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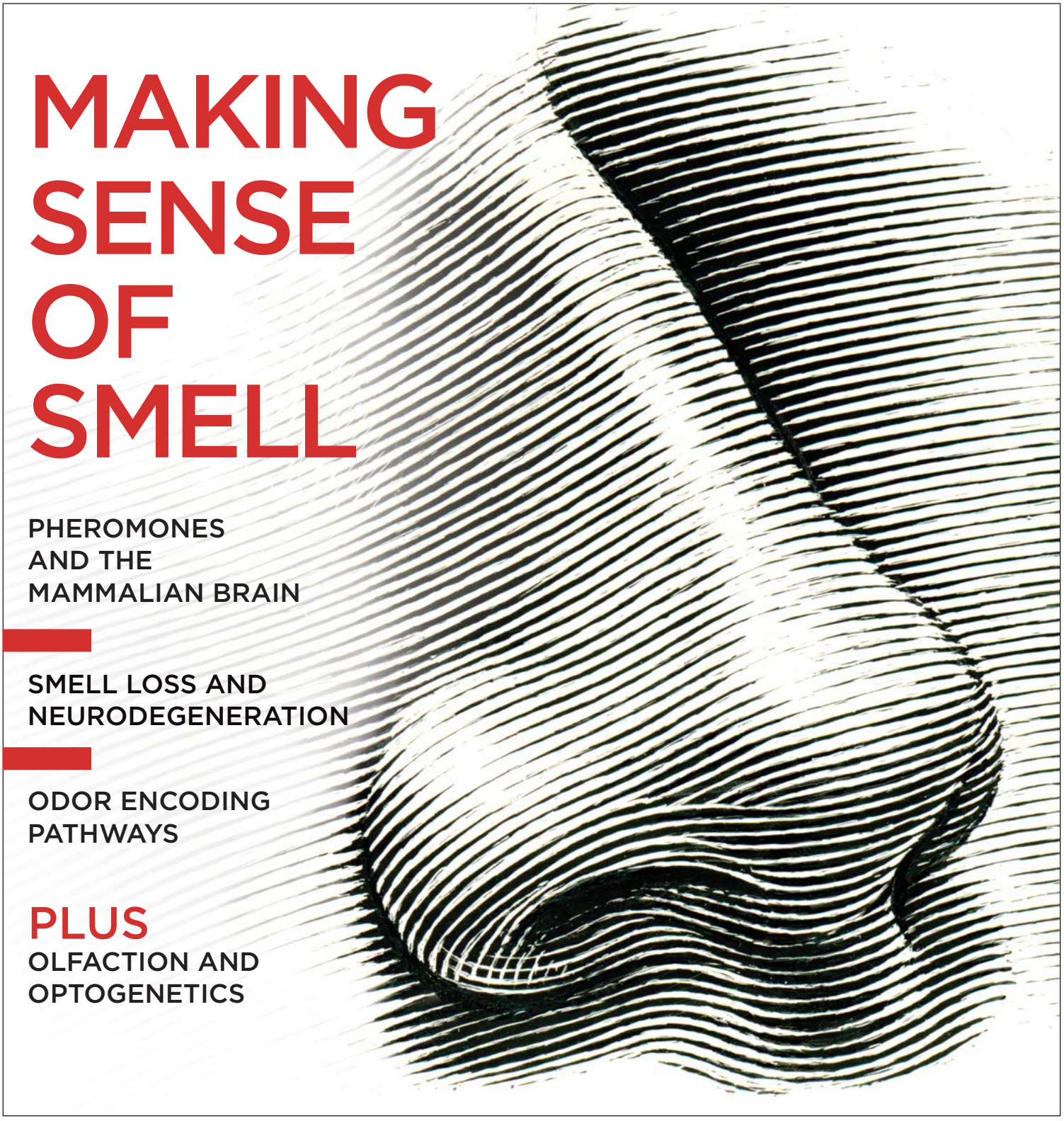

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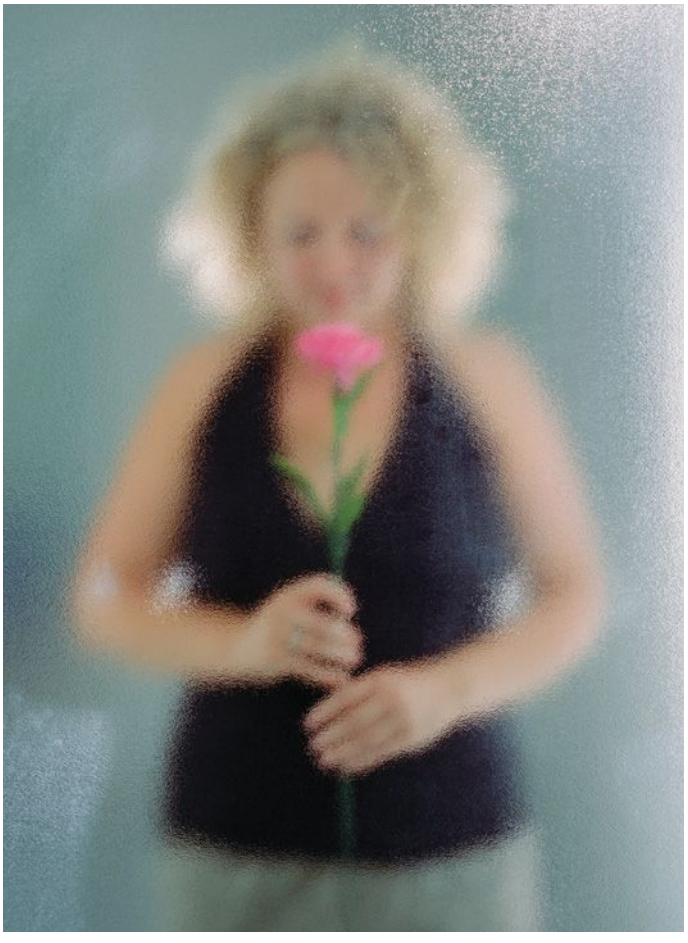

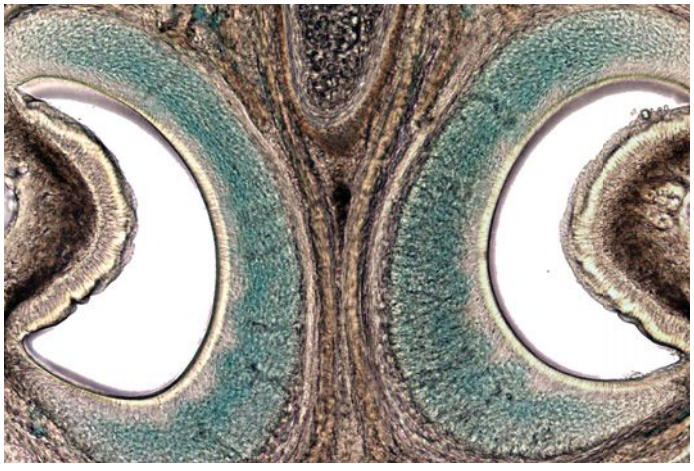

Features

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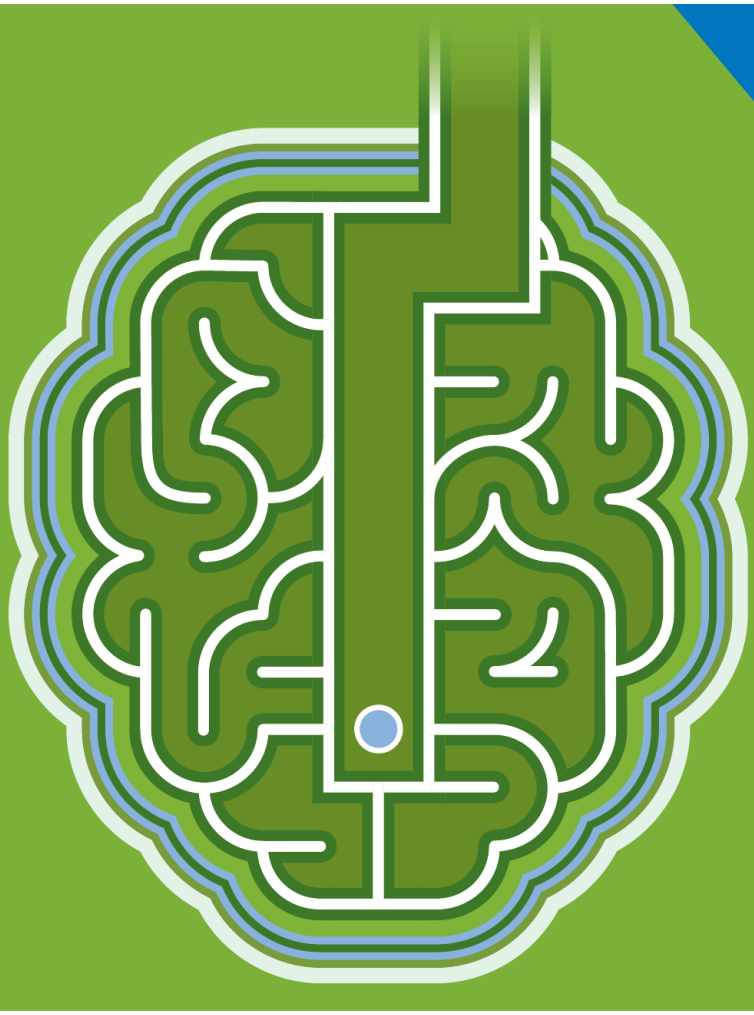

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Department Contents

CORRECTIONS:

In "A Hair-Raising Solution?" (September 2013), David Steinberg's name was omitted from the list of PureTech Venture recruiters who signed on dermatologist George Cotsarelis.

In "Microbial Fuel Factories" (September 2013), the outdated term archaebacterium was used to describe two microbes instead of the current preferred designation archaeon.

"Going Viral" (September 2013) incorrectly noted the names of the first authors in three of the listed references (Ref. 2, N.A. Moran; Ref. 4, S.R. Modi; and Ref. 10, A.M. Comeau, H.M Krisch).

The Scientist regrets the errors.

Online Contents

THIS MONTH AT WWW.THE-SCIENTIST.COM:

VIDEO
Croak-O-Matic
Watch University of Wisconsin researcher Barrett Klein's robotic túngara frogs calling to females.

SLIDE SHOW
We Are Family
See denizens of tight-knit lab groups living it up at social functions.

VIDEO
Organic Gardeners Extraordinaire
Observe the amazing symbiosis between leafcutter ants and the fungal gardens they tend.

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

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

- The rise of deep-brain stimulation—in the clinic and in the lab
- The genetics of schizophrenia and bipolar disease
- Annual Salary Survey results
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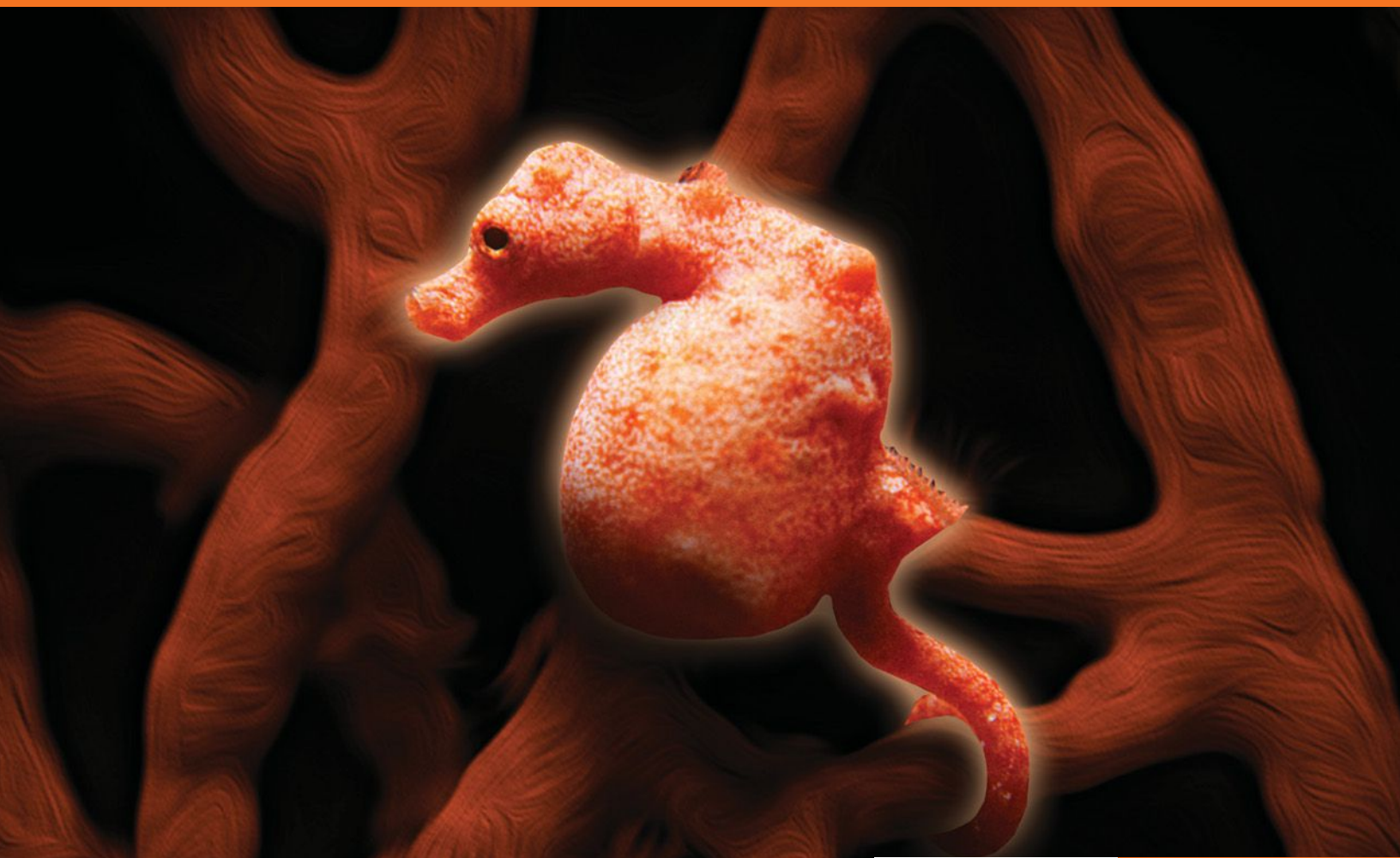

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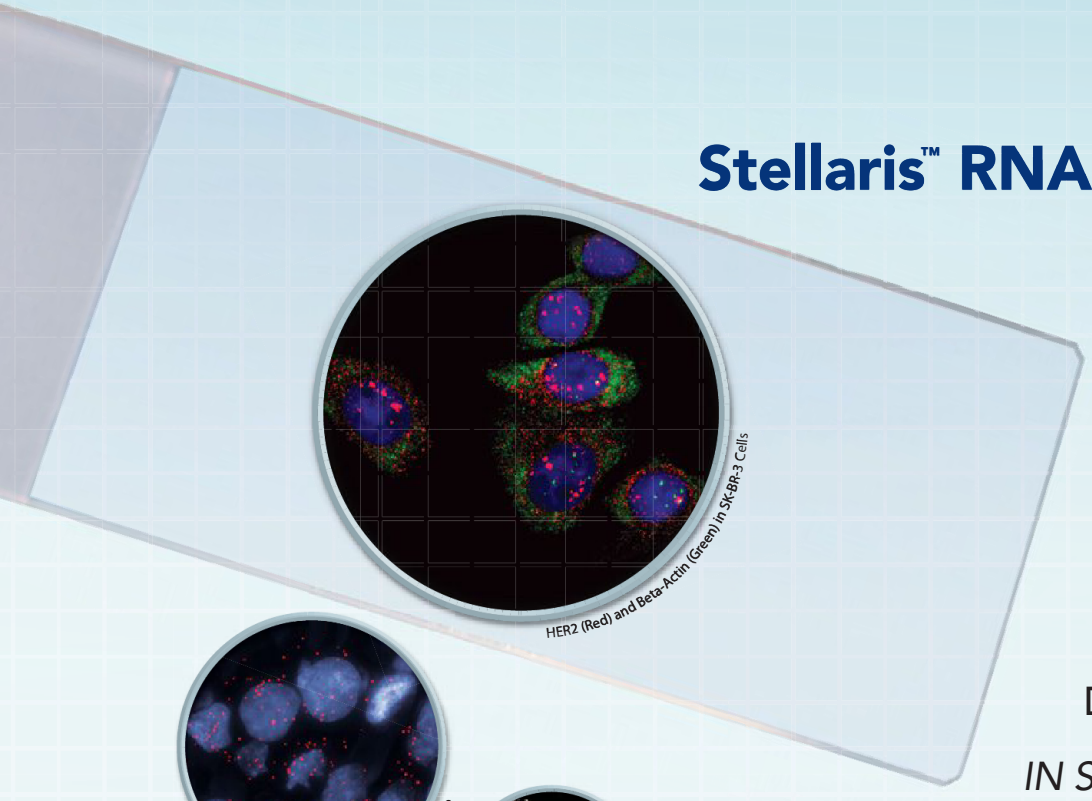

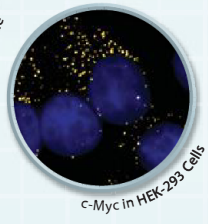

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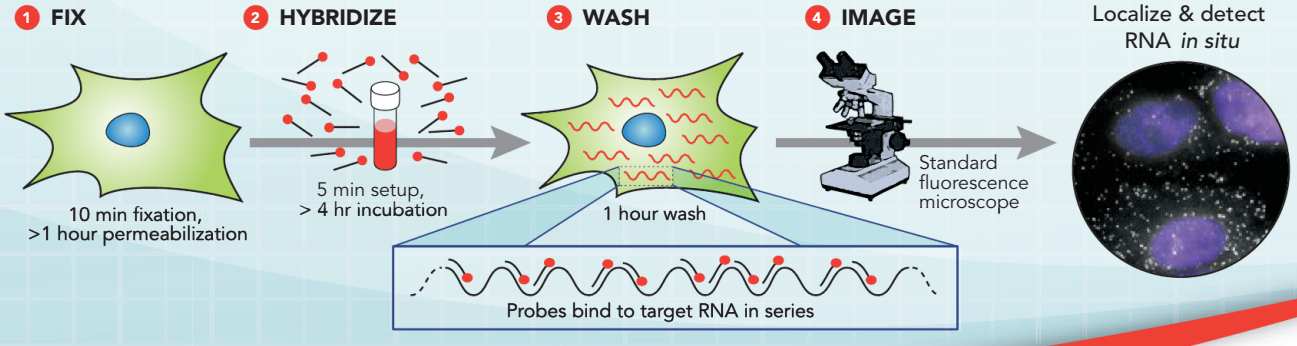

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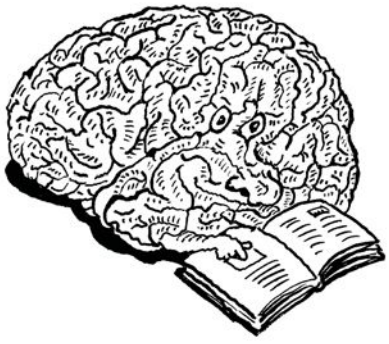
Contributors

C. Ron Yu's first passion was not biology. Growing up in the city of Hangzhou, near Shanghai, Yu was fascinated with quantum mechanics and relativity. He fondly remembers the first experiments he performed in his high school's physics lab using instruments that dated to the 1940s. "The lab was nothing compared to what you can find in US schools," he says. As an undergraduate at Tsinghua University in Beijing, Yu worked to embed biosensors into lipid membranes. The project spurred his interest in applying physics to biological systems, and he went on to major in biology and minor in physics. From there, he studied the biophysical properties of nicotine receptors as a graduate student at Columbia University, using an electrophysiology rig that he built from the ground up. Although he contemplated a postdoc in Germany, Yu stayed at Columbia while his wife wrapped up her PhD work in the U.S. One of his dissertation committee members, Richard Axel, who shared the 2004 Nobel Prize for cloning the first odorant receptor, invited Yu to try out a project with him in the meantime. "Before I knew it, I was his postdoc," says Yu. The position—investigating the formation of neural circuits in the olfactory system—lasted eight years, after which he started his own lab at Stowers Institute for Medical Research in Kansas City, Missouri. He now studies mammals' behavioral responses to pheromones, which is the topic of his feature article "A Pheromone by Any Other Name" on page 38.

