMIs offers a more radical and informative vision of humanity and brain function. As I recount in the *The Brain Electric*, Nicolelis has for years been using BMI research to push the boundaries of neuroscience, describing an expansive view of a brain that readily adopts new modes of interacting with the world.

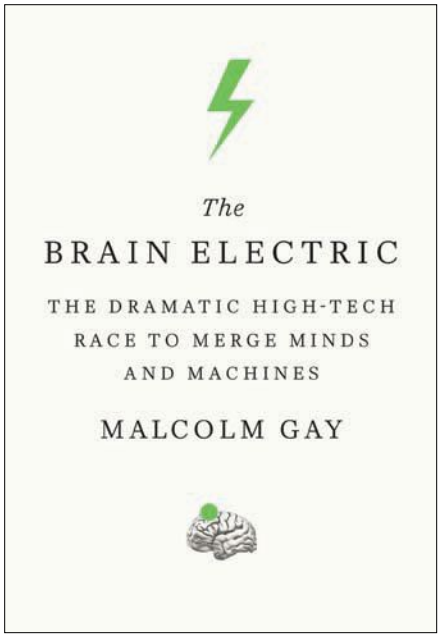
In the past, that's meant linking brains to various robotic devices. More recently, however, Nicolelis has moved into ever-more fantastical realms as he's enabled animal subjects to perceive infrared light, and, more to the point, given them direct neural access to the minds of their fellow research subjects.

"It's not about moving an arm. It's about suggesting that the brain is so plastic that it can incorporate another body as its source of information to probe the world," Nicolelis told me while discussing his early efforts to build a brainet. "That touches on theories of self, theories of identity. Once you connect brains like that, who is to say there's not another level of emergent properties that materialize by the interaction of the two brains?"

In many ways we have no idea, and a thicket of technical and scientific obstacles must be overcome before BMI researchers can examine more than keyhole-size regions of the brain.

Nevertheless, as BMIs and related devices progressively enter consumer and clinical markets, the research also offers a powerful tool to probe the brain's deeper mysteries, shedding light on its sensory systems, while also describing links among the motor system, perception, and even consciousness itself.

The revolution that BMIs promise, then, is not one merely of cyborg futurism and a brave new world. Rather, it is something infinitely more profound: a more precise articulation of the world we've always known. ■



Farrar, Straus and Giroux, October 2015

Malcolm Gay is an arts reporter for The Boston Globe. *He has won numerous national journalism awards, including honors from the James Beard Foundation, the Association of Alternative Newsmedia, and the National Association of Black Journalists. Read an excerpt of* The Brain Electric *at the-scientist.com.*

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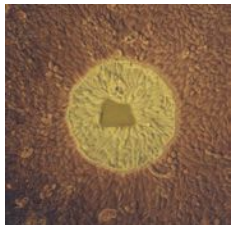

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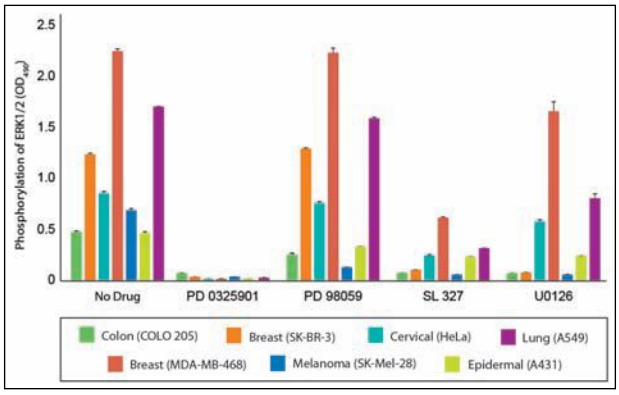

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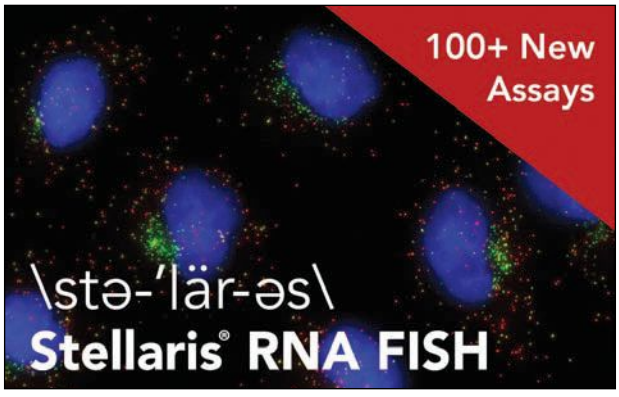

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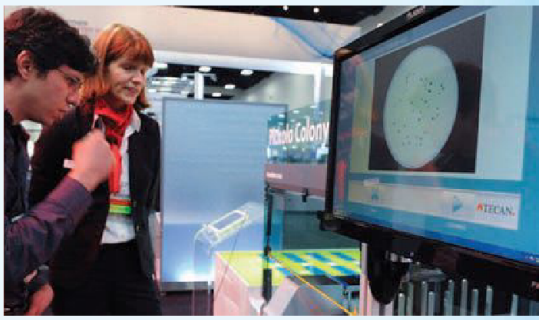

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The First Neuron Drawings, 1870s

BY AMANDA B. KEENER

It's 1873 at a hospice hospital near Milan, and a young Italian physician named Camillo Golgi is surrounded by brains and nervous tissue from animals including cows and dogs, as well as from recently departed patients. Unaware that he will one day become famous for, among many things, discovering the "Golgi body," the 30-year-old scientist currently has a single goal: to capture the mysterious details of neurons by staining them with a concoction of his own design.