Sensory scientist **Richard Doty** first became excited about science when he took a psychology class at Whitworth College in Spokane, Washington. "I enjoyed the concept of the field—it was a nodal point for all kinds of exciting things," he says, "from animal behavior to the human brain." He subsequently graduated from Colorado State University, and went on to do a master's degree at California State University, San Jose, in conjunction with NASA's Ames Research Center, where he studied space flight and the sensitivity of the vestibular system to low-levels accelerations. During his PhD studies at Michigan State University, Doty discovered a scent gland in the belly of field mice and other wild mouse species that secretes sebum used in territorial marking. This led him to the University of California, Berkeley, where he studied odor communication in dogs. In 1980, he and his colleagues received a grant from the National Institutes of Health to set up the first clinical research center focused on the study of smell and taste at University of Pennsylvania. Doty is well-known for his invention of the University of Pennsylvania Smell Identification Test. Among other applications, the self-administered scratch-and-sniff test has shown how smell loss can herald Alzheimer's and Parkinson's disease, which Doty writes about in his feature "Smell and the Degenerating Brain" on page 32. He is also author of seven books, including *The Great Pheromone Myth*.

Robert Perlman grew up around the University of Chicago, studied there, and is now a professor emeritus at the same institution. His father was a surgeon and faculty member at the university's medical school. Perlman started college after his sophomore year of high school as part of a University of Chicago program for young entrants. He enrolled in his alma mater's medical school immediately after graduation to avoid the draft, and stayed on at the university for a few years to do research before heading off for his internship and residency at Bellevue Hospital in New York City. He then got a commission in the public health service and studied bacterial genetics at the National Institutes of Health (NIH). At the NIH, he helped to discover the role of cyclic AMP in regulating bacterial gene expression. Perlman never made it back into clinical medicine. He taught at Harvard Medical School and the University of Illinois at Chicago, where he did research on the biology of adrenal chromaffin cells, before returning to the University of Chicago. In the 1990s, while serving as dean of biological sciences, Perlman became hooked on thinking about evolution and its connections to human biology and medicine. That topic became the focus of his recently published book *Evolution and Medicine*, discussed in his essay "Dr. Darwin at the Bedside" on page 74.

Get a Whiff of This



An issue devoted to the latest research on how smells lead to actions

BY MARY BETH ABERLIN

For Proust, it was madeleines; for my kids, pizza. The Brooklyn neighborhood in which they grew up was dotted with pizzerias from which wafted the mouthwatering smells of newly baked pies. Eating pizza was a definite pleasure for them, but evoking the experience was another thing altogether. One of their favorite books was a scratch-and-sniff book that let the reader smell his way through the construction of a pizza—tomato sauce, cheese, mushrooms, oregano, the finished pie—odors remarkably like the real ones. Even scratching the pages now, some 30 years later, you can still inhale faint traces. Or maybe that's just the scent of memories . . .

Once a year, *TS* devotes the better part of an issue to one of the senses. In 2011 it was taste; 2012, touch; this year, smell. As we were pulling content together, the staff realized that there were many, many ways to cover this particular sense. Olfaction is complex. It's less acute in humans than in most animals, however, and is processed somewhat differently by other species—insects, fish, rodents—commonly used as models for studying smell. We decided to concentrate mostly on the reports of researchers using the latest tools and techniques to probe into the nitty-gritty of how the olfactory system is wired to deliver an odor message to the brain, and how that delivery translates into behavior.

Humans don't use antennae to parse odors, as insects do, nor do humans seem to have special organs that respond to a class of compounds called pheromones. While there is little debate about the role pheromones play in eliciting insect reproductive behaviors, and general agreement about their role in certain mammals, there's plenty of contention when it comes to humans. In "A Pheromone by Any Other Name" (page 38), C. Ron Yu lays out what is currently known about the vertebrate vomeronasal organ (found in vestigial form in human infants), the molecular machinery it uses to translate the perception of pheromones into action, and the developing realization that these elusive molecules are involved in more than just sexual behavior.

Behavioral changes are hallmarks of a number of neurodegenerative diseases. And so are disturbances in the ability to smell. In "Smell and the Degenerating Brain" (page 32), Richard Doty describes how olfactory loss often accompanies Alzheimer's and Parkinson's diseases, and how diagnosing smell dysfunction early could help differentiate between these and other disorders. Doty marshals evidence to support the hypothesis that "cholinergic dysfunction plays a significant role in the olfactory loss seen in a number of neurological diseases."

This month's profile, "An Olfaction Odyssey" (page 56), describes John Hildebrand's contributions to the study of smell. He pioneered using the giant sphinx moth as a model to study the development of the insect antennal olfactory system, including a gender-bending experiment that he describes as "one of the greatest 'wow, gee-whiz' discoveries" to come out of his lab. And this past January, he and colleagues published in *Science* what he considers the "culmination" of his work on olfaction: the discovery that action potentials are sparked deep in the brain when a sphinx moth smells an odor that is "behaviorally significant," and that learned odor preferences can be added to the moths' innate repertoire.

You can read about optogenetics in many places, but here Amber Dance ("Scents in a Flash," page 62) covers the technique's use in sorting out different aspects of olfaction's complexity.

Finally, the entire Literature section (pages 54–55) is devoted to recent scientific publications that relate to olfaction. Two studies use calcium imaging: one to follow scent selectivity by individual neurons in olfactory glomeruli; another to show that a network of as few as 25 nerve cells activated in a *Drosophila* mushroom body encode enough odor information to explain a fly's behavior. The third paper reports that odors evoke a neural afterimage that may play a role in memory formation. Madeleines? Pizza?

Next year: the sense of sight. ■

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

ANDRZEJ KRAUZE

Speaking of Science

Just think, during the day the mosquito is sleeping and doesn't need to smell you. But when the sun goes down, the mosquito's olfactory system becomes extra-sensitive, and she is ready to smell and then bite you.

—**Samuel Rund,** University of Notre Dame PhD candidate, discussing the results of a paper he recently published in *Scientific Reports* reporting that mosquitoes smell human odors better at night (Aug. 29)

It could be physiology, it could be the DNA and the molecular structure of his ligament as opposed to somebody else's.

—Mets General Manager **Sandy Alderson,** opining in *The New York Times* about why 30-year-old Detroit Tigers pitcher Justin Verlander has avoided injury while pitching so many innings (Aug. 27)

The way we teach [science] now, with an hour of instruction here and a laboratory class there, it doesn't allow for what has been my experience: that immersion is the essence of scientific discovery.

—Nobel Laureate **Elizabeth Blackburn,** on how to improve science teaching in the United States (*The New York Times*, Sept. 3)

Ultimately, it would be ideal to see Lego offer many more non-stereotyped female characters like the Scientist in their sets, and it would be even better to see them go back to marketing such sets to both boys and girls.

—University of California, Davis, PhD student **Elizabeth Sweet,** on the release of Lego's first-ever female scientist figurine, Professor C. Bodin (*LiveScience*, Sept. 4)

It appears that what we currently call "schizophrenia" may comprise disorders with quite different trajectories. For some people, remaining on medication long-term might impede a full return to wellness. For others, discontinuing medication can be disastrous.

—**Tom Insel,** director of the National Institute of Mental Health, in a blog post on benefits and drawbacks of long-term antipsychotic medications (Aug. 28)

With rare exceptions, it is hard to think of a single truly novel psychotropic drug that has emerged in the last 30 years.

—Weill Cornell Medical College psychiatrist **Richard Friedman,** in a *New York Times* piece, "A Dry Pipeline for Psychiatric Drugs" (Aug. 19)

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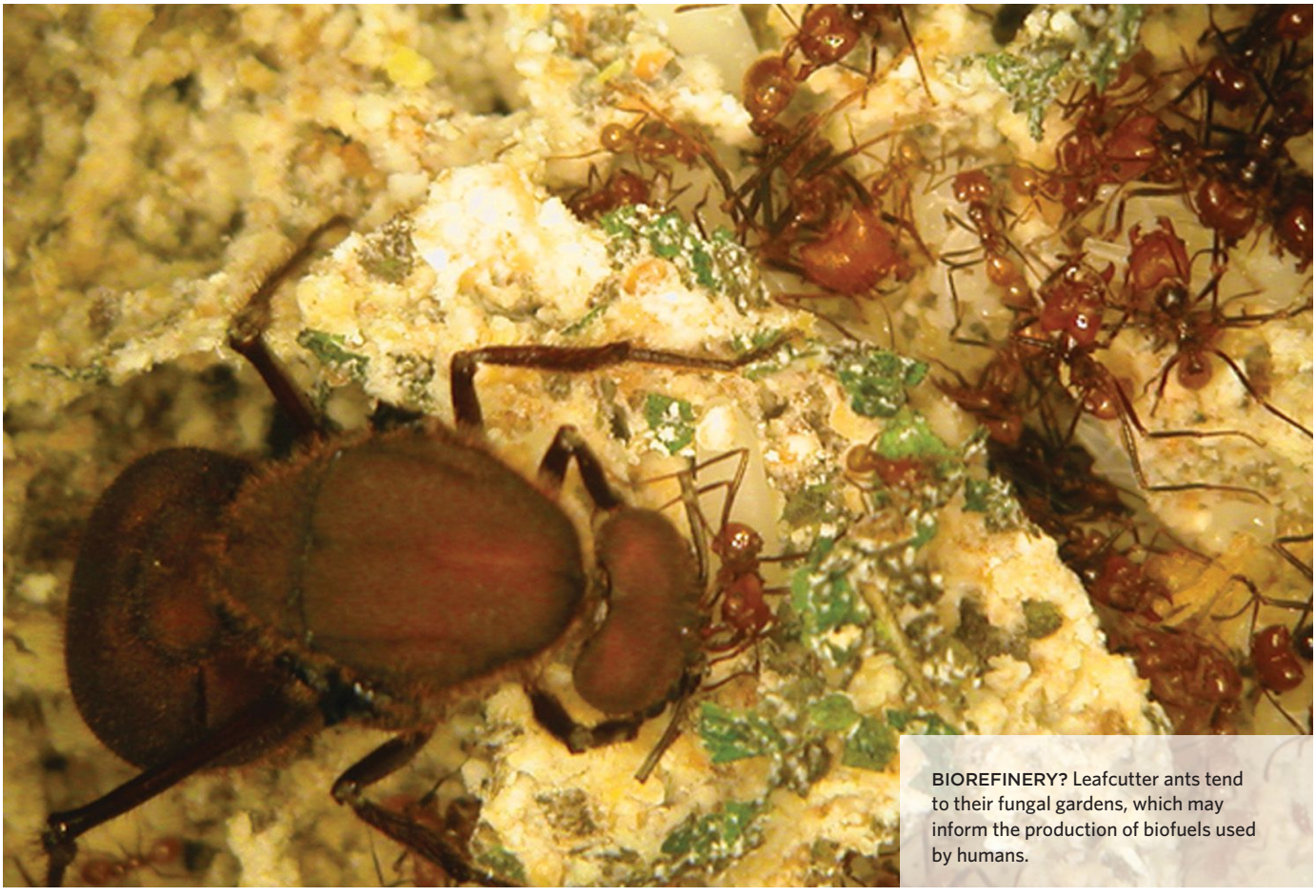


BIOREFINERY? Leafcutter ants tend to their fungal gardens, which may inform the production of biofuels used by humans.

MICHAEL POULSEN (UNIVERSITY OF COPENHAGEN)

Biofuel Mimicry

In a humid room at the University of Wisconsin–Madison (UW), large Tupperware boxes hold thick beds of gray fungi, pockmarked with holes and crawling with leafcutter ants. The boxes are home to colonies of two leafcutter species, *Atta cephalotes* and *Acromyrmex echinatior*, brought back from the tropical forests of Panama and Costa Rica by bacteriologist Cameron Currie and his colleagues, who study these insect agriculturalists and the fungus gardens they tend.

Leafcutter ants create the largest colonies of any ant, with some comprising 8 million individuals. To sustain themselves, they march across the forest carrying vast quantities of leaves, piece by piece, in great green convoys, back to the nest. The ants use the leaves as fertilizer to cultivate gardens sown with bacteria and *Leucoagaricus gongylophorous*, a fungus that produces fruiting bodies packed with nutrients for the ants to feast on.

But these fungus gardens are more than just fascinating examples of insect agriculture and symbiosis; they could also provide a model for the more efficient production of renewable biofuels. Currie and his team are using genomic and proteomic techniques to unravel the molecular secrets underlying this ancient symbiosis between fungus, bacteria, and ants. In the process, they are identifying novel enzymes that could be integrated into industrial processes to help convert abundant nonfood biomass into ethanol.

"This symbiosis has evolved over millions of years," says Frank Aylward, a graduate student in Currie's lab. "So it's a great place to look for enzymes that could be useful in biofuel production."

But the process by which the ants' fungus gardens convert biomass into nutrients is not well understood. It has long been assumed that *L. gongylophorous* drives the degradation of otherwise indi-

gestible cellulose, but some researchers aren't convinced, because the fungus can't always grow on cellulose in pure culture.

"This symbiosis has evolved over millions of years. So it's a great place to look for enzymes that could be useful in biofuel production." —Frank Aylward, University of Wisconsin–Madison (UW)

To address this issue, a team led by Currie sequenced the genome of *L. gongylophorous*. The sequences revealed around 200 genes potentially encoding lignocellulases—a class of enzymes that break down woody, or lignocellulosic, biomass. (*Appl Environ Microbiol*, 79:3770-78, 2013) "That showed that the fungus has the capacity [to break down plant biomass]," says Aylward, the first author of the paper. But just because the fungus has the genes doesn't mean that those genes are expressed, he adds.

So Aylward and colleagues also performed metaproteomic analyses of samples from the fungus gardens of both leafcutter species, and confirmed that 145 lignocellulases were actually present. "That's pretty definitive proof that the fungus is the primary driver of biomass breakdown," says Garret Suen, a bacteriologist at UW and a coauthor of the study. Indeed, very few of the lignocellulases appeared to come from the bacteria that live alongside the fungus, though the microbes play their part in the symbiotic relationship by fixing nitrogen for the fungus. The bacteria may also help the fungus access cellulose by breaking apart plant polymers that encase it, such as hemicellulose, adds Suen.

"Describing these dynamics at the molecular level offers new insights into the contributions made by the fungus to plant degradation," Michael Poulsen, who studies insect-fungus symbioses at the University of Copenhagen, writes in an e-mail to *The Scientist*. "[The study] adds an important piece of the puzzle of our understanding of one of the key features that has made this ancient symbiosis extremely successful."

The identification of enzymes capable of degrading lignocelluloses could also be a boon for the biofuels industry. Biofuel companies already use enzyme blends to break down starches from plant biomass, most commonly corn, into sugars to be fermented into ethanol. But the enzymes currently available can't break down tough-to-degrade molecules such as lignocelluloses on an industrial scale.

Armed with this suite of new enzymes capable of breaking down lignocelluloses, Aylward says biofuel companies may be able to use the leaves and stalks of the corn plant—rather than just the cobs, which inflates food prices—or other nonfood crops and waste products, such as grass, sawdust, or leaf litter. That would make ethanol production far more sustainable.

The idea is that the genes coding for these enzymes in the fungus gardens of leafcutter ants can be mass-produced in the lab by inserting them into *E. coli* or yeast, then used to break down feedstocks that require less land, fewer resources, and that don't compete with food crops.

"This is a highly evolved system, so the hypothesis is that the enzymes would be highly tuned to break down plant biomass," says Suen. "If we purify all of the enzymes we've identified, we should, in theory, get a highly valuable mix."

But first the team has to work out just how effective these new enzymes really are, and, since they work synergistically, to test countless combinations. Aylward himself is moving on to the Massachusetts Institute of Technology, where he will study microbial oceanography. But Currie's lab will carry on with characterizing the new lignocellulases, teasing apart the synergistic interactions, and finding the optimal combinations.

By analyzing samples from different strata within the fungus gardens, the team also demonstrated that the fungus produces different sets of enzymes at different stages in the degradation process. "I think that could be important for biotech applications," says Aylward. "A lot of people are using one enzymatic cocktail at the moment; perhaps we need to start thinking about doing it in stages."

To make matters even more complicated, researchers will also need to take into account the role of bacteria. "It could be that bacteria are producing some enzymes that enhance the efficacy of the fungal enzymes," says Aylward. "Integrating the bacterial component is likely to be important, because the synergism we see with leafcutter gardens is on the level of the entire symbiosis."

—Dan Cossins

Trouble in the Heartland

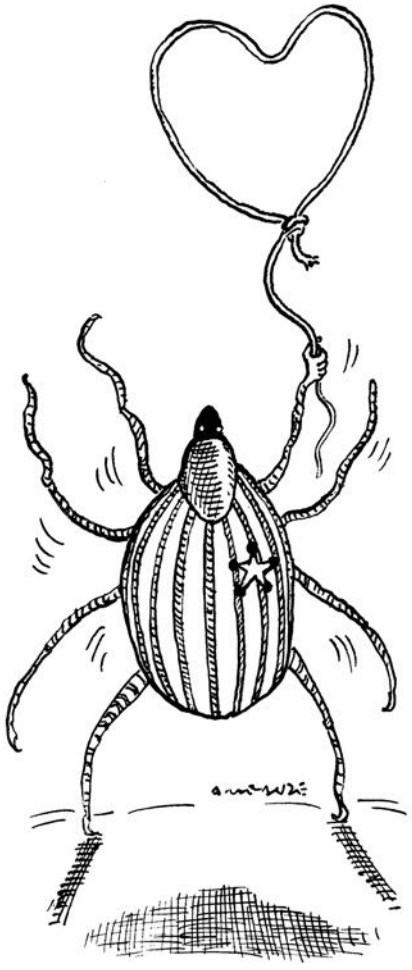
In June 2009, two male patients were independently admitted to the Heartland Regional Medical Center in northwestern Missouri with fever, headache, muscle pain, nausea, and diarrhea—all classic signs of ehrlichiosis, a common tick-borne disease in the region. Although both men reported having recently been bitten by ticks, blood and serum samples sent to microbiologist William Nicholson, chief of Pathogen Biology and Disease Ecology at the Centers for Disease Control and Prevention (CDC), came back negative for *Ehrlichia chaffeensis*, the disease-causing bacterium.

Nevertheless, Nicholson says, when the researchers plated the samples over a culture of canine tumor cells, they started to see signs of a pathogen. First, they noticed increased vacuole formation in the cells. "When we see that, within a day or two we usually see *Ehrlichia*," Nicholson explains. But in this case, no *Ehrlichia* appeared, and the cells eventually began to fall apart. Then, the single layer of cells that lined the bottom of the flask started to detach earlier than normal—within 6–7 days, instead of 2 weeks. Nicholson and his colleagues continued to transfer the cells to fresh media, "and then it'd do it again," he says. "That was an indication

that we have something in there, we just can't see it."

After finding none of the various pathogen culprits familiar to the researchers, Nicholson's group turned to their colleagues in the CDC's electron microscopy (EM) department for help. When they got the transmission EM images back, "you could clearly see the cell just loaded with virus," he says. "That was a nice bit of detective work," says Sam Telford, an epidemiologist at Tufts University.

Based on the virus's shape and size, the researchers suspected it belonged to the family Bunyaviridae. To get a more precise identification, Nicholson turned to Laura McMullan in the CDC's viral special pathogens group, which had recently purchased a 454 sequencer. Its sequence revealed the virus to be a novel Bunyaviridae species belonging to the genus *Phlebovirus*, and the researchers named it the Heartland virus (HRTV) following the convention of naming viruses after their region of origin, which was coincidentally the name of the hospital where it was discovered (*N Engl J Med*, 367:834-41, 2012).



The next step was to determine the virus's vector. Interestingly, the closest known relative of HRTV was the severe fever thrombocytopenia syndrome virus (SFTSV), a tick-borne *Phlebovirus* identified in 2011 after causing several cases of severe fever in China. Indeed, with both Missouri patients having reported tick bites, the researchers suspected that HRTV might also be carried by the arachnids.

In April, June, and August 2012, Nicholson and his colleagues collected more than 56,000 ticks of various species and life stages from several sites in northwestern Missouri, including the farms of both HRTV patients. They froze them in vials and sent them off to the CDC center at Fort Collins, Colorado, for molecular analysis. Sure enough, some of the ticks—specifically nymphs of the lone star tick *Amblyomma americanum*—carried HRTV, including those found at the farm of one of the patients (*Am J Trop Med Hyg*, doi:10.4269/ajtmh.13-0209, 2013). All told, however, the virus was relatively rare, Nicholson says, estimated to be present in about 1 in every 500 ticks. For comparison, *Ehrlichia* is found in some 10 percent of ticks. This rarity could explain why no virus was found in the ticks at the second farm, where the researchers were not able to collect nearly as many animals.

The researchers suspect that the ticks are becoming infected from the blood meal they ingest as larvae, after which they fall to the ground and burrow into the soil, where they will develop and molt into the nymphal stage. Then, when the nymphs emerge in the spring looking for their next meal, they can pass the infection on to people. Of course, "this is speculation based on the fact that we're getting these hot ticks in the spring," Nicholson says.

To get more answers, the team has been out in the field again this year, and is expanding its search for the virus from just ticks to the vertebrates that *A. americanum* generally feeds on, such as wild turkey, deer, raccoons, and gray squirrels.

"It just goes to show that the diversity of potential pathogens carried by ticks is fairly large."

—Sam Telford, Tufts University

As for the virus's origin, "none of us believe that this is a new introduction," says Telford. More likely, "it's been under our noses all along. It just goes to show that the diversity of potential pathogens carried by ticks is fairly large."

One possible explanation for the virus's recent emergence as a disease-causing pathogen, then, is the country's changing demographic. "The American population as a whole is aging," Telford notes. "Previously, maybe something like this was infecting perfectly healthy younger farmers in Missouri, and they just sort of shrugged it off." Indeed, the two case patients were 57 and 67 years old. "It's a pattern that we've seen in infectious biology all along—that as people age they become immune-compromised and far more susceptible to severe disease," says Telford, who in 1997 discovered a flavivirus carried by deer ticks—which also transmit Lyme disease—that has shown up on the radars of epidemiologists only in the last 5 years.

"It's much more than just a story of pathogen discovery and a new threat from ticks," he adds. "I think the more interesting stuff is how these [pathogen] communities evolved, where they come from, and what are the things that lead us to recognize them as potential causes of disease." —Jef Akst

Viral in Valencia

Something was amiss in the Spanish coastal city of Valencia. A dozen cases of hepatitis C, a potentially fatal blood-borne viral infection that causes cirrhosis of the liver, had turned up within a short time span in early 1998. As more cases popped

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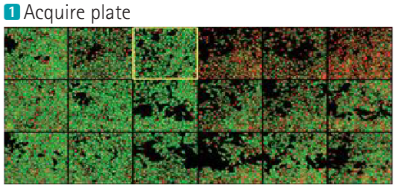

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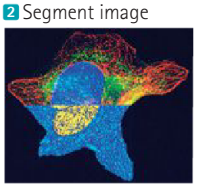

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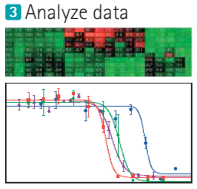

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ON TRIAL: Spanish anesthetist Juan Maeso (middle) with his lawyer in a Valencia court room, where he was charged with infecting 275 patients with the Hepatitis C virus over a 10-year period

up over the ensuing weeks, one fact linked virtually all the cases: the patients had at one time or another been admitted to one of two local hospitals.

Valencian public health department officials set up a committee of local scientists and epidemiologists to get a handle on the outbreak. One tool the health department planned to use to identify the source of the infections was a genetic analysis that was just starting to be employed in court cases related to HIV transmission. The forensic tool, based on the principles of molecular phylogenetics, could help infer the most recent common ancestor of virus strains from any two people based on the estimated rate of accumulated viral mutations.

Because of his experience in molecular biology, Fernando González-Candelas, an evolutionary geneticist at the University of Valencia, was tapped to head the health department's phylogenetic testing. As the investigation expanded, the number of possible cases of infection soared into the hundreds. "We had no idea when we were contacted that it was going to be such a big and complicated problem that it turned out to be," says González-Candelas. Ultimately, 275 people—almost all of them patients at one or both of two hospitals in Valencia—were determined to be victims of the outbreak, which stretched back to 1988.

When the Valencian provincial court learned of the health department's scientific committee, it asked to use the findings of the phylogenetic analysis as evidence for a criminal case against Juan Maeso, an anesthetist who worked regularly at the two hospitals (and occasionally at others) and who had administered painkillers intravenously to all of the known hepatitis C patients following surgical procedures.

González-Candelas and his team spent the next 2 years comparing 4,000 sequences of the hepatitis C virus (HCV) genome from 322 patients who had contracted HCV during Maeso's tenure to more than 100 genome sequences from 28 HCV haplotypes that Maeso carried.

But virus genomes evolve rapidly—about one million times faster than the human genome. "There is a race between the virus and the immune system, with one trying to control the other and the other trying to escape," says González-Candelas.

This means that viral sequences from the source and even a recently infected individual are almost never identical,

according to Anne-Mieke Vandamme, an epidemiological virologist at Katholieke Universiteit Leuven in Belgium who was not involved in the research. However, the rate at which mutations accumulate is relatively constant, so recently infected individuals should have viruses with higher sequence similarity to the source than those infected in the distant past.

"We had a small subgroup of patients with only one point of contact with the source, and we used those patients to calibrate the [molecular] clock," says evolutionary biologist Borys Wróbel, González-Candelas' former student, who is now at Adam Mickiewicz University in Poznan, Poland. The clock helped confirm that of the 322 patients tested, 275 were likely infected by Maeso. But the timing of 47 of those infections was inconsistent with the molecular clock the team had established.

Another consequence of the high viral mutation rate is that there are millions of variants of HCV kicking around in each infected person's liver, and only a very small fraction of those are present in any given blood sample, making matches between viral sequences in the source and those suspected to have been infected by that source even harder to pin down.

"[González-Candelas's] phylogenetic analysis was very well done," says Vandamme, who has testified in more than 20 cases involving molecular phylogenetics. However, she says that because so much uncertainty exists in molecular clock estimates, this type of analysis can never be definitive proof of guilt; rather, it can only be used to bolster other evidence.

Once the analysis was complete, another five years passed before Maeso's trial began. "Spanish justice is very slow," says González-Candelas. "One of the tactics of the defense was to make it even slower." González-Candelas' testimony began in September 2005 and lasted for three weeks. "It's very strenuous," he says of the experience.

A panel of three judges pronounced Maeso guilty in May 2007, by which time the virus had killed four of the infected patients. The prosecution theorized that Maeso had been injecting himself with tiny bits of the painkillers meant for his patients, then giving them the shots without changing the syringe. However, Maeso never admitted guilt, claiming that he was a scapegoat for the outbreak. He was sentenced to 1,933 years in prison and ordered to pay USD $27 million to the patients' families.

But González-Candelas's involvement in the case did not end with the conclusion of the trial. He says that patients who fear they were exposed still occasionally pop up wanting to be tested. Also, he and his team waited five years to publish the paper, which appeared this July in *BMC Biology*, reporting their findings only after Maeso exhausted the appeals process.

Molecular phylogenetics is still used sparingly in court cases regarding HCV infection, primarily due to the expense of the analysis. However, as sequencing costs continue to plummet, González-Candelas expects the technique to play a larger role in such trials in the future.

—Chris Palmer

A Briny Paradise

On a late-November morning in 2011, microbial ecologist Virginia Edgcomb of the Woods Hole Oceanographic Institute (WHOI) departed Piraeus, the port of Athens, Greece, with a few dozen other scientists on the R/V *Atlantis*, a US Navy research vessel operated by WHOI. Their destination: a group of super-salty, anoxic basins on the floor of the Mediterranean Sea several hundred kilometers away. These unique habitats, which can be more than 10 times the salinity of normal sea water and depleted of dissolved oxygen, were created as tectonic activity in the Mediterranean region exposed buried salt deposits that had formed when the sea dried up some 5.5 million years ago.

"They are among the most extreme environments on Earth," says deep-sea

biologist Roberto Danovaro of the Polytechnic University of Marche in Ancona, Italy. "They put together anoxic conditions, hypersaline conditions, and also concentrations of specific ions that are almost incompatible with some physiological functions of cells."

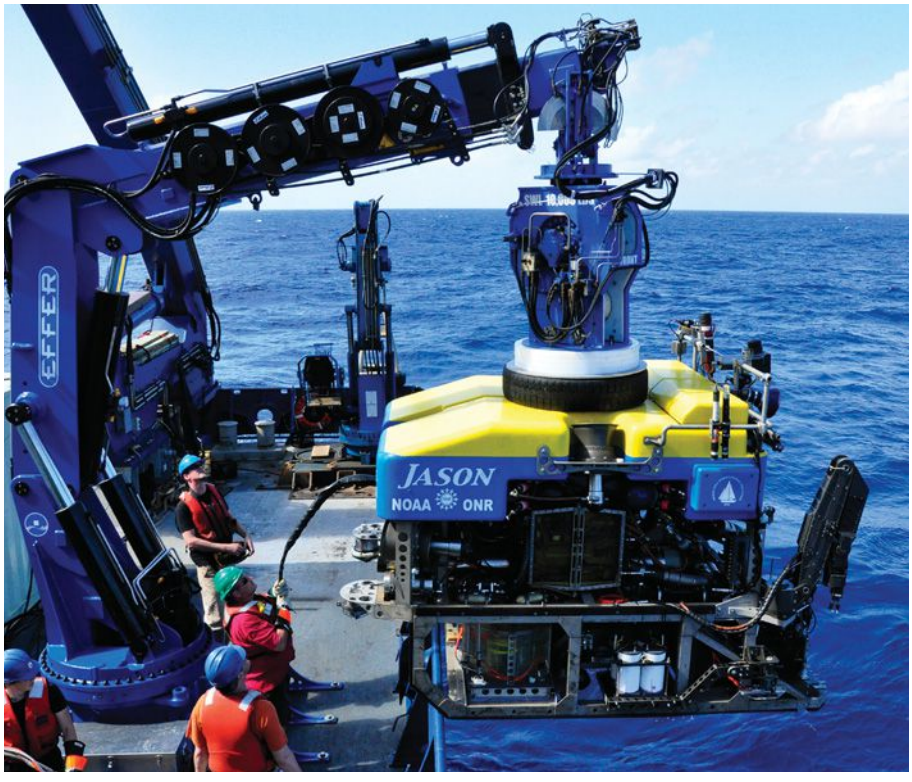
A few years earlier, however, Michail Yakimov at the Institute for Coastal Marine Environment in Messina, Italy, had sent Edgcomb samples that he had collected from two of the basins in 2003, in which she detected protist DNA (*Extremophiles*, 13:151-67, 2009). "In almost every extreme hypersaline habitat examined to date . . . protists were either completely absent or very rare," she says. "So it was surprising that we were recovering the signature of so many protist groups."

Intrigued by those initial findings, Edgcomb received National Science Foundation funding to further explore the Mediterranean hypersaline basins. Onboard the late 2011 expedition was *Jason*, a remotely operated vehicle (ROV) that could give the researchers "eyes, so to speak, on the sea floor," Edgcomb says—3,000 to 4,000 meters below the surface.

And it was an interesting sight, indeed. The hypersaline water of the basins is so dense that it mixes very little with the surrounding water. "We could see tin cans, bottles, floating on top of the brine," Edgcomb recalls. "*Jason*, in fact, had a hard time going down into the brine to sample because it was too buoyant."

PHOTO BY SHELLEY LAUZON, WOODS HOLE OCEANOGRAPHIC INSTITUTE

MOTHER SHIP: The R/V *Atlantis*, seen here on its maiden voyage to the Woods Hole Oceanographic Institute in 1997, ferried Virginia Edgcomb and her collaborators to areas in the Mediterranean Sea that harbor hypersaline environments deep under the surface.



DEEP DIVER: *Jason*, a remotely operated vehicle, plumbed the depths at and around the hypersaline basins, collecting samples as it went.

PHOTO BY JILL MCDERMOTT © WOODS HOLE OCEANOGRAPHIC INSTITUTION

"It looks like a lake surface," agrees microbiologist Alexandra Stock, a postdoc in Thorsten Stoeck's lab at the Technical University of Kaiserslautern in Germany and a researcher on the 2011 cruise.

Despite *Jason*'s difficulties, the ROV and other methods were successful at helping the researchers collect samples. After more than two weeks at sea, the crew returned to shore with dozens of water and sediment samples from four different hypersaline basins—including samples from the basins themselves and from the buffer zones, or haloclines, that sit just outside the perimeter of the dense, super-salty water. Back in the lab, Edgcomb and her colleagues are analyzing gene expression patterns of different water and sediment samples to compare metabolic activities within the communities; estimating the biodiversity of bacteria, archaea, and eukaryotes in the haloclines and the basins themselves; and exploring the ecosystem effects that protist grazing is having on the halocline of one basin and on the Mediterranean Sea in general.

By analyzing samples collected on earlier research cruises, Edgcomb, Stock, and their colleagues have already learned, among other things, that the basins harbor diverse communities of large unicellular eukaryotes known as ciliates (*BMC Microbiology*, 13:150, 2013). Not surprisingly, they found that the ciliate communities in the haloclines immediately above the brines, where mixing with seawater occurs, were more similar to one another than the communities within the brines themselves. For Edgcomb, the results confirmed her suspicions. "Even though they're close together—some of these basins are only a few kilometers apart—the chemistry of those habitats is so different, I would think that that would select for different types of organisms that could tolerate each set of conditions," she says.

Indeed, the fact that the basins are isolated from each other—and considerably different in terms of their chemical composition—is intriguing to evolutionary biologists, who have compared the hypersaline basins to isolated island ecosystems. "These are like reversed islands," says Danovaro. "Instead of emerging from the sea surface, they go down into the seafloor."

The basins, many of which are only 2,000 to 3,000 years old, are still too young for researchers to get a sense of how their resident populations are evolving, says Edgcomb, who is more interested in how the eukaryotes there are surviving at all. "[We're] trying to understand the limits of life for eukaryotes, and whether we can extend what we know as the limits of life for protists," she says. In addition to being hypersaline and anoxic, the basins are also "the only known Earth environments that are similar to suspected magnesium-rich habitats on Mars, Europa, Titan, and Enceladus," she adds. In some areas, the concentrations of magnesium are so high, they "appear to just mummify everything," Edgcomb says. Indeed, "it is debatable . . . whether [the] cells are alive or simply preserved in the brines, since high salts and particularly high magnesium are excellent preservatives." But the new findings suggest that microbes are indeed living in the haloclines, which also harbor relatively high levels of magnesium. "Protists appear to cope with higher magnesium concentrations than I thought," she says.

The researchers still don't know exactly what components of the environment explain the varying species compositions of the different basins, however. Stock and her colleagues are reviewing the data and asking what other factors—perhaps some that they didn't measure—might play a role. In addition, the researchers are working to get more sequence data, examining the physical features of the organisms themselves, and preparing samples for scanning electron microscopy. "These basins have already demonstrated [that they] hide important stories to be told," Danovaro says. —Jef Akst

Proceed with Caution

While genomic data sharing is essential for research, scientists must work to keep sensitive, potentially damaging information under wraps.

BY MARK GERSTEIN AND DOV GREENBAUM

The news continues to bring unprecedented revelations describing the US government's machinations to mine personal information and snoop on enemies and allies alike. In June it was the ongoing National Security Agency leak saga, spurred by the release of protected federal surveillance information by former defense contractor Edward Snowden.

Other news stories further suggest that the government is trawling more than our personal communications. Cables uncovered by WikiLeaks indicate that Big Brother's interests include exploring the DNA of foreign diplomats and officials.

But it's not just the government compiling databases of genetic information. With the precipitous drop in DNA sequencing costs, entire human genomes can now be deciphered for around a millionth of the price 10 years ago. Altogether, the personal genomics industry, grassroots patient projects, and academic research efforts will end up putting hundreds of thousands of genetic sequences online—and soon.

It's known that each genome sequenced could potentially compromise the privacy of multiple family members in addition to the actual "owner" of the code. Left unchecked, a number of nightmare scenarios could result from the government—or the public—having access to vast genomic databases.

For example, DNA extracted from bits of sloughed-off hair or skin could be used to follow a person's movements, to reveal evidence of stigmatized medical conditions or illegitimate children, or even to plant an incriminating and/or synthesized DNA sample at a crime scene. While these scenarios likely represent the far limits of current technology, as more governments and corporations gain the technical know-how to perform large-scale personal-information mining, we should carefully consider the consequences of making large amounts of data, particularly genomic data, universally available.

Of course, big data isn't just for spying. It's also crucial for the future of medicine, and especially for translating genomics research. Thus, potential abuses notwithstanding, we should promote the accumulation of vast collections of DNA as powerful tools to combat disease. To this end, the public needs not only to be assured that threats of government exploitation are kept in check, but that the more pedestrian concerns of leaks to insurance providers, employers, or even friends are also prevented.

The yin and yang of genomic data access are exemplified by the National Institutes of Health's August announcement regarding access to the sequenced HeLa genome. Henrietta Lacks, the progenitor of the famed quasi-eponymous HeLa cell line, was a poor African American woman whose cervical cancer tumor cells live on 60 years after she died from the disease. Lacks did not provide consent for any of the hundreds of thousands of experiments that have been conducted using cell lines derived from the original HeLa line. When the HeLa genome sequence was published without consent from the Lacks family, the story made front-page news.

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Ethical issues aside, the HeLa genome is an incredibly useful tool for the biomedical community: having access to the sequence helps researchers better interpret experiments carried out using HeLa cells. Still, the availability of this genome partially exposes close relatives within the Lacks family to an invasion of their privacy.

NIH's solution balanced the desires to make the information available for biomedical research and to protect it to a reasonable degree. The data, kept in a protected environment, will be made available to researchers whose applications to use it have been approved by a data-access committee. In some respects, this was a landmark decision. However, it is not clear that this type of solution scales to the thousands, even millions of genomes that must be analyzed to substantiate statistically sound biomedical research.

What can we do?

Technological solutions such as anonymization and encryption are unlikely to work on their own. To date, biomedical researchers have been greatly stymied by the time-consuming and technically difficult tasks of de-identifying and encrypting terabytes of genomic data. Moreover, in the race between overbearing, research-stifling encryption tactics and hackers, technical solutions inevitably become de facto challenges that the latter predictably overcome.

We envision a hybrid social-technological solution wherein codes of conduct, regulatory oversight, and punitive threats that keep data-mining corporate organizations in line could be combined with technical approaches for use in genomics research.

For example, a nongovernmental agency overseeing a limited-access, cloud-based database could be incentivized to protect our genomic data. Such an agency could store most of the genomic information for biomedical research in an extraterritorial cloud repository assembled with consent from the global scientific community and ad hoc standards committees. Researchers seeking access to the data would contribute funds to support this entity, and be bound by the repository's rules and standards. These regulations would contractually supersede many of the weaker genomic data privacy protections in place across the vastly different local jurisdictions. Individual researchers would be granted a personal license to access this information, which would depend on continuing education.

A cloud-based system would enable all of the information in the repository to be maintained in a standardized format so that researchers could develop their own analytical programs and move them up to the cloud to scale to large volumes of data. Integral to the cloud proposal is the idea that a fraction of the data would be made freely available by genomic "test pilots," who would bear the risk of making their personal information public. This public information would be the basis for "stub" data sets, which researchers could use to benchmark and develop their programs. (See "Data Drive" on page 67.)

Further, the cloud could be set up in a way that would restrict the outbound flow of data and log all use of secure data sets. And if a researcher did violate the privacy of the consented genomes, he or she would be punished. Just as the threat of losing their license often prevents less-scrupulous attorneys from violating client confidentiality, a licensing system for genomics researchers could provide meaningful penalties to prevent intentional leaks.

We need to move quickly to implement practical solutions for genomic privacy. The ethical issues are clear, the medical benefits of DNA data mining are real, and most importantly, more genomic data are being produced every day. ■

Mark Gerstein is a professor of biomedical informatics, molecular biophysics, biochemistry, and computer science at Yale University, where Dov Greenbaum is a professor of molecular biophysics and biochemistry.

Three-Way Parenthood

Avoiding the transmission of mitochondrial disease takes a trio, but raises a host of logistical issues.

BY JOHN D. LOIKE, MICHIO HIRANO, AND YEHEZKEL MARGALIT

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When first used in humans in the 1970s, in-vitro fertilization (IVF) raised significant ethical, legal, and philosophical concerns. The ability to manipulate human reproduction was viewed in many circles as an attack on the traditional family and an odious attempt to assert human dominion over nature. Terms such as "designer babies" and "playing God" were commonly applied to IVF. Nevertheless, much of the scientific community touted the potential benefits of these technologies, viewing them as the start of a new era of medicine. Indeed, despite those dire predictions four decades ago, IVF is now widely accepted and has enabled infertile couples to conceive more than 5 million healthy babies.