He impregnates formalin-fixed nervous tissue sections with potassium dichromate and silver nitrate, causing microcrystals of silver chromate to fill in the delicate dendrites and axons of neurons. The technique paints the structures black and makes them visible in situ for the very first time. Golgi would spend several years perfecting the reaction, which likely started out as an accidental discovery. According to Paolo Mazzarello, director of the Golgi Museum in Pavia, Italy, Golgi may have first noticed the reaction's potential to mark neurons while staining connective tissues surrounding the brain's blood vessels with silver nitrate, a commonly used compound at the time. "He probably saw some partial staining of the [nerve] cells," and from there, a combination of chance and intuition led Golgi to develop the invaluable method.

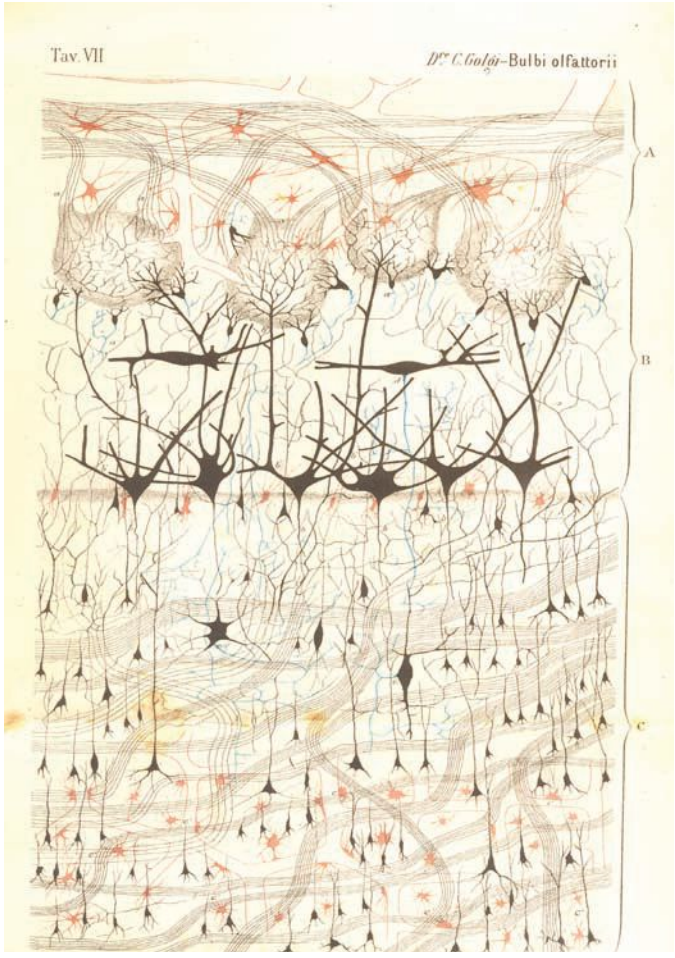
Still in use today, Golgi's black reaction, now called the Golgi stain, made it possible to see the fine structures of neurons and their branching processes, enabling Golgi and many scientists after him to draw conclusions about how neurons interact with each other.

"By using this method, it was possible for neuroscientists and neuroanatomists to lay down the microscopic anatomy of the nervous system," says Mazzarello.

Golgi's first published drawing of neurons stained using the black reaction featured the nervous structures of a dog's olfactory bulb. Golgi used a camera lucida to project an image of the stained tissue onto paper so that he wouldn't miss any details in his drawing. He categorized the tissues of the dog olfactory bulb into three layers: a thin outer layer of bundled neuronal axons, or nerve fibers; a middle layer of gray matter made of cell bodies and dendritic processes; and a dense layer of white matter containing alternating layers of nerve fibers and glial cells.

Of the dendrites he saw in the middle layer, Golgi wrote, "they cross and lie over each other in a most complicated manner, but never enter into any real connections or anastomoses." The question of connections was an important one to Golgi. Mazzarello says that when Golgi examined his preparations, "he had in mind a sort of holistic conception of the brain, [an] idea that the brain worked as a unit."

Ironically, Golgi's method would be used several years later by Santiago Ramón y Cajal to support the latter's view of the nervous system as a collection of individual elements. "With the same method, one saw network everywhere and the other saw single cells interacting everywhere," says Mazzarello. ■



A FIRST LOOK: Camillo Golgi's original black-and-white drawing of a dog's olfactory bulb appeared in a paper in the *Rivista Sperimentale di Freniatria e Medicina Legale* in 1875. Colored plates appeared in reprinted versions distributed by Golgi and in the German translation of his paper. Golgi indicated three layers in his drawing. The superficial layer (A) is composed of nerve fiber bundles made of myelinated axons (black lines), a branching blood vessel (outlined in red), and glial cells (red). The nerve fiber bundles enter spheres of cell bodies called glomeruli in the middle layer (B), which consists of gray matter (nerve cell bodies and dendrites). Large mitral cells lining the middle layer (black) are arranged such that their dendrites reach toward the glomeruli, where they form synapses with the axons extending from the superficial layer. The mitral cell axons (some of which are shown in blue) aim for the inner layer (C) of the olfactory bulb. The inner layer also contains granule cells, blood vessels, glial cells, and nerve fibers.

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