Fourteen years ago, my Columbia University colleagues and I (JL) examined the mitochondrial origins of Dolly, the cloned sheep, and proposed the concept of a "three-parent" fertility procedure to treat mitochondrial disorders (*Nat Genet*, 23:90-93, 1999). The unique genetic information within mitochondria enables these organelles to function as the biochemical engines of the cell. However, sometimes deleterious mutations occur in mitochondrial DNA (mtDNA) that cause myriad human pathologies—such as heart problems, liver failure, brain disorders, blindness, hearing loss, myopathy, and in the most extreme cases, death. These mitochondrial disorders are incurable and are passed down maternally from generation to generation. One in 6,500 children worldwide is affected with mtDNA defects. (See "Power Failure," *The Scientist*, May 2011.)

To prevent defective mtDNA from being passed from mother to child, scientists in the U.K. are planning to offer a "three-parent" fertility procedure. Based in part on protocols developed by scientists at the New York Stem Cell Foundation and at Columbia University Medical Center (*Nature*, 493:632-39, 2013), this procedure modifies standard IVF technology to create an embryo from the eggs of two women and sperm obtained from one man. Specifically, nuclear DNA from the egg of a woman carrying mitochondrial defects is transferred into the enucleated cytoplasm of a donor egg that harbors nonmutated mtDNA. This genetically reconstituted egg is then fertilized in vitro by sperm from a male partner, and the resulting embryo is implanted into the uterus of the woman with the mitochondrial disorder. This embryo will contain genetic material from three donors, but will not express any symptoms of the mitochondrial disorder.

The potential for creating children from multiple parents is not limited to the halting of the passage of mitochon-

drial disorders. In May 2013, Shoukhrat Mitalipov and his colleagues at the Oregon Health and Science University published a milestone article describing the use of IVF technology to transfer genetic material from any nonsperm cell into a human egg, thereby generating a preimplantation embryo from which human embryonic stem cells can be readily isolated and maintained in the laboratory (*Cell*, 153:1228-38, 2013). One of many potential outcomes of this research is the ability to create a human embryo without any male genetic contribution—by transferring the nucleus of a somatic cell from one woman into an enucleated egg of another. Embryos could also be made from more than three genetic parents by merging multiple embryos into a single chimeric infant, as has already been achieved in rhesus monkeys (*Cell*, 148:285-95, 2012).

All of these genetic engineering procedures raise both legal and ethical concerns. Legal issues include: Who are the legal parents of a child generated from genetic material obtained from multiple donors? Would such a child have the right to know the identity of all his gene donors? In an article to be published in the *Harvard Journal of Law and Gender* (in press), we propose a legal solution to address some of these issues. We propose that intentional parents—i.e., individuals who will assume responsibility of child care and agree to act as parents to the child—should be recognized as the legal parents of the child. We also propose that it is necessary to legally validate and define the parental intent and responsibilities of all parties involved in a pre-authorized contractual agreement. These proposed definitions of parenthood should supplement and expand biological and genetic considerations resulting from advances in molecular biology.

One of many ethical concerns raised by such technologies is whether these advances in reproductive medicine could lead to the creation of "designer babies," in which parents select the genetic composition of their children for enhancement or for health reasons. The fear in creating designer babies is that it may herald a new era of "consumer eugenics" with potentially unknown consequences for humankind. From an ethical perspective, any procedure involving genetic engineering should require that all genetic donors submit a medical history (and perhaps their complete genome sequences as well) to provide an early warning of future health risks for the child. We also believe that as children reach legal maturity, they have the right to know their genetic origins. We recognize that, as with any new technology, there is always the fear of abuse. But we argue that the potential reproductive benefits of these technologies will trump those ethical fears.

We argue that the potential reproductive benefits of these technologies will trump associated ethical fears.

That the road from scientific innovation to societal acceptance is often rocky is a given, and is emblematic of scientific innovation. As these genetic engineering technologies develop and become safer and less expensive, the potential to enable people with genetic defects to conceive a healthy child is a dream that should be vigorously pursued. ■

John D. Loike serves as the director of Special Programs, Center for Bioethics, Columbia University College of Physicians and Surgeons. Michio Hirano is a professor in the Department of Neurology at Columbia University College of Physicians and Surgeons. Yehezkel Margalit is a faculty member in the Law School of Ono Academic College in Kiryat Ono, Israel.

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Monitoring Magnetic Bugs

Diamonds are a biomagnetologist's best friend.

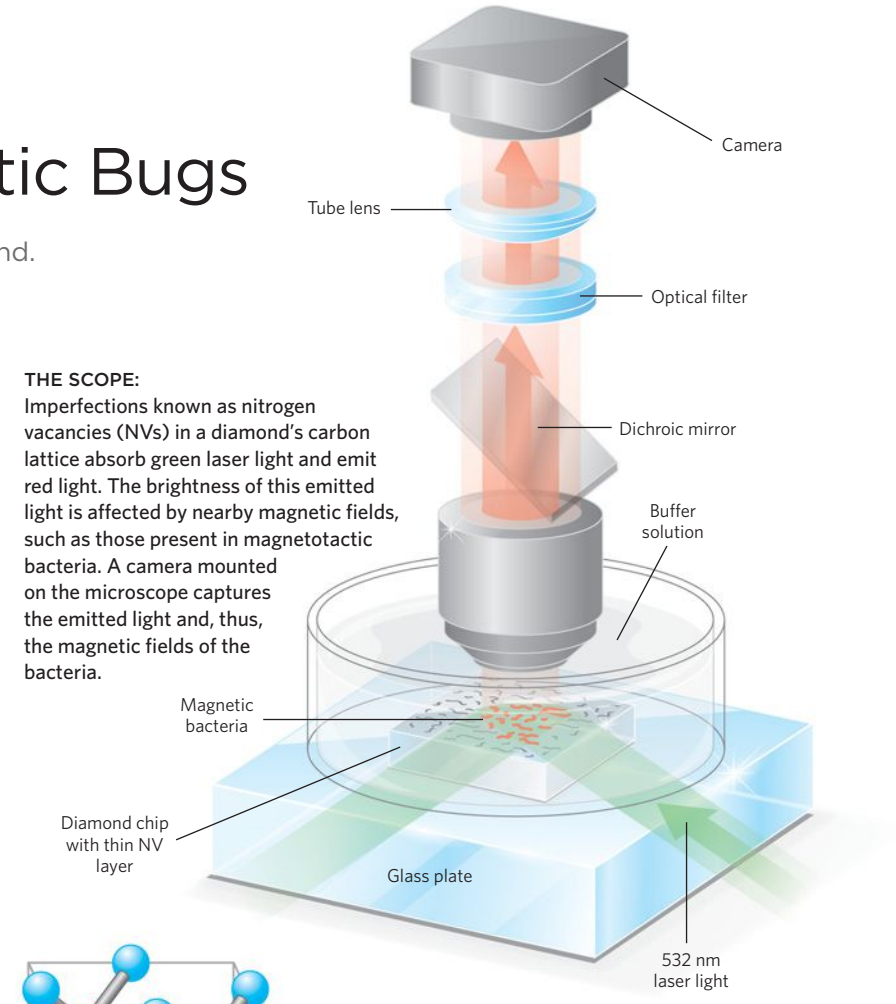
BY RUTH WILLIAMS

Some bacteria build intracellular nanoscale magnets and use them to travel by orienting themselves to the Earth's magnetic lines. Researchers are interested in these magnet-building microbes (who wouldn't be?) not just because they might have implications for higher organisms that use magnetically guided migration, but also because such magnetic nanoparticles could have medical applications. They might be used to enhance the contrast of patients' cells in MRI images, for example, or even to kill cancers.

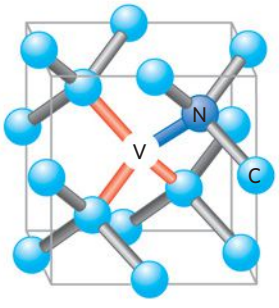
Now, for the first time, studying magnetic fields at high resolution inside living bacteria is possible, thanks to a gem of an idea from Ronald Walsworth, a physics professor at Harvard University, and colleagues.

The key is diamonds, or, to be precise, imperfections in diamonds called nitrogen vacancies (NVs). NVs are disruptions to the diamond's carbon atom lattice whereby two neighboring carbons are replaced with a single nitrogen atom and an adjacent gap. Importantly, these NVs have a couple of properties that make them perfect for magnetic imaging, explains Walsworth. First, their associated electrons have a particular way of spinning that is affected by nearby magnetic fields. Second, NVs absorb green light and emit red light, the intensity of which increases or decreases in relation to the spin of their electrons. Magnetic fields emitted by the bacteria would therefore affect the spinning electrons, resulting in a dimming or brightening of the red light.

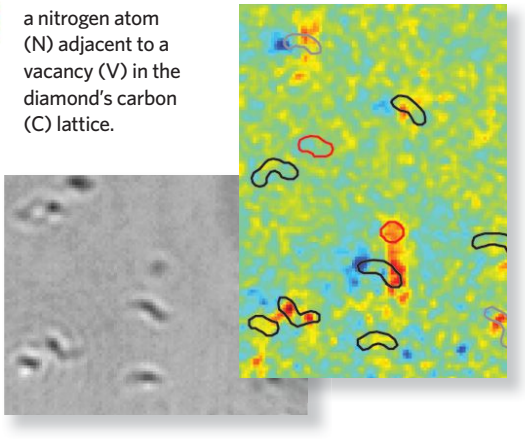
Walsworth and colleagues have constructed a microscope in which living magnetotactic bacteria placed on an NV-containing diamond chip can be viewed under both normal light conditions and under conditions that detect the bacteria's magnetic fields—via the diamond's emitted red light. "The new technique will be excellent" for figuring out the biological pathways controlling magnetic particle growth in these bacteria, says Mihály Pósfai, a magnetotactic bacteria expert at the University of Pannonia in Hungary. (*Nature*, 496:486-89, 2013)



THE SCOPE: Imperfections known as nitrogen vacancies (NVs) in a diamond's carbon lattice absorb green laser light and emit red light. The brightness of this emitted light is affected by nearby magnetic fields, such as those present in magnetotactic bacteria. A camera mounted on the microscope captures the emitted light and, thus, the magnetic fields of the bacteria.



THE CHIP: An NV consists of a nitrogen atom (N) adjacent to a vacancy (V) in the diamond's carbon (C) lattice.

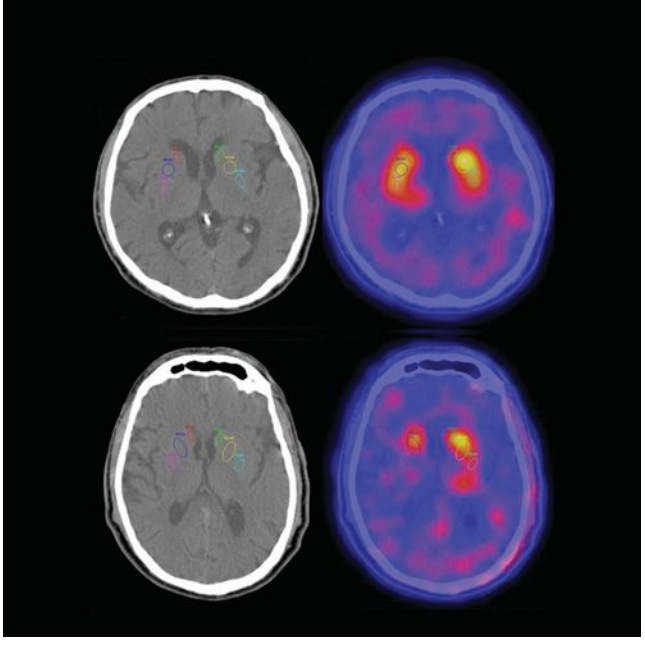


THE IMAGES: The microscope captures both regular light images (right) and magnetic images (far right) of the same live bacteria (outlined in the far right image).

AT A GLANCE

MAGNETIC IMAGING METHOD	OPERATING CONDITIONS	RESOLUTION	LIVE CELLS	EXPERTISE REQUIRED
Scanning super-conducting quantum interference device (SQUID) microscopy	Device must be in a vacuum and cooled to −269 °C	~5 μm	No	Advanced electron spin resonance (ESR) techniques
Diamond chip-based wide-field optical imaging	Ambient; room temperature	0.4 μm	Yes	Regular light microscopy skills, and some basic ESR knowledge

OLFACTORY DIAGNOSIS (opposite page): Patients with Parkinson's disease (PD; bottom row) have fewer dopamine tranporters (labeled with radioactive ligands in brain scans on right) than healthy controls (top row). Because PD patients have associated olfactory loss, smell testing can help diagnosticians differentiate between PD and other neurodegenerative diseases that also show a decline in brain dopamine receptors.



COURTESY OF JACOB DUBROFF

Smell and the Degenerating Brain

An impaired sense of smell is one of the earliest symptoms of Alzheimer's, Parkinson's, and some other neurodegenerative diseases. Could it be a useful diagnostic tool?

BY RICHARD L. DOTY

James Black, a 62-year-old London taxi cab driver, went to his doctor complaining of memory difficulties and intermittent periods of confusion that he'd been experiencing for 2 years. A minor road accident caused by poor concentration and vision problems had forced him to retire. His wife reported that for more than a decade James had also experienced difficulty smelling—a condition, called hyposmia, that was confirmed by olfactory testing. His neurological examination revealed he was suffering from damage to the brain's frontal lobe. Ultimately, James was diagnosed with Alzheimer's disease (AD), the most common dementia-causing disorder.[1]

James's situation is far from unique. Olfactory loss is not only an early warning sign of AD, but also of Parkinson's disease (PD) and some other neurological disorders, presenting long before their classic clinical symptoms. Once such symptoms become evident, evaluation of olfactory ability—which is easily performed using commercially available smell tests—can help ensure the correct diagnosis and treatment strategy. Indeed, a number of diseases often misdiagnosed as AD or PD, such as severe depression or progressive supranuclear palsy, are accompanied by little or no smell loss. Thus, olfactory testing can be useful in differentiating between such oft-confused disorders.

Importantly, some disorders commonly misdiagnosed as PD do not respond well to L-DOPA and other drugs that increase dopamine, a neurotransmitter involved in the control of motor function. Such agents are the most effective treatments available for PD patients. Thus, olfactory testing can aid physicians in predicting whether patients can derive meaningful benefit from such drugs. In patients with mild to moderate AD, olfactory testing indicates responsiveness to donepezil, a drug that improves cognitive function in some patients.[2] In light of these and other findings, the Quality Standards Subcommittee of the American Academy of Neurology and other professional organizations have endorsed smell testing as an aid in the diagnosis of AD and PD. Nevertheless, the importance of olfaction in these diseases is largely overlooked, and such testing is not routinely performed in neurology clinics.

Predicting decline

Numerous studies have used quantitative smell tests in an attempt to identify asymptomatic older persons who are most likely to develop cognitive or motor symptoms indicative of neurodegenerative disease. In a pioneering study published in 1999, Amy Bornstein Graves and her associates at the University of South Florida administered a 12-item version of the University of Pennsylvania Smell Identification Test (UPSIT), termed the B-SIT, to 1,604 community-dwelling senior citizens who showed no signs of dementia.[3] Over the course of the two-year study, the olfactory test scores proved to be a better predictor of cognitive decline than scores on a global cognitive test. Overall, individuals who had no sense of smell and who possessed at least one *APOE-4* allele—a genetic risk factor for AD—were nearly five times more likely to develop cognitive decline than those of the same age who had no smell dysfunction and who carried no such allele. This risk was increased nearly tenfold in women, whereas in men it went up approximately threefold. Possessing at least one *APOE-4* allele in the absence of smell loss did not significantly increase a person's risk for future cognitive decline.

A more recent study of 1,092 older persons with no signs of dementia (average age 80 years) from a multiethnic community in New York City also observed an association between smell loss and cognitive function. Those individuals with both mild cognitive impairment (MCI) and memory loss had lower scores on the 40-odor UPSIT than those with MCI but no memory loss. The UPSIT scores were also correlated with age, several cognitive measures, and the volume of the hippocampus, a brain structure associated with memory.[4]

Research has also elucidated a link between smell dysfunction and PD. In the 1990s, G. Webster Ross and his colleagues at the University of Hawaii administered the B-SIT to 2,276 nonsymptomatic elderly men of Japanese ancestry (average age at the beginning of the study was 80 years). After adjusting for age, smoking behavior, and other confounders, those subjects whose initial olfactory test scores fell within the bottom 25 percent of the group were five times more likely to develop PD than those whose test scores fell within the top 25 percent. Over a four-year period, 35 were clinically diagnosed with PD.[5]

Evaluation of olfactory ability can help ensure the correct diagnosis and treatment strategy for neurodegenerative disease. Nevertheless, the importance of olfaction is largely overlooked, and such testing is not routinely performed in neurology clinics.

Further support for olfactory involvement in PD came in 2004, when Mirthe Ponsen and her associates at Vrije Universiteit in Amsterdam published a study of 361 asymptomatic first-degree relatives of PD patients, finding that those whose olfactory test scores were significantly below normal were more likely to develop PD over a two-year period than those with no smell impairment.[6]

Additionally, Ponsen measured the extent of degeneration of brain regions associated with PD-related motor dysfunction. The team injected patients with a radioactively labelled agent that binds to the dopamine transporter responsible for moving dopamine back into neurons in the brain following its release into the synaptic cleft, then measured the amount of such binding using gamma-ray cameras—a technique known as single-photon-emission computerized tomography (SPECT). The less binding detected, the greater the damage in the cells of interest.

At the two-year assessment, four of the 40 relatives with the lowest olfactory test scores—all of whom exhibited substantial reduction in the amount of dopamine transporter binding at the start of the study—were diagnosed with clinical PD, while none of the 38 relatives with the highest olfactory test scores developed the disease. After five years, those relatives with initial smell loss had at least a 10 percent risk of developing clinically defined PD.[7] When the degeneration in the brain regions producing dopamine as measured by SPECT were taken into account, the investigators suggested that the risk of developing PD in the presence of hyposmia may be as high as 22 percent.

Such studies suggest that olfactory testing can sometimes predict future development of cognitive and, in the case of PD, motor dysfunction in those at risk for degenerative disease. Although the predictive power of olfactory testing alone is not high, it rivals and even exceeds that of a number of other biomarkers of PD, including disease-related metabolites found in the cerebral spinal fluid and SPECT imaging of the dopamine transporter. Importantly, novel methods are being developed that enhance smell testing's predictive power. For example, intranasal application of atropine, a drug that accentuates cognitive dysfunction in patients with AD, may induce a greater degree of smell loss in symptomless individuals who are at risk for future dementia—in effect "unmasking" the incipient disease.[8]

The root of the problem

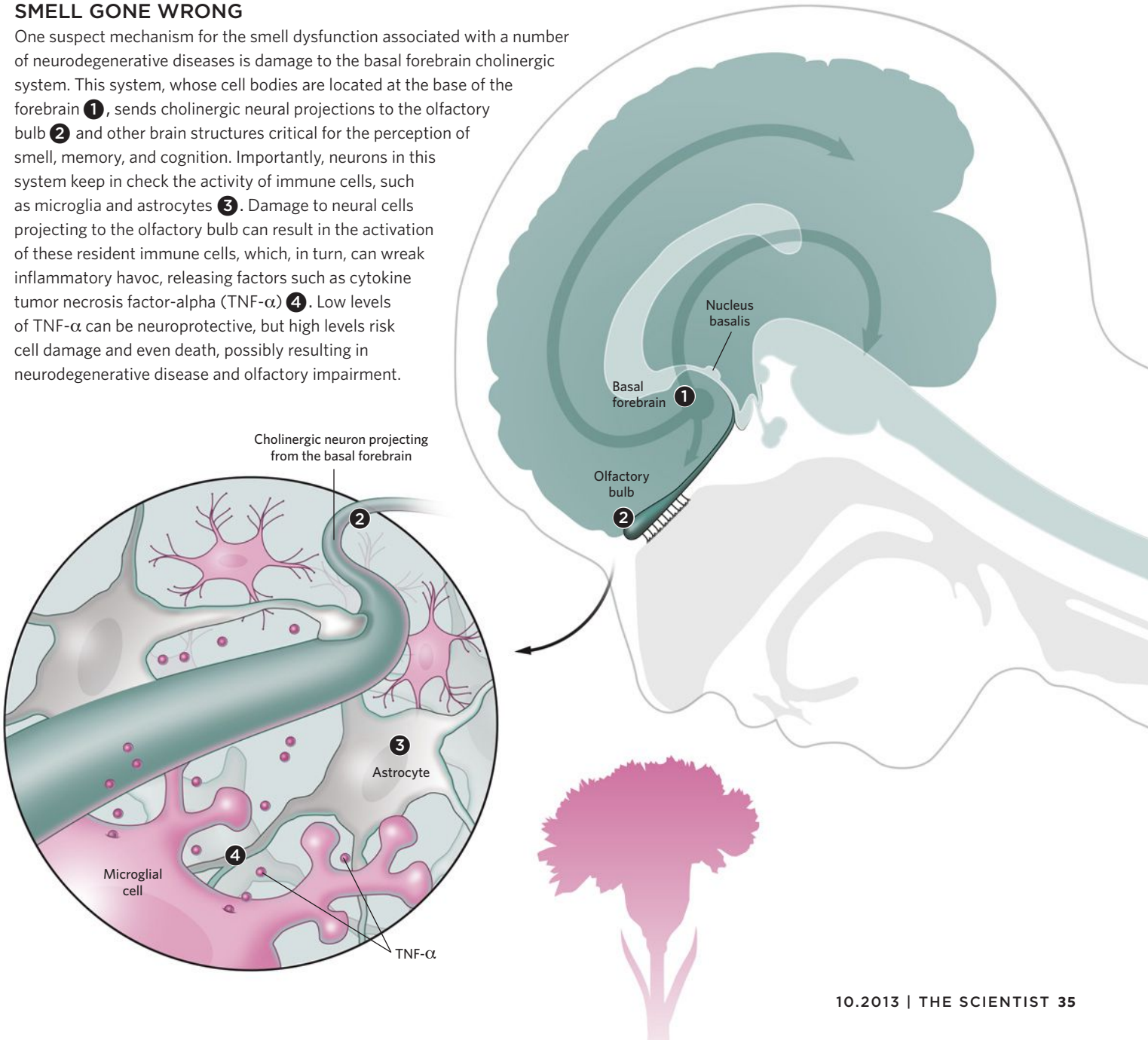
While smell dysfunction can be useful in differential diagnosis, the fact remains that many neurological diseases exhibit essentially equivalent olfactory loss, including disorders with neuropathologies completely distinct from those of AD and PD, such as myasthenia gravis, an autoimmune disorder characterized by muscle weakness.[9] Hence, while olfactory dysfunction is a sensitive indicator of some neurological diseases, it is not specific to any single disease. Is it possible that the same pathological process is involved in the olfactory loss associated with most or all of these disorders, or is the olfactory system simply sensitive to damage from a range of disease-specific factors?

One hypothesis is that deficits in certain neurotransmitter systems are largely responsible for smell dysfunction that occurs in conjunction with neurodegenerative disease. A major player in this regard is the basal forebrain cholinergic system, which is involved in the secretion of the neurotransmitter acetylcholine (ACh) in many areas of the brain. ACh plays a significant role in attention, memory, and the facilitation of cortical plasticity, including functional recovery following brain injury.

Cholinergic neurons that project from the basal forebrain to the olfactory bulb also directly modulate neural activity and inhibit the activity of cells critical for immune responses to brain damage and foreign agents, including microglia, astrocytes, and oligodendrocytes.[10] When cholinergic and other neural cells that project to the olfactory bulb are damaged, inhibition of microglia can be suppressed, resulting in immune activation and the secretion of inflammatory mediators such as the cytokine tumor necrosis factor–alpha (TNF–α).[10] (See illustration on this page.) Although low levels of TNF–α are neuro-

SMELL GONE WRONG

One suspect mechanism for the smell dysfunction associated with a number of neurodegenerative diseases is damage to the basal forebrain cholinergic system. This system, whose cell bodies are located at the base of the forebrain ❶, sends cholinergic neural projections to the olfactory bulb ❷ and other brain structures critical for the perception of smell, memory, and cognition. Importantly, neurons in this system keep in check the activity of immune cells, such as microglia and astrocytes ❸. Damage to neural cells projecting to the olfactory bulb can result in the activation of these resident immune cells, which, in turn, can wreak inflammatory havoc, releasing factors such as cytokine tumor necrosis factor-alpha (TNF-α) ❹. Low levels of TNF-α can be neuroprotective, but high levels risk cell damage and even death, possibly resulting in neurodegenerative disease and olfactory impairment.



HOW WELL CAN YOU SMELL: The University of Pennsylvania Smell Identification Test (UPSIT) is comprised of four booklets of "scratch-and-sniff" odorants, which subjects must identify from a list of alternatives.

protective, high levels induce damaging inflammation and even cell death. Activated glial cells and local inflammatory processes are believed to contribute to the development of a number of neurodegenerative diseases, and may also contribute to the degeneration of the basal forebrain cholinergic system, resulting in olfactory impairment.

Several lines of evidence support the concept that cholinergic dysfunction plays a significant role in the olfactory loss seen in a number of neurological diseases.

First, the ability of patients with PD to identify odors has recently been shown to be correlated with the degree of cholinergic denervation of the forebrain, as measured by functional imaging of radioactively labeled agents that bind to acetylcholine receptors.[11]

Second, autopsy studies show that disorders with an olfactory dysfunction element are typically accompanied by significant damage to the forebrain cholinergic structures. Such damage, which in most cases is reflected by gliosis (the glial response to brain damage) and cell loss, is less severe or absent in diseases whose olfactory function is spared or less compromised, including depression and essential tremor. Although cell loss within basal forebrain cholinergic structures is minimal in patients with Huntington's disease, another disorder with marked olfactory loss, their cholinergic system is nonetheless dysfunctional. Specifically, patients exhibit changes in the expression of choline acetyltransferase (ChAT), which is involved in ACh synthesis, and of vesicular acetylcholine transporter, a protein critical for transporting ACh from the cytoplasm into the synaptic vesicles.[12]

Third, another measure of cholinergic circuit health, called short-latency afferent inhibition (SAI), also correlates with relative olfactory function differences in patients with neurodegenerative disease.[13] SAI is measured by electrically stimulating a sensory nerve in the hand immediately before activating the motor cortex by transcranial magnetic stimulation (TMS), a noninvasive procedure in which magnetic coils on the surface of the scalp are used to stimulate cortical neurons. When electrical stimulation is applied to the sensory nerve just before the onset of TMS, the subsequent activation of motor neurons in the muscles of the arm is delayed or inhibited. In AD and PD, particularly PD with dementia, such inhibition is less marked.

Finally, a large literature based on animal studies clearly links olfactory function to cholinergic processes. For example, mouse strains that express fewer α7-nicotinic cholinergic receptors perform poorly on odor detection/discrimination tasks relative to strains that have more of these receptors. Other studies have shown that physostigmine, a drug that inhibits an enzyme that decreases the amount of acetylcholine at synapses, increases the ability of rats to discriminate between low concentrations of odors.

Much remains to be learned about the factors that initiate cholinergic dysfunction and the degree to which dysregulation of noncholinergic neuromodulators, such as serotonin and norepinephrine, contributes to olfactory loss. For example, in diseases associated with abnormal aggregates of α-synuclein, tau, and β-amyloid (Aβ), it is unclear whether the olfactory deficits precede or follow the development of such neuropathology. Relatively strong correlations have been found between olfactory test scores very late in life and the number of such pathological structures in the postmortem brains of both healthy older persons and in older persons with AD, PD, and Lewy body disease.

Additionally, various steps in acetylcholine synthesis and release can be altered by Aβ-related peptides associated with AD. For example, exposure of brain slices from the hippocampus and cortex of rats to very low concentrations of Aβ and Aβ fragments can inhibit potassium-evoked release of acetylcholine. This does not occur in brain slices from the rat striatum, suggesting regional selectivity of such effects. Researchers have also shown that Lewy bodies, the defining α-synuclein-comprised pathological features of PD and Lewy body dementia, sequester precursor enzymes that are critical for the expression of acetylcholine and some other neurotransmitters, presumably disrupting neurotransmitter production.[14] Whether these aggregates are contributing to olfactory dysfunction remains to be determined.

Environment and behavior

Many environmental and behavioral risk factors for a number of neurodegenerative diseases are also risk factors for smell dysfunction. For example, age is a risk factor for AD and PD, and smell loss is common in healthy older persons. Furthermore, viral and bacterial infections, notably those of the upper respiratory tract, are

COURTESY OF SENSONICS

the most frequent cause of chronic, often permanent, smell loss in the general population, and a number of viruses and bacteria have been indirectly implicated in the etiology of neurodegenerative diseases.[15] Decreased smell function can also result from head trauma and chronic exposure to ionized metals and air pollution, known risk factors for AD and PD.

It is well established that airborne toxins, viruses, nanoparticles, and other foreign substances—collectively called xenobiotics—can enter the brain through the nose via the olfactory epithelium, either damaging receptor cells directly or initiating harmful inflammatory responses, ultimately altering olfactory function.[16,17] The olfactory epithelium is protected to a large degree by detoxification enzymes, including some encoded by members of the P450 gene superfamily. However, protection provided by such detoxifying enzymes—which in some instances are found at higher levels in the nose than in the liver—can be compromised. Postmortem studies have identified nanoparticles and inflammatory mediators in the olfactory epithelia and bulbs of children and young adults living in highly polluted areas of Mexico City.[17] In some cases, AD- and PD-like pathology has been observed in their brains. Many young people living in these highly polluted areas also exhibit subtle olfactory dysfunction. Furthermore, iron deficiency enhances the uptake of manganese through the olfactory epithelium in rats, and in humans, anemia has been found to be a risk factor for both AD and PD.

It is not beyond the realm of possibility that damage to neurotransmitter systems from pathogens or other xenobiotic agents could be instrumental in altering basic metabolic and immunological activity in numerous brain regions. Such damage might then cause, catalyze, or hasten the formation of Lewy bodies, neuritic plaques, neurofibrillary tangles, and other pathologic entities that, in turn, alter the functioning of cells within the olfactory pathways. It is also possible that such damage, which might alter smell function, exacerbates nascent or latent disease-related brain pathology that otherwise would not be expressed.[16]

Ultimately, the expression of most neurodegenerative disease neuropathology and symptoms, including olfactory dysfunction, depends on myriad factors involving health, genetic predispositions, sex, age, and environmental exposure to disease-related risk factors. The complexity of neurodegenerative pathologies—which often span a number of diseases—remains a challenge not only for the diagnostician, but also for those seeking to develop treatments that target elements of such pathologies.

Is it possible that the same pathological process is involved in the olfactory loss associated with most or all neurodegenerative disorders, or is the olfactory system simply sensitive to damage from a range of disease-specific factors?

Clearly, future research is needed to better define the connection between olfaction and the pathologic processes associated with neurodegenerative disease. Is olfactory dysfunction a result of damage associated with classic markers of neurodegenerative disease, such as abnormal aggregates of α-synuclein, tau, and Aβ? Or does olfactory loss precede such damage? Can damage to the olfactory system, per se, induce neurodegenerative disease in those genetically or otherwise predisposed to such disease? These and a host of other questions await clarification. ■

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A Pheromone by Any Other Name

Long known to play a role in sexual attraction, pheromones are revealing their influence over a range of nonsexual behaviors as researchers tease apart the neural circuitry that translates smells into action.

BY C. RON YU

Following a trail of smell, a male fruit fly zeroes in on a banana peel. For the fly, the banana is not only a fantastic food source, but also fertile ground for finding mates. Sure enough, a virgin female is already feasting on the banana peel. He approaches her, taps her with his forelegs, and flutters his wings to sound a staccato love song, all in the hopes of securing her as a mate. But there is more to this scene than meets the eye or ear. The success of this courtship ritual critically depends on a single substance: an organic ester, 11-cis-vaccenyl acetate (cVA). CVA is found on the male's cuticle, or exoskeleton, and in his ejaculatory bulb, a structure similar in anatomy and function to the human prostate. To mature female fruit flies, cVA is an aphrodisiac that induces their receptivity to an approaching male. To males, however, cVA is an antiaphrodisiac, even capable of inducing aggression. Although females do not produce the compound, residual cVA transferred from previous mating partners during copulation remains on their bodies. If a female reeks of the compound, new suitors are repelled.

CVA is a pheromone, classically defined as a substance secreted by an animal that elicits a specific reaction in other members of the species. Although best understood in insects, pheromones are also known to play important roles in mammalian behavior and physiology, from territorial marking in mice to the induction of mating in elephants.

The powerful effect a pheromone can exert on an animal captures the popular imagination. The idea of irresistibility is so ingrained in our psyche that the mention of pheromones immediately conjures up images of love potions, whiffs of which instantly make the wearer more sexually attractive. Indeed, Googling "human pheromone" will lead you to companies trying to sell you one of these "scientifically proven" attractants. (See "Something Smells Funny," page 44.) While such marketing has deepened the sensual mystique surrounding pheromones, so far there is no substantial evidence that such perfumes can induce mate-seeking behavior in men or women. However, decades of research have revealed a fascinatingly wide range of pheromones across the animal kingdom that are not limited to affecting reproductive behaviors. And in the last 10 years or so, scientists have unveiled some of the neural mechanisms of pheromone processing in the brains of both fruit flies and mice, identifying clues to how these compounds work at the molecular, neural, and behavioral levels.

Researchers have made rapid progress in our understanding of the neural circuits in the fly brain. Do the same principles also govern the processing of pheromone information in the mammalian brain?

Lessons from the fly

Thanks in part to the fantastic genetic tools developed in the last decade, research on the fruit fly, *Drosophila melanogaster*, has uncovered many details of pheromone pathways, from the antennae to the brain. CVA, the only volatile fly pheromone so far identified, is detected in the antennae by olfactory receptor neurons (ORNs) that express a G protein-coupled receptor called OR67d. These neurons project their axons into bulb-shape structures called glomeruli in the antennal lobe of the brain, where olfactory information is initially processed. Each glomerulus is

innervated by a distinct set of projection neurons (PNs) that then transmit the information into deeper brain regions. (See "Odor Encoder," page 55.)

How does cVA, a single compound emitted by male flies, trigger behaviors that differ so widely between males and females? The answer lies within the neural pathways that parse the information into different neural circuitries in male versus female brains. The sexually dimorphic circuitry begins in the antennal lobe, where the glomeruli that receive input from OR67d neurons are larger in the male brain than in the female brain. From the antennal lobe, the PNs that receive input from glomeruli project their axons into two other brain areas: the mushroom body, where information about odors is associated with other sensory inputs, forming the basis of learned behaviors; and the protocerebrum, which is similar to the hypothalamus in the mammalian brain and is the origin of stereotypic behaviors, such as courtship and mating, in flies. Whereas the projection patterns from the PNs are similar in the male and female brains when they reach the mushroom body, they differ when the axons reach the protocerebrum.

Thanks in part to the fantastic genetic tools developed in the last decade, research on the fruit fly, *Drosophila melanogaster*, has discovered many details of the pheromone pathway, from the antennae to the brain.

From the protocerebrum, the male and female circuitries further diverge and connect to different downstream neurons. The divergent patterns in the two sexes are thought to underlie the sexually dimorphic responses to cVA.

The mapping of this neural circuitry in the fruit fly brain reveals two important features of pheromone detection. First, information about a pheromone passes through a highly specific neural pathway, which is often referred to as a labeled line. The labeled line connects the sensory input, in this case a single chemical compound, to the behavioral output. Second, the labeled line differs between the sexes, allowing a single compound, cVA, to serve as an attractive sex pheromone for females and an antiaphrodisiac for males.

The short generation time of fruit flies and the availability of new genetic tools have enabled rapid progress in our understanding of the neural circuits in the fly brain. An immediate question, then, is whether the same principles also govern the processing of pheromone information in the mammalian brain.

Mammalian pheromones

Researchers have long recognized the roles that pheromones play in many mammals. In some species, such as cats and ungulates, a particular sniffing behavior has evolved that is believed to facilitate the exposure of sense organs to pheromones. The behavior, known as the flehmen response, is characterized by the curling of the upper lip and the exposure of the front teeth. In elephants, the flehmen response is characterized by the repeated tucking of the trunk into the open mouth. Female elephants release the urinary pheromone (Z)-7-dodecen-1-yl acetate, which induces strong flehmen responses in males. In pigs, sows in estrus respond to 3α-androstenol and 5α-androstenone, two steroid pheromones enriched in the saliva of male pigs, by exhibiting the "standing response," a rigid, motionless pose that signals reproductive readiness.

But such behavioral responses to pheromones, called releasing effects, are relatively rare in mammals. More common are so-called priming effects, in which pheromone blends cause a change in the signal receiver's physiology that does not manifest itself as an immediate behavioral response. A large body of literature in the last century, mostly rodent studies, has identified the effects of mammalian pheromones on reproductive physiology, for example.[1] Rodent urine is rich in chemicals, including pheromones, that function in intraspecies communication. For example, the urine of a male mouse can accelerate the onset of puberty in young females. On the other hand, females housed in groups have delayed estrus onset and prolonged reproductive cycles. Interestingly, these latter effects can be reversed by the presence of a sexually mature male or his urine. Moreover, it was discovered that the presence of a strange male or his urine may cause a newly impregnated female to abort implanted embryos. Territorial marking and intermale aggression, as well as maternal aggression displayed by new mothers, are also found to be induced by urine and urinary compounds.

These numerous pheromone-induced responses in mammals contrast with the relatively simple behaviors observed in insects, but there is yet another layer of complexity built into mammalian pheromone communication. The pheromone-elicited responses in mammals often depend upon the context of pheromone exposure and the experience of the animals. For example, a female mouse can switch from being attracted to a male following pheromone exposure to being aggressive, depending on whether she is in estrus or has just given birth. On the other hand, a sexually naive male kills young pups he encounters, but if a male has mated and then cohabited with a pregnant female in the past 3 weeks, he will instead exhibit paternal behaviors, such as helping to return wayward pups to the nest.[2]

Complicating matters even further is the fact that researchers don't have a good understanding of what the mammalian pheromone compounds are. In mammals, pheromones are found in urine and exocrine gland secretions including sweat, tears, and secretions from the preputial glands near the genital area. Chemical isolation experiments have identified a number of molecules that may serve as mammalian pheromones. Some chemicals have been shown to alter sexual maturation, mating behavior, and aggression, for example, when presented in conjunction with urine, but these do not directly trigger behaviors or physiological changes on their own. In contrast to insect systems, individual chemical compounds rarely evoke behavioral responses or

SECONDARY OLFACTION: The mouse vomeronasal organ, shown here in cross section, is located just above the roof of the mouth and comprises sensory epithelia (blue crescents) that convert the chemical signal of pheromones into electrical nerve pulses that carry messages to the brain.

endocrine changes in mammals, and this has made it difficult to pinpoint whether a compound is serving as a pheromone or as a modifier of pheromone responses.

Furthermore, the neural mechanisms the mammalian brain uses to process complex olfactory chemical cues and to generate stereotypic responses remain largely unknown. But recent studies in mice, combining molecular biology, genetics, imaging, and behavioral studies, have started to shed light on this problem. In addition to sex identification, it is now thought that mammalian pheromones convey information about an animal's social status, reproductive status, genetic background, and individual identity. The specific compounds that serve as pheromones, their cognate receptors, and the neural circuits that process the information remain subjects of intense investigation.

The vomeronasal system

Instead of a single or a few receptors that detect a single pheromone, as is the case with insects, many vertebrates have evolved a dedicated organ to detect a much larger variety of chemical substances that may serve as pheromones. While pheromones may be detected by other sensory organs, including the main olfactory system and the taste buds, a major contribution to pheromone detection in vertebrates comes from the vomeronasal organ (VNO). Discovered in nonhuman mammals by Ludwig Jacobson in 1813, the VNO is a tubular structure in the nasal cavity embedded above the palate, or roof of the mouth. It is found in most amphibians, reptiles, and nonprimate mammals, but is absent in birds and most primates. The VNO opens to the base of the nasal cavity, and in carnivores and ungulates, connects with the oral cavity through a passage known as the nasopalatine duct. The sensory epithelia of the VNO form curved layers along the septum that divides the two sides of the nose, with large blood vessels on either side. (See image on this page.) The sensory epithelia surround the VNO lumen, which is filled with fluid from the vomeronasal glands. This is where pheromone molecules interact with their neuronal receptors.

The intricate anatomical structure of the VNO has long fascinated scientists, but it was not suspected to be a sensory organ until early in the 20th century. In fact, its distinct function from that of the main olfactory system in mammals was not demonstrated until 1970, when the VNO was found to be essential for transmitting intraspecies information important for sexual maturation and aggressive behaviors in rodents.[1] Surgical removal of the VNO eliminates territorial aggression and territorial marking in male mice and male hamsters. And in numerous species, including hamsters, rats, ferrets, and lemurs, VNO removal leads to a decrease in sexual investigation and copulation by males.

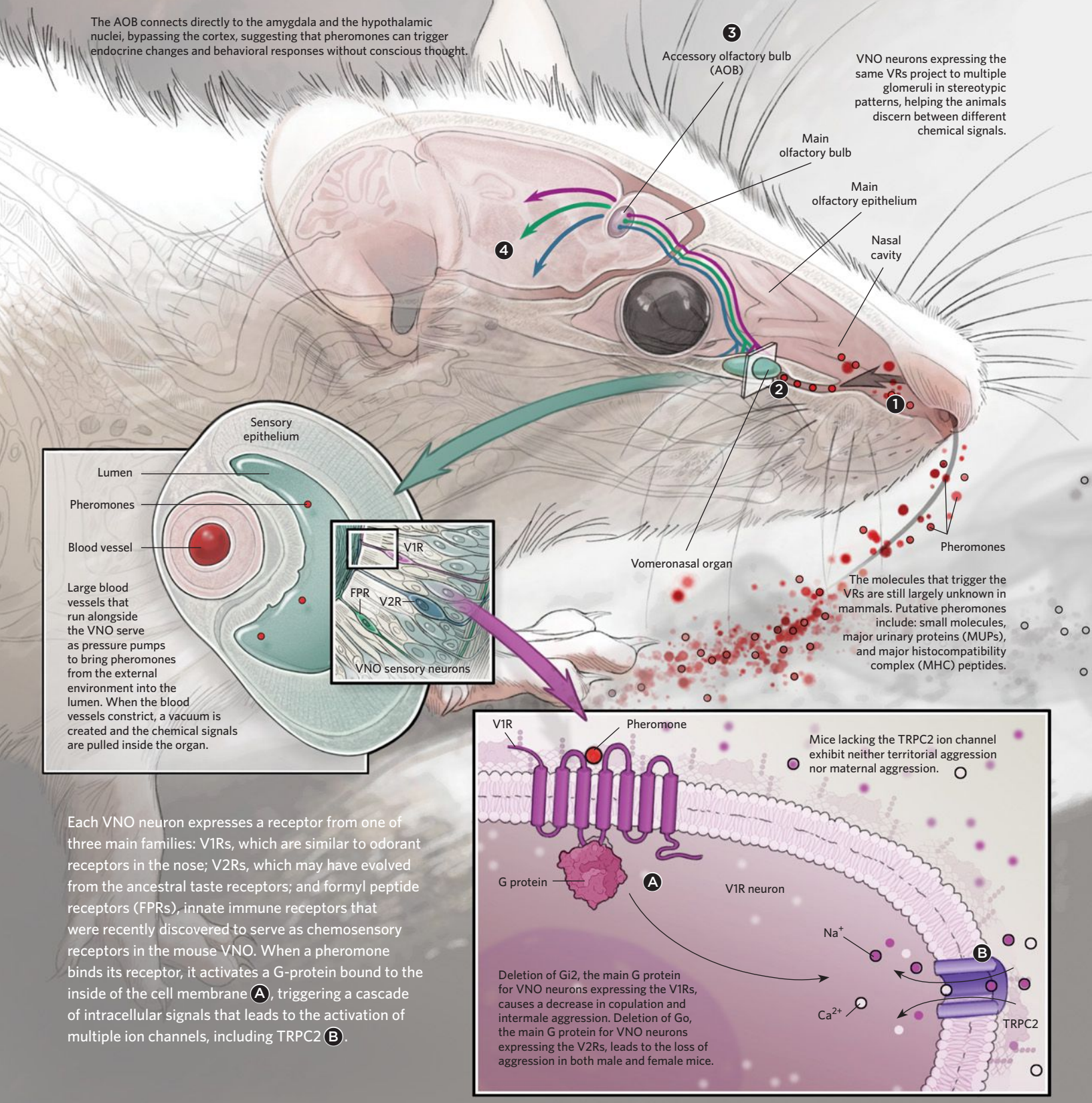
Female sexual behaviors are also affected when the VNO is removed. Back arching, which signals mating readiness in some female rodents, is reduced in hamsters, rats, and mice whose VNO has been removed. Puberty onset and pregnancy are affected in mice without VNOs as well. Moreover, a mouse's ability to recognize members of their own strain and to distinguish individuals is affected. And in recent experiments, also with mice, researchers have shown that response to predator signals, parental behaviors, and the infanticidal to parental behavioral transition are also dependent on a functional VNO.

Researchers have identified three main families of vomeronasal receptors (VRs) in the VNO, G protein-coupled receptors with seven transmembrane domains. Ligand binding to VRs triggers a cascade of intracellular signaling events that transform the chemical signals into electrical nerve impulses. The V1R family consists of more than 200 receptors, each with short extracellular N-terminal domains, that are similar to the odorant receptors found in the nose. The V2R family also has nearly 200 members, which may have evolved from ancestral taste receptors. The third family of VRs, the formyl peptide receptors (FPRs), contains only seven members. They are innate immune receptors, only recently determined to serve as chemosensory receptors that in the mouse VNO appear to recognize a set of cues that signal the health of individuals.[3] (See illustration on following page.)

PROCESSING PHEROMONES

The mouse vomeronasal organ (VNO) is a tubular structure located in the nasal cavity just above the roof of the mouth. Chemical signals enter the VNO through an opening at the base of the nasal cavity ❶, where they enter the organ's lumen. Projecting into the lumen are sensory neurons outfitted with vomeronasal receptors (VRs), G protein-coupled receptors that initiate intracellular signaling cascades when bound by a ligand, converting pheromone signals into electrical nerve pulses ❷. Those signals are then sent to glomeruli in the accessory olfactory bulb (AOB), where they are relayed to mitral cells that project into deeper brain regions ❸. In areas like the amygdala and the hypothalamic nuclei, that information is further processed and used to effect changes in the animal's behavior and physiology ❹.

The AOB connects directly to the amygdala and the hypothalamic nuclei, bypassing the cortex, suggesting that pheromones can trigger endocrine changes and behavioral responses without conscious thought.



Each VNO neuron expresses a receptor from one of three main families: V1Rs, which are similar to odorant receptors in the nose; V2Rs, which may have evolved from the ancestral taste receptors; and formyl peptide receptors (FPRs), innate immune receptors that were recently discovered to serve as chemosensory receptors in the mouse VNO. When a pheromone binds its receptor, it activates a G-protein bound to the inside of the cell membrane Ⓐ, triggering a cascade of intracellular signals that leads to the activation of multiple ion channels, including TRPC2 Ⓑ.

The pheromone molecules that trigger these receptors, however, are still unknown. Some small synthetic molecules, including 2-heptanone, several dimethylpyrazines, and sulfated compounds, activate the VNO neurons, but these compounds do not elicit behavioral effects by themselves. In addition to these low-molecular weight compounds, mouse urine also contains high levels of proteins, many of which belong to the major urinary protein (MUP) family. These proteins have been shown to bind small molecules, possibly serving to retain in the urine volatile chemicals that act as pheromones. Recent experiments have suggested that MUPs may even act as pheromones on their own. Genetically engineered MUPs made by *E. coli* bacteria can trigger defensive behavior in mice.[4]

Another group of molecules that has been implicated in VNO activation is the major histocompatibility complex (MHC).[5] MHC peptides are remnants of proteins that are broken down during normal cellular metabolism. These peptides are presented on the cell membrane as a complex of surface antigens. Because MHCs are highly divergent molecules between species, and because the peptides they present are determined by the genetic background of the individual animal, it was once thought that MHC peptides could serve to portray information about the genetic background of the transmitting animal. However, individual VNO neurons appear to respond to peptides from a variety of genetic backgrounds, making it unlikely that animals can distinguish between individuals based on activation by MHC peptides alone.

Finally, while the large families of VRs have been identified and some of the putative ligands found, scientists are often at a loss when it comes to matching the receptor with its ligand. So far, only one ligand-receptor pair that triggers specific behavior has been identified.[6,7] Kazushige Touhara's group at the University of Tokyo has identified a tear gland peptide (exocrine gland-secreting peptide 1, or ESP1) that activates V2Rp5 and triggers robust lordosis, or back-arching, behavior in female mice. Labeling of the V2Rp5 circuit suggests that ESP1 may activate a predetermined labeled line, as cVA does in fruit flies.

Pheromonal pathways

Pheromone-triggered behaviors are inborn, requiring no learning or prior exposure to the chemical cues. This suggests that the neural circuits are genetically specified. The labeled-line circuit in the insect brain represents a relatively simple mode of signal processing, but the pheromone circuits in the mammalian brain are much more complex. Signals detected by the VNO must be parsed and integrated to induce the proper response. The holy grail of pheromone research is therefore to identify the neural logic of signal processing in the mammalian brain.

A primary site of neural computation is likely to be the accessory olfactory bulb (AOB). VNO neurons expressing the same VRs project to multiple glomeruli in stereotypic patterns. From there, individual mitral cells project into deeper brain regions—specifically, the amygdala and the hypothalamus. Tracing experiments have established that the AOB is directly connected to these brain areas—bypassing the cortex, which mediates higher cognitive processes—thus allowing pheromones to directly trigger endocrine changes and behavioral responses without conscious thought. (See illustration on opposite page.)

Ongoing research continues to probe the nature of how pheromones are processed in the mammalian brain. Meanwhile, my group and others have focused on better understanding pheromone compounds and their receptors. To make headway studying this problem, my colleagues and I generated a transgenic mouse line that expresses the genetically encoded calcium sensor, G-CaMP2, in the VNO. Using these mice, we can image pheromone-triggered responses in VNO neurons. Stimulating VNO neurons with urine samples from individual mice of both sexes—with different genetic backgrounds or at different hormonal statuses, for example—we profiled the response patterns and were able to identify cells specifically tuned to sex, estrus signal, genetic makeup, and individual identity.[8] This approach could eventually lead to the identification of receptors that convey specific information, as well as their ligands, the pheromones.

The holy grail of pheromone research is to identify the neural logic of signal processing in the mammalian brain.

Other investigators are using genetic means to interfere with VNO function. In the early 2000s, Peter Mombaerts, then at Rockefeller University, and colleagues deleted a cluster of V1R genes, causing a reduction in male copulation and maternal aggression.[9] More recently, researchers demonstrated that the genetic deletion of Gαi2, the main G protein for VNO neurons expressing the V1R odorant receptors, causes a decrease in both maternal and intermale territorial aggression.[10] Deletion of Gαo, the main G protein for VNO neurons expressing the V2Rs, also leads to the loss of aggression in both male and female mice.[11]

Even more intriguing experiments involve mice lacking the TRPC2 ion channel, which is uniquely expressed in the VNO and was thought to be exclusively responsible for VNO function. TRPC2-knockout mice exhibit a set of fascinating behaviors: they show neither territorial aggression nor maternal aggression. Instead of vigorously attacking intruder males, TRPC2-mutant males display sexual mount behaviors toward the intruders.[12,13] In large arenas, TRPC2-mutant females act as if they were males themselves, and display chasing and mounting behaviors toward intruder males.[14] These behaviors demonstrate that despite reduced responses to pheromone stimulation in the TRPC2 mutants, the residual responses may be sufficient to transmit signals to the brain and trigger behavioral responses—albeit abnormal ones.

Furthermore, though the behavioral patterns exhibited by such mutant mice are "inappropriate" for the conditions, they

SOMETHING SMELLS FUNNY

The existence of human pheromones is a controversial topic. Some studies have found that extracts from human sweat have a calming effect on the opposite sex, but they do not appear to induce sexual arousal.[1] Others have tested the effect of steroids secreted from the armpit sweat glands and reported that one such steroid, androstenone, is attractive to some people, while others find it repugnant. Still others, however, do not consciously detect it.

Because the effects observed for these putative pheromones rely on psychophysical tests that require the subjects to report their feelings, they do not strictly fit the classic pheromone definition in that no direct action or change in physiology is observed. Moreover, the reported effects are usually subtle.

More direct evidence of the existence of human pheromones comes from a study of menstrual cycle synchronization published in the late 1990s by a team led by Martha McClintock at the University of Chicago. By collecting body secretions from women at different times in their menstrual cycles and presenting the substance under subjects' noses, the authors reported that the test subjects either accelerated or slowed their cycles to synchronize with the donor's, even without conscious perception of the odor.[2] However, the conclusion has been called into question by other studies, and the phenomenon of menstrual synchronization itself is disputed, even when women are living together.[3]

Furthermore, even if human pheromones do exist, how their effects are mediated remains mysterious. The VNO is a vestigial organ in humans. The molecular machinery that is essential for VNO function in other mammals, including the ion channel TRPC2 and the V1Rs and V2Rs, have largely become nonfunctional pseudogenes in humans. Any processing of a human pheromone signal would have to occur in other systems.

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are not novel behaviors. In other words, although alterations to the VNO can reduce mating and aggressive behaviors, the behavioral patterns themselves are not altered. For example, in TRPC2 mutant mice, females may display a male-like behavior. Similarly, the parental behavior circuit also exists in the male brain, but it is not apparent until the male VNO has been activated when he mates with a female. These observations suggest that animals have a limited behavior repertoire. The function of the VNO, therefore, is to detect and integrate a multitude of signals in the environment, and, in turn, activate one of a few preset neural circuits to elicit a stereotyped behavioral output.

Many questions remain about how pheromone signals elicit such regimented behavioral patterns—namely, what are the compounds that stimulate the VNO, what are their primary receptors, and what are the details of the brain circuits that translate these signals into behavioral output? But as constantly evolving genetic and physiological approaches allow us to trace the anatomical connection from the AOB to deeper brain structures, we will continue to elucidate the pheromone circuits in the mammalian brain as we have in the insect brain. ■

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Send in the Bots

Animal robots have become a unique tool for studying the behavior of their flesh-and-blood counterparts.

BY JEF AKST

COURTESY OF JOSÉ HALLOY, FRANCESCO MONDADA EPFL GROUP

As a PhD student at the University of Toulouse in France, Simon Garnier was fascinated by the chemical signposts used by Argentine ants—an invasive species from the Mediterranean to California—to navigate their savanna environment. As the insects traverse complex terrain, they leave traces of pheromones that other ants will then follow, reinforcing the trailblazers' path. "In nature, they will create these big networks of pheromone trails, sort of like the road system for us," Garnier explains. And despite their wide-ranging and convoluted habitats, the ants always seem to construct highways that carve the shortest route back to the nest from a food source. Such navigational efficiency might suggest an advanced intelligence in these tiny-brained insects. The ants, which tend to take the path with the smallest angle of deviation at each fork in a complex maze, could be computing the angles at each bifurcation. But

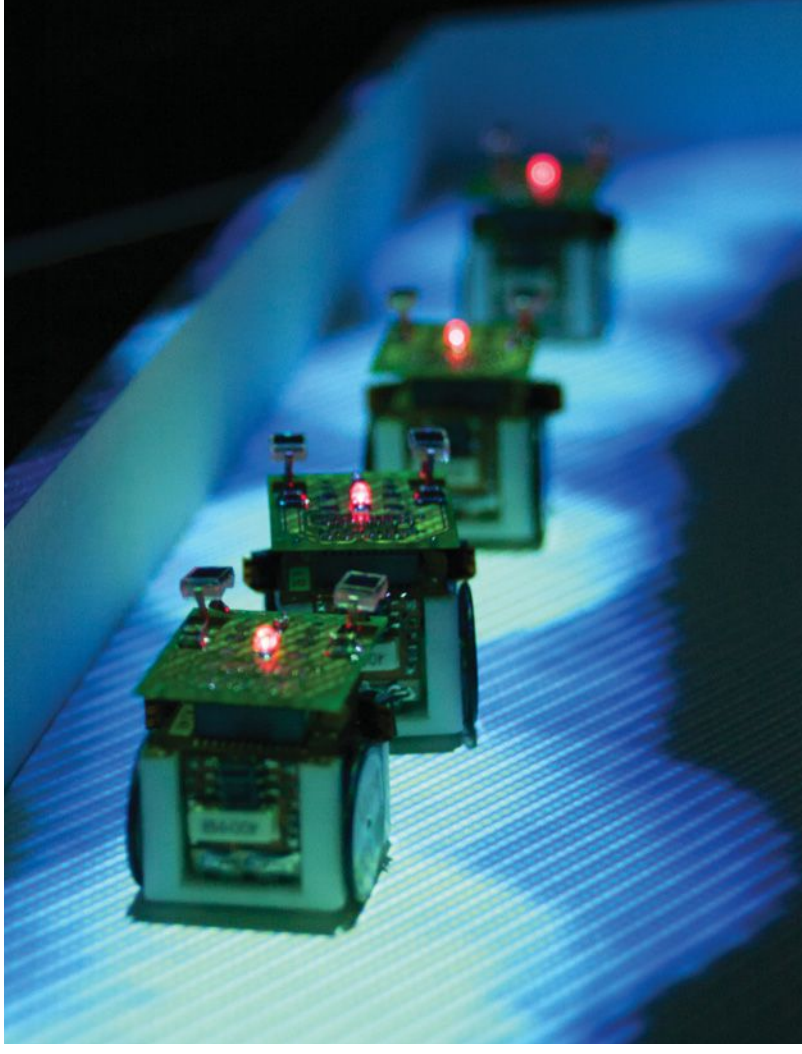
FOLLOW THE ROBOTS: More and more, researchers are turning to robots to answer questions in animal behavior. Here, young chickens following a robotic mother shed light on the process of imprinting.

Garnier knew there might be a simpler answer: by just trying to head straight, the ants would have a greater chance of taking the less deviant path—no complex angle measurements required.

Like any hypothesis, his idea needed to be tested. But measuring brain activity in a moving ant—the most direct way to determine cognitive processing during animal decision making—was not possible. So Garnier didn't study ants; he studied robots. Using a small fleet of dice-size machines, rolling on wheels powered by wristwatch motors, he and his colleagues tested the robots' ability to navigate artificial networks, using whatever computational capability the researchers programmed. A camera detected the location of the robo-ants as they moved through an arena and relayed the information to a video projector, which shone a bit of blue light just behind a trail-laying bot. As more robots moved about, the more-frequented areas glowed brighter. The robots then navigated the environment by sensing light intensity through two sensors on their "heads."

In making an artificial organism, you discover new constraints on what it is to be an animal and move around in the environment. I really think there is a lot to be discovered by doing the engineering side along with the science.

—Jeffrey Schank, University of California, Davis



ROBOTIC ANTS © CNRS PHOTOTHÈQUE/CRCA TOULOUSE/SIMON GARNIER

FOLLOW THE LIGHT: These robotic ants sense and follow the blue light that is projected on the arena floor. As more robo-ants traverse a path, the brighter the light shines. Groups of these robo-ants mimicked the behavior of real ants—following the less deviant path at a fork in the road—without the need for complex cognitive processing.

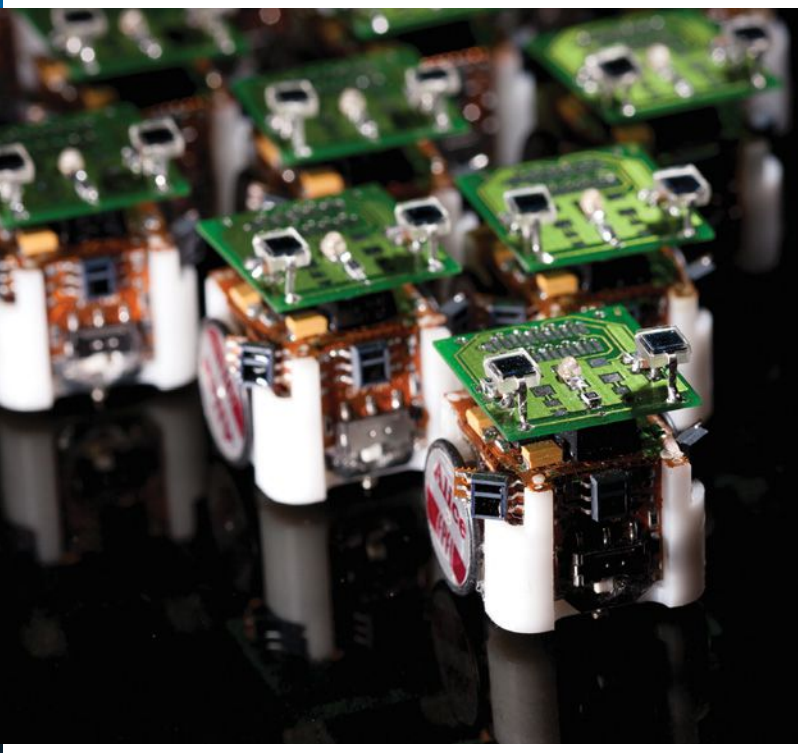
As Garnier had suspected, no higher intelligence was necessary for the robo-ants to take the less wayward turn, and when released en masse, the bots built and followed light roads that mimicked the real ants' pheromone highways, albeit in a simpler environment.[1] The only rule the robots had to follow was "Go straight," and their behavior matched that of real ants "almost perfectly," says Garnier. "To explain the behavior of the ants, we don't need to have any form of complex cognitive processing; for this particular decision, it [can be] decided by the shape of the environment."

Indeed, several groups have used autonomous robots that sense and react to their environments to "debunk the idea that you need higher cognitive processing to do what look like cognitive things," says Andrew Philippides of the University of Sussex in the U.K. As early as the mid-1990s, early biorobotics pioneer Barbara Webb of the University of Edinburgh was developing robots to mimic the phonotaxis of female crickets—which are adept at localizing and moving toward calling mates. In so doing, Webb showed that no complex processing was required: the cricket's auditory system, absent any cognitive processing, was sufficient for a robot to identify and approach a male's call.[2]

Today, a growing number of scientists are using autonomous robots to interrogate animal behavior and cognition. Researchers have designed robots to behave like ants, cockroaches, rodents, chickens, and more, then deployed their bots in the lab or in the environment to see how similarly they behave to their flesh-and-blood counterparts. In some cases, experimenters have thrown robots in with animals to see how they interact, and have even programmed robots to influence group decisions.

Among their many benefits, robots give behavioral biologists the freedom to explore the mind of an animal in ways that would not be possible with living subjects, says University of Sheffield researcher James Marshall, who in March helped launch a 3-year collaborative project to build a flying robot controlled by a computer-run simulation of the entire honeybee brain. "Running experiments, especially neuroscience experiments with animals, is a very costly, time-consuming process," he says. "There's much less scope for curiosity-driven research there."

Furthermore, designing and programming robots to recapitulate specific behaviors forces scientists to think about animals in an entirely different way. "[In making] an artificial organism . . . you discover new constraints on what it is to be an [animal] and move around in the environment," says Jeffrey Schank, who has built robots to study the behavior of rat pups at the University of California, Davis. "I really think there is a lot to be discovered by doing the engineering side along with the science."



ROBOTIC ANTS © CNRS PHOTOTHÈQUE/CRCA TOULOUSE/SIMON GARNIER; FROG COURTESY OF BARRETT KLEIN

A real-world experience

As Garnier's efficiently navigating robo-ants demonstrated, complex behaviors are not always what they seem. Schank came to the same realization completely by accident, while investigating how young rats huddle together with their nest mates during the first week of life. He and his colleagues built self-propelled robot rats, about four times larger than real rat pups, but constructed to have the same general body shape. Each robot was equipped with a ring of pressure sensors that allowed it to respond to contact. Schank coded the rules of aggregation he'd developed from a computer simulation into the robots, and when he set them loose in an arena four times the size of the space he'd given to real rat pups, he thought it was a smashing success. Not only did the bots move around the space like the rat pups did, they aggregated in remarkably similar ways to the real animals.[3] Then Schank realized that there was a bug in his program. The robots weren't following his predetermined rules; they were moving randomly.

"It turned out that a lot of what the pups were doing in this context could be explained by the shape of their bodies and how they interact with the arena," says Schank. And the experience taught him a valuable lesson. "You can't just investigate what's going on in the brain of an organism," he adds. "Cognition and behavior are a function of the environment, the body, and the brain."

Of course, that doesn't mean the animals don't have higher processing skills. Predictions derived from robotics-based inquiries will always have to be tested in animals, emphasizes Tony Prescott, a cognitive neuroscientist at the University of Sheffield in the U.K., who develops rodent-mimicking whiskered robots. "Animal experiments are still needed to advance neuroscience." But, he adds, robots may prove to be an indispensable new ethological tool for focusing the scope of research. "If you can have good physical models," Prescott says, "then you can reduce the

SOPHISTICATED DUMMIES

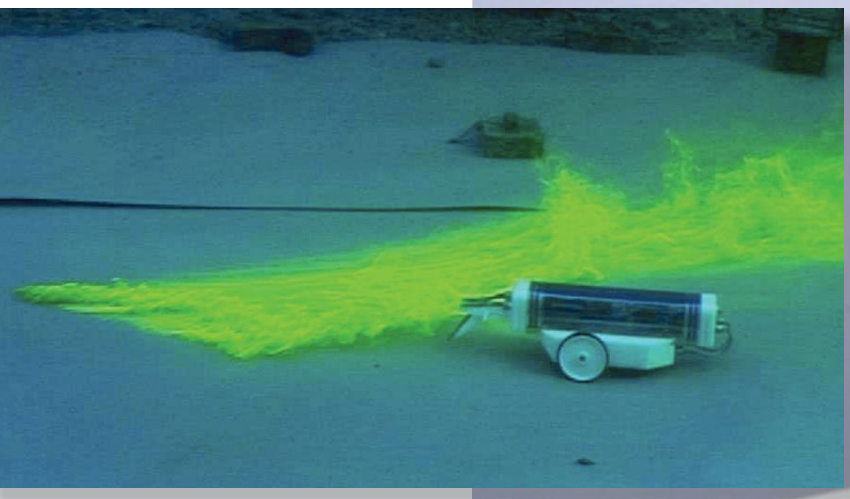
Autonomous, sensing-and-reacting robots have only begun to prove their worth in behavioral ethology in the last decade or two, but robotics and biology have been intertwined for much longer. Since its inception, robotics has been taking its cues from biology, drawing inspiration from nature's proven solutions for sensing and moving about a complex environment. And in biology, researchers have long been using robotics techniques to animate "dummies"—physical models of animals that can be presented to animals in and out of the lab. "[Robots] can be a tool like a special social microscope to study animal behavior," says José Halloy of the Université Paris Diderot. Here are some examples—past and present—of how researchers have used robotic models to ask questions about animal behavior.

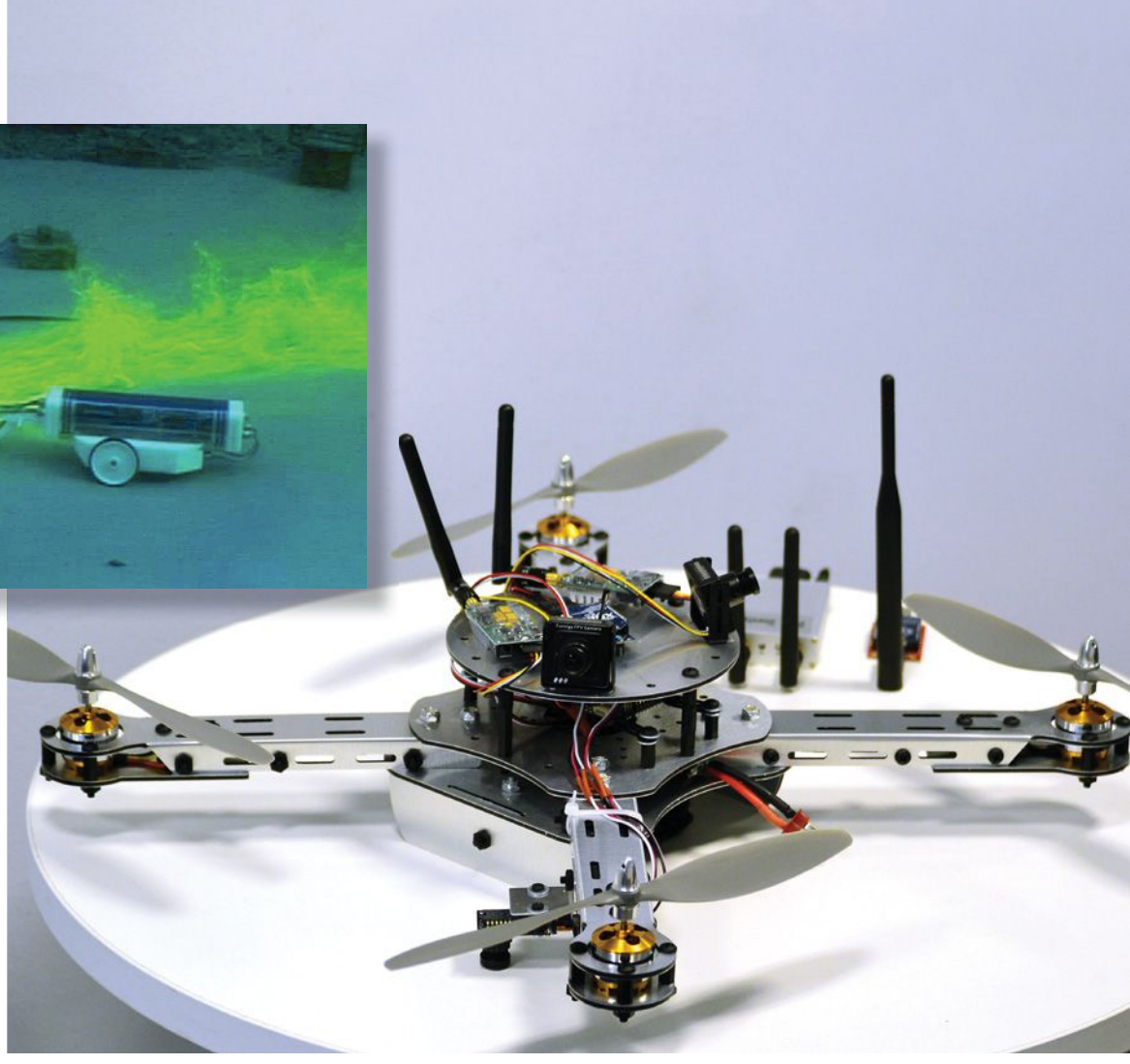
FROGS

Amorous túngara frogs of the Panamanian rain forests inflate a large vocal sac in their throat, but "the vocal sac is way bigger than it needs to be for actual sound production," says Ryan Taylor of Salisbury University in Maryland. Knowing that frogs see well at night—peak mating time—he wondered if the vocal sac might be serving as a visual cue, in addition to the call it helped produce. To isolate sound from vision in their experiments, Taylor and his colleagues turned to robots.

They built their robo-frogs using a rubber model, then hooked the robots up to a machine that pumps air into faux vocal sacs, causing them to inflate like those of a real frog. They can then program the device to coordinate the audio signal from the speakers with inflation of the vocal sac, or to dissociate those stimuli. Sure enough, the timing of the vocal sac inflation, relative to the two notes of the male's call, "seems to be really important for the females," says Taylor.

"By creating something like a doppelgänger, a kinematic model, you can try to elicit behaviors in a standardized fashion," says collaborator Barrett Klein, now at the University of Wisconsin–La Crosse. "And since the research direction has to do with looking at multiple modalities—vision and acoustics at this point, and possibly tactile sensations in the near future—this allows, you could say, an unprecedented level of control into the realm of the impossible."





PLUME TRACKING (ABOVE): To better understand how lobsters track scents through the water to find their prey, Frank Grasso of the City University of New York in Brooklyn and his colleagues designed a tube-like robot to detect and follow odor trails.

ROBO-BEE FLIGHT (RIGHT): This "quadcopter," which measures 50 cm across, will embody a supercomputer simulation of the entire honeybee brain, responding autonomously to olfactory and visual cues.

QUADCOPTER COURTESY OF CHELSEA SABO; LOBSTER ROBOT COURTESY OF FRANK GRASSO

number of experiments and only do the ones that answer really important questions."

One commonly cited benefit of robotics-based inquiries is that they are a step closer to reality than a straight computational approach. The robots, though still simulations themselves, interact in a real physical space, rather than in an environment simulated by a computer program—a necessarily incomplete depiction of the real world. For example, in Philippides's work on visual navigation in ants and bees, "the thing that's just so hard to simulate with any sort of realism is what the world looks like and how the world changes when the sun goes behind a cloud," he says. "A simulation just doesn't cut it."

Furthermore, there are still open questions about what parts of the environment are important to animals, and virtual simulations are inherently biased by the researchers' knowledge. "People say simulation is doomed to succeed," says Webb. "If you build the simulated world and the simulated animal to live in that world, then what you put into the simulated world is all the things that *you* think are important . . . so there's a certain circularity" in the logic, she says. As a result, it should not be surprising that the simulated animal "works" in the simulated world, she explains, but "in the real world, you nearly always get caught out with something that you didn't expect."

Building animal-mimicking robots is not easy, however, particularly when knowledge of the system's biology is lacking. For example, when Prescott and his collaborators went to program whisker movements in their motorized robo-rats, which they use to test theories about cognition and motor control, they realized they didn't know how the whiskers should react when they touch objects. "No one had asked that question," Prescott says. (For more on Prescott's research, see "Robo Rat," *The Scientist*, April 2012.)

But by asking such engineering questions, researchers often get biological answers. When Frank Grasso, director of the Biomimetic and Cognitive Robotics Lab at the City University of New York in Brooklyn, began designing robots to investigate lobster navigation, he soon learned that having the robots recognize and follow scents wasn't sufficient. Grasso first programmed his robo-lobsters—which consisted of a cylindrical body on two large wheels and fiber-optic antennae that detected chemicals in the water—to head toward high concentrations of an odor, the general principle believed to be used by real lobsters to locate their prey. (See photograph on this page.) But this rule failed to recapitulate natural lobster behavior. However, when the researchers also gave the robots a sense of flow, and programmed them to assume that odors come from upstream, the bots much more closely mimicked real lobster behavior. "That was a demonstration that the animals'

brains were multimodal—that they were using chemical information and flow information," says Grasso, who has since worked on robotic models of octopus arms and crayfish.

University of Sheffield evolutionary biologist Marshall is tackling a similar problem as he tries to simulate how honeybees process visual input. Part of a multi-institution collaboration to model the entire honeybee brain on a supercomputer and use the simulation to control two flying robots, Marshall is developing the algorithms that will dictate how the robots see through their camera eyes. While the neural circuitry underlying the olfaction system has been well studied in bees, the vision system has "not been described to anywhere near the same extent," says Marshall.

Robots often debunk the idea that you need higher cognitive processing to do what look like cognitive things.

—Andrew Philippides, University of Sussex

"So there we're working much more in the dark," gathering clues from what's known about vision in other insect species, including *Drosophila* and bumblebees, while making logical assumptions from an engineering perspective as well.

Of course, Marshall emphasizes, the critical test will come when the researchers implement the cognitive model they've developed in the flying robots—currently being adapted from premade 50-cm X-shaped "quadcopters"—which will communicate wirelessly with the supercomputer running the honeybee brain simulation. (See photograph on opposite page.) "The [model's] embodiment in the robot is a really important part of the project," Marshall says. "You get richer sensory information from the real world . . . than you could ever hope to achieve in a simulated world."

Mixed societies

In some sense, the use of robotics in animal-behavior research is not that new. Since the inception of the field of ethology, researchers have been using simple physical models of animals—"dummies"—to examine the social behavior of real animals, and biologists began animating their dummies as soon as technology would allow. "The fundamental problem when you're studying an interaction between two individuals is that it's a two-way interaction—you've got two players whose behaviors are both variable," says Gail Patricelli, a behavioral ecologist at the University of California, Davis, who has animated taxidermied bowerbirds and sage grouse to study their courtship behavior. Dummies allow biologists to control one side of the interaction, and robotics is equipping the dummies with ever more-advanced behaviors. (See "Sophisticated Dummies" on page 47.)

With the advent of autonomous, sensing-and-reacting robots, however, the introduction of robots into animal societies has taken on a whole different meaning. "The idea here is to build mixed groups of animals and robots that interact [and] show

BEES © AXEL MICHELSEN; BOWERBIRD COURTESY OF GAIL PATRICELLI

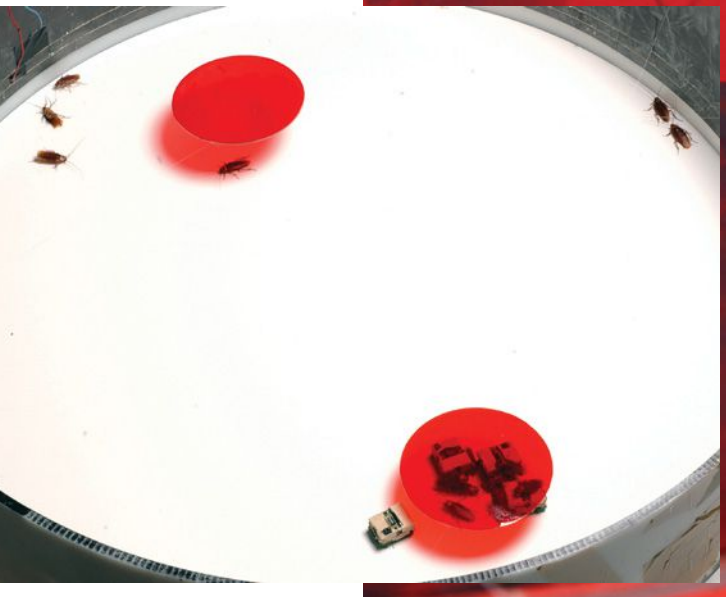
HONEYBEES

Commonly cited classics of biorobotics are the bee experiments of the early 1990s by Axel Michelsen of Odense University in Denmark (now the Odense campus of the University of Southern Denmark). He built a wax-coated brass honeybee model, slightly larger than a real bee and having only a single wing, and sent it into a beehive, programmed to perform various renditions of the waggle dance, which the bees interpret to reveal the location of a new food source. "The model simulates the dance, carries a scent, and has an acoustic near-field similar to that of live dancers," Michelsen and his colleagues wrote in a paper published in 1992.[7]

"It was moderately successful," says Gail Patricelli, a behavioral ecologist at the University of California, Davis—though "some bees attacked it." Of course, the honeybee waggle dance is "one of the most complicated forms of communication outside of humans, so it was a pretty hard place to start."

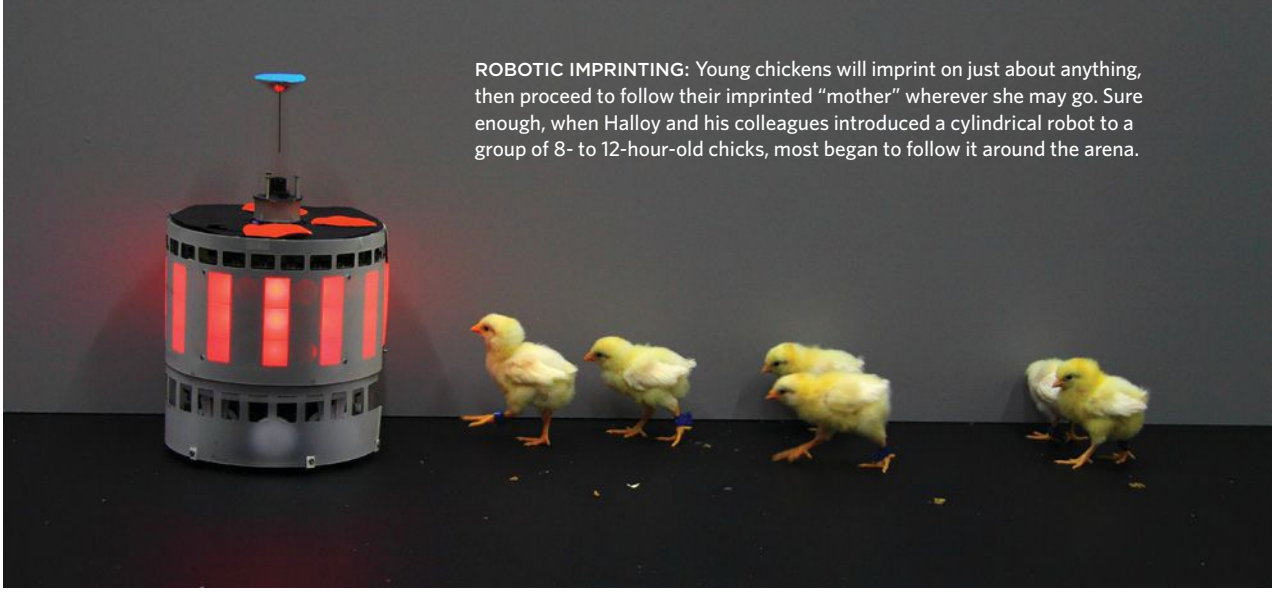
BOWERBIRDS

As a PhD student in Australia, Patricelli decided to tackle something she thought would be a little easier—building a model of a female satin bowerbird (*Ptilonorhynchus violaceus*) to study male courtship behavior. She covered a sheet-metal frame with the skin and feathers of a female bowerbird, and developed custom electronics to fit inside and control the robot's behavior. She and her colleagues operated the robot by remote control, instructing it to occasionally display a startle response to a courting male. As they hypothesized, the males lowered the intensity of their courtship displays following a startle response, presumably to avoid scaring the female any further and losing a potential mate.[8]



CHICKS COURTESY OF JOSÉ HALLOY, FRANCESCO MONDADA, EPFL GROUP; COCKROACHES COURTESY OF THE LEURRE PROJECT

BOT BUDDIES: Cockroaches like to hide out in darker shelters, but they also prefer to hang out with their peers, rather than remain alone. By covering small, autonomous robots with cockroach-scented filter paper, José Halloy of the Université Paris Diderot and his colleagues were able to convince the real insects to follow the bots into more well-lit shelters.



ROBOTIC IMPRINTING: Young chickens will imprint on just about anything, then proceed to follow their imprinted "mother" wherever she may go. Sure enough, when Halloy and his colleagues introduced a cylindrical robot to a group of 8- to 12-hour-old chicks, most began to follow it around the arena.

social interaction in the long term," says Université Paris Diderot researcher José Halloy, who has tested the ability of robotic cockroaches to interact with the real insects.

But building a robot that animals will accept as one of their own is complicated, to say the least. Robots employed to explore theoretical concepts of behavior and cognition don't necessarily have to look exactly like the animals they're mimicking. But to develop social relationships with real animals, robots have to look, smell, and act the part at least well enough to fool the research subjects. "It's a very challenging task . . . to build a device that's capable of being part of the group," says Halloy.

So he started simple. While at Université Libre de Bruxelles in Belgium, Halloy and colleagues developed robots that could successfully integrate with a group of cockroaches. Cockroach cognition is relatively straightforward to program, and it takes just a drop of cockroach pheromone to convince real cockroaches that the robot is a member of their species. "What is important in animal-robot interactions is to send the correct signals," says Halloy. "And in the case of the cockroaches, it doesn't matter that you look like a cockroach, but it does matter that you smell like a cockroach."

If you have machines and animals interacting, then the question is, what kind of collective intelligence can they display?

—José Halloy, Université Paris Diderot

After programming the robots to select between lighter and darker shelters just as cockroaches do, the researchers allowed them to interact with the real insects. Sometimes they kept the robots programmed to the natural cockroach behavior of preferring darker shelters, and watched as the robots seamlessly integrated into the group. Other times, the team programmed the robots to frequent more well-lit ones, and even when there were only four robots in a group with 12 cockroaches, the researchers saw a dramatic shift in the group's behavior: many of the insects would follow the robots into the lighter shelters as a result of live cockroaches' tendency to aggregate.[4] (See photographs on opposite page.)

The robot is translating a message, Halloy says. "We want to say to the cockroach, 'Okay, guys, you prefer the dark shelter, but we'd like you to be in the lighter one.' By programming the robot, [we] were capable of switching the whole group decision."

A handful of other researchers have also successfully integrated robots with live animals—including fish, ducks, and chickens. There are several notable benefits to intermixing robots and animals; first and foremost, control. "One of the problems when studying behavior is that, of course, it's very difficult to have control of animals, and so it's hard for us to interpret fully how they interact with each other," says Iain Couzin of Princeton University, who has used autonomous fish robots, controlled by a mag-

SAGE GROUSE COURTESY GAIL PATRICELLI; SQUIRREL COURTESY OF RULON CLARK

SAGE GROUSE

Now at UC Davis, Patricelli is delving into the courtship behavior of sage grouse (*Centrocercus urophasianus*), males of which congregate in competitive displaying groups called leks on the plains of Wyoming. Once again, she enclosed a robotic device in the skin of a female bird, and in this case, the robot could traverse the field along model-train tracks. It takes a bit of "experimental arts and crafts . . . to make them move naturally," says Patricelli, but "when she's out there moving around on the lek across the rough ground, it's remarkably lifelike."

After setting a robot loose in the lek, Patricelli's team observed the males' reactions. As the grouse-bot approached, males that generally were more successful at securing mates increased the frequency of their courtship display—which consists of inflating two air sacs on their necks to produce a loud whooping call—without sacrificing any volume or intensity.[9]

SQUIRRELS

In addition to creating realistic models of animals' peers, some researchers are using robots to mimic predator-prey interactions. The late psychology professor Donald Owings of UC Davis, for example, launched a project to build robotic squirrels in order to investigate the signals they send to hunting snakes. Noticing that squirrels heated up their tails when waving them at threatening rattlesnakes, which can "see" in the infrared part of the light spectrum, Owings and his colleagues equipped a taxidermied female California ground squirrel (*Spermophilus beecheyi*) with a motorized tail that could produce heat. Sure enough, experiments with real rattlesnakes showed that the combination of signals was more effective at getting the snakes to back off than tail flagging alone.[10]

net carried by a wheeled robot below the tank, to test responses of sticklebacks and golden shiners to both robotic peers and faux predators.[5] "One advantage of employing a robot is you can have control over one or even a number of different individuals within groups, so you can set up scenarios—repeatable scenarios—[to reliably] test the responses of individuals." (See "Crowd Control," *The Scientist*, July 2013.)

Of course, with more discerning species, the task becomes more difficult, says Couzin, who has found that while sticklebacks seem to display fairly natural responses to the faux fish, golden shiners are more sensitive to the acoustic vibrations caused by the robot's motor. "To convince them that your model fish is really a fish can be quite tricky," he says.

From an engineering perspective, this challenge raises some fundamental questions. "What do you need to be capable of doing if you want to be a social animal?" Halloy says. "We don't really understand that fully on the animal side, and we certainly don't understand that on the artificial side."

So for his second venture into mixed animal-robot societies, Halloy turned to another well-studied system: imprinting in birds. Inspired by the classic imprinting experiment in which a group of goslings hatched to see Austrian biologist Konrad Lorenz hovering over them, and then proceeded to follow the father of ethology instead of their real mother, Halloy taught hundreds of 8- to 12-hour-old chickens to imprint on a robot that he controlled. (See photographs on pages 50 and 52.)

By and large, the experiment seemed to work, but it did prove a more difficult task than getting a group of cockroaches to follow a pheromone-spiked robot into a lit shelter. Most of the baby chickens imprinted strongly; however, for a minority the imprinting failed. In some cases, the chicks were even afraid of the robot—something Halloy hadn't expected. Nevertheless, the work once again showed that robots can alter the behavior of the group: when robot-imprinted chicks were mixed with chicks that had failed to imprint, the group displayed some interesting dynamics, with the animals getting closer to, then farther from, the robot as they explored the space. Groups of six strongly imprinted chicks had, of course, no problem faithfully following their robot mother.[6]

Now in Paris, Halloy continues to probe animal group dynamics using robots. In February, he began a project to design robots to interact with schools of fish. The robots will be situated outside of the tank, where they can carry some sort of lure, Halloy says—"a fake fish or anything else that can send signals to the group of fish." In a parallel project, researchers at the University of Graz in Austria are aiming to do something similar with juvenile honeybees, using a static array of devices that can send a moving signal to the bees.

In addition to the basic science—namely, understanding why animals behave the way they do—another aspect of the research motivates Halloy: "What kind of artificial collective intelligence can the animals produce when they're interacting with machines?" he wonders. "Are the animals capable of using machines?" It feels a bit like science fiction, Halloy admits, but animals can do plenty of things that robots cannot—most simply, they can effectively navigate complex landscapes. At the same time, robots, equipped with powerful computers and wireless communication technologies, can do many things animals can't. So, says Halloy, the question is, "Can they do more together than they can do separately?" ■

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The Literature

NEUROBIOLOGY

Scent Sorting

THE PAPER

S. Kikuta et al., "Odorant response properties of individual neurons in an olfactory glomerular module," *Neuron*, 77:1122-35, 2013.

Although the human sense of smell is feeble compared to that of many animals, it is acute enough to distinguish between very similar odors. Researchers know a lot about how our 400 or so distinct types of odor receptors combine to differentiate roughly 10,000 odors. But the neuronal architecture underlying our ability to precisely discriminate between slightly different odorant molecules picked up by the same receptor is less well understood.

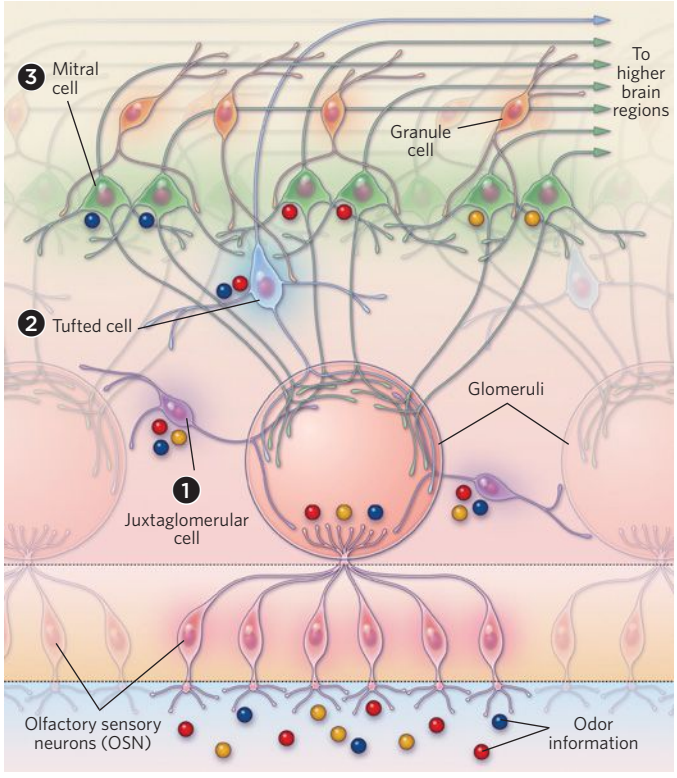
In humans, the outer layer of the olfactory bulb, the most forward part of the brain, which lies atop the back of the nasal passage, is comprised of roughly 5,500 ball-like neural junctions called glomeruli. An individual glomerulus receives input from olfactory sensory neurons (OSNs), each of which expresses a particular odorant receptor that binds a range of molecules, and thus is activated by a specific set of odors. Within the glomerulus, those OSNs connect to a network of deeper, downstream neuronal layers to form a glomerular module, which somehow parses individual odors before signaling to higher brain regions.

The architecture and activity of this downstream network has so far eluded observation, however—so how the glomerular modules actually parse the information was unclear.

To tease apart the inner workings of these odor decoders, Shin Nagayama of the University of Texas Medical School in Houston and colleagues injected green calcium dye into the various individual cells that make up a single glomerular module in a living mouse. With a series of injections, they visualized the anatomical connections between different types of neurons. The team then compared the activity of the different types of neurons in each layer of the module.

Immediately surrounding each glomerulus is a mixed population of inhibitory and excitatory cells called juxtaglomerular neurons. After exposing OSNs to a combination of odors, the researchers noticed that the juxtaglomerular cells responded to a much wider range of odors than the next layer of neurons, made up of tufted cells, which were more discriminating. The last cell type, mitral cells, located furthest from the glomerulus were most selective, responding to just a few specific odor molecules.

The odor selectivity of neurons, or the range of individual odors they respond to, in the glomerular module "is sharpened in a gradient from surface neurons to those in deeper layers," says Nagayama.

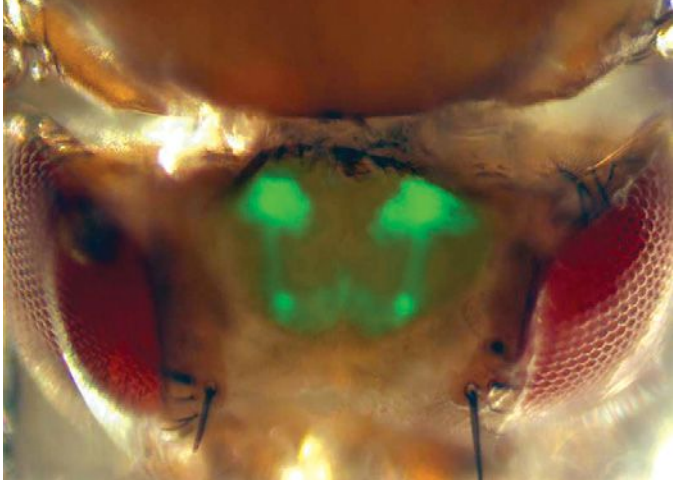


PARSING SMELL: Within every glomerular module, olfactory sensory neurons (OSN) activated by a particular range of odorants connect to a network of deeper, downstream neurons. Juxtaglomerular cells, located in the first layer 1, respond to a wider range of odorants than tufted cells, neurons in the next layer that relay signals to higher brain areas 2. Mitral cells, a type of neuron located in the deepest layer of the module, respond to even fewer odorants 3. The particular set of odorants that activate the mitral cells depends on the lateral location of each cell within this layer.

The mechanisms that govern odor response profiles remain unclear, but the results indicate that neurons in different layers of the module may undergo differing degrees of inhibition by surrounding interneurons called granule cells, says Nathan Schoppa, a neurobiologist at the University Colorado School of Medicine who was not involved in the study. "Juxtaglomerular cells, which display odor responses as broad as [those of] OSNs, may not be affected at all by this intrinsic circuitry," Schoppa says, "whereas tufted cells and, then, mitral cells are increasingly affected."

Regardless of the mechanisms, in terms of solving the problems confronted by the olfactory system, "the narrowing of the odor-tuning profile seen the most for mitral cells is likely to be important for helping the brain distinguish different but similar odors," says Schoppa.

—Dan Cossins



VISUALIZING SMELL: Neurons in the mushroom bodies of *Drosophila*, shown in this composite image, help the flies discriminate among a large number of odors.

NEUROSCIENCE

Odor Encoder

THE PAPER

R.A.A. Campbell et al., "Imaging a population code for odor identity in the *Drosophila* mushroom body," *J Neurosci*, 33:10568-81, 2013.

THE FINDING

In the fruit fly (*Drosophila melanogaster*), pheromones and other highly salient odors have direct neural links to the brain from antennal olfactory receptor neurons. However, there are more smells that the flies must distinguish than there are odor-processing neurons, says Glenn Turner, a neuroscientist at Cold Spring Harbor Laboratory in New York. Imaging the brains of flies exposed to different smells, Turner and colleagues discovered that many odors are encoded by small, nonlocalized ensembles of neurons, allowing the insects to discriminate among vast numbers of closely related scents.

THE DISCRIMINATION

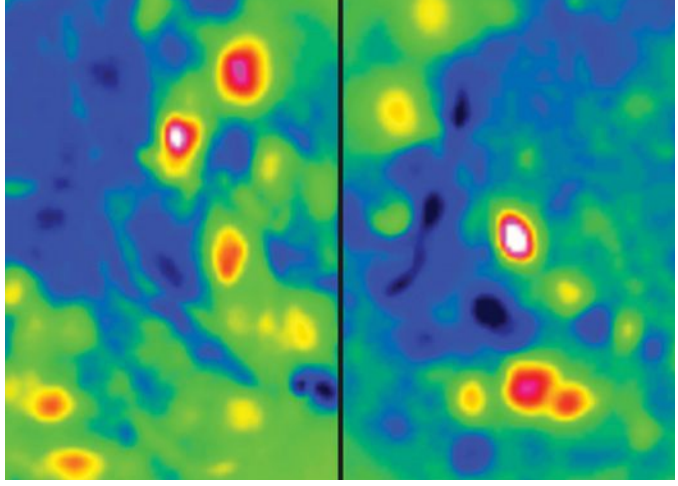
The researchers used two-photon calcium imaging to record neural activity in immobilized flies exposed to various odors. Focusing on the mushroom body, an area that plays a role in olfactory learning and memory, the experiments revealed sets of neurons that encoded particular odors. Behavioral experiments demonstrated that the less the neural ensembles overlapped, the better the flies were able to differentiate the odors.

THE NEURAL NETWORK

As few as 25 of the fly's 2,000 or so mushroom body neurons encode enough information about an odor to account for the fly's performance in the behavioral discrimination task. The researchers also demonstrated that flies could generalize a learned aversive association when smells were represented by largely overlapping neural sets.

THE LINK

"This work provides a powerful correlation between population cell activity and behavior," says Vivek Jayaraman, a neuroscientist at the Janelia Farm Research Campus in Ashburn, Virginia, who was not involved in the study. **—Chris Palmer**



ODOR PATTERNS: These two panels show mouse olfactory bulb neurons responding to two different odors. In afterimages, patterns like these persist despite the odor being removed.

NEUROSCIENCE

Let It Linger

THE PAPER

M.A. Patterson et al., "Odor representations in the olfactory bulb evolve after the first breath and persist as an odor afterimage," *PNAS*, 110:E3340-49, 2013.

THE FINDING

Afterimages are the lingering sensations of a stimulus that is no longer present. Taste, vision, hearing, and touch can all induce afterimages, and now, Alan Carleton of the University of Geneva and his colleagues have described the phenomenon in mouse olfaction. They showed that even after an odor is removed, some neurons persist in their odor-specific activity.

THE DETAILS

Carleton's group found little activity in olfactory glomeruli, where incoming sensory neurons terminate, once an odor was removed. However, activity continued in some olfactory neurons located downstream from the sensory neurons, called mitral/tufted (M/T) cells. When the researchers directly stimulated the M/T cells with light, using optogenetics, they observed lingering cellular activity in the form of neuronal firing, without invoking activity in the sensory neurons.

THE LOCATION

The findings combine to implicate the brain, rather than the nose, as being responsible for the afterimages. "The post-odor response of mitral cells is not due to the odor molecules remaining in the nasal cavity, but presumably reflects the neuronal circuit activity generated centrally in the olfactory bulb and/or olfactory cortex," Kensaku Mori of the University of Tokyo, who was not part of the study, said in an e-mail.

THE FUNCTION

Carleton said his next step is to understand whether there is any functional relevance of odor afterimages. "Maybe these afterimages can be useful to form traces or memories in the brain so that when you have [neural activity simulating] a longer odor presentation . . . the brain may memorize it better," he said. **—Kerry Grens**

COURTESY OF ROB CAMPBELL AND GLENN TURNER; COURTESY OF ALAN CARLETON

PROFILE

An Olfaction Odyssey

Thanks to a book, a war, and a big green caterpillar, John Hildebrand found himself mapping the exquisite and surprising wiring of the insect olfactory system.

BY MEGAN SCUDELLARI

It began with a fateful encounter with a praying mantis. In 1965, a young John Hildebrand, then a biochemistry PhD student at Rockefeller University in New York City, spent his evenings in the university library catching up on the latest publications ("You know—before there was an Internet," he says).

One night, long after the sun had set and only a few people still milled around the library, Hildebrand was perusing the New Books shelf and noticed a slim volume with a vivid color photograph of a praying mantis on the cover. "I really liked praying mantises—as a kid I used to keep them as pets—so I picked up the book," says Hildebrand. He settled into a large easy chair and read the book from cover to cover.

"If I hadn't liked praying mantises, I wouldn't have picked up the book, and I don't know that I would ever have found what I've loved doing ever since then."

"When I put it back on the shelf, the little voice in my head said, 'That's it. You've just found what you want to do,'" says Hildebrand. The book was *Nerve Cells and Insect Behavior* by Kenneth Roeder. Hildebrand hunted down all of Roeder's papers, then those of other researchers referenced in the book. Using that network of papers and scientists as a foundation, he identified prospective universities where he might study insect neurobiology. "If I hadn't liked praying mantises, I wouldn't have picked up the book, and I don't know that I would ever have found what I've loved doing ever since then."

What Hildebrand has loved doing—and has built a successful career around—is investigating the insect olfactory system. He pioneered the use of the giant sphinx moth *Manduca sexta* as a model laboratory species for neurobiological research, and has shown how the insect detects various odors and processes them in the brain.

Here, Hildebrand harkens back to how the Vietnam War dictated his early career, why he refused to give up music for science, and how he was tricked into moving from New York to Arizona.

HILDEBRAND ON THE HUNT

Beantown. "I grew up in Boston and got into science very early thanks to my father, who was an engineer with a background in chemistry. As a young student, I worked in the fledgling Museum of Science in Boston as a volunteer in the live animal room for several years. But though I especially liked biology, starting at the age of four I was a fanatical musician. I came to the point of choosing going to college or going to a conservatory or becoming a performer. One way or another, I was going to be a musician. The short of it is that I ended up going to Harvard because it was the nearest university to where I lived. Nobody believes it, but that was the reason. It was only 3 miles from my house. I had this rationale in my mind that since I was already active in the music scene in Boston, if I went to Harvard I could keep doing all the things I was doing. And that proved to be true." But Hildebrand had overlooked one major problem.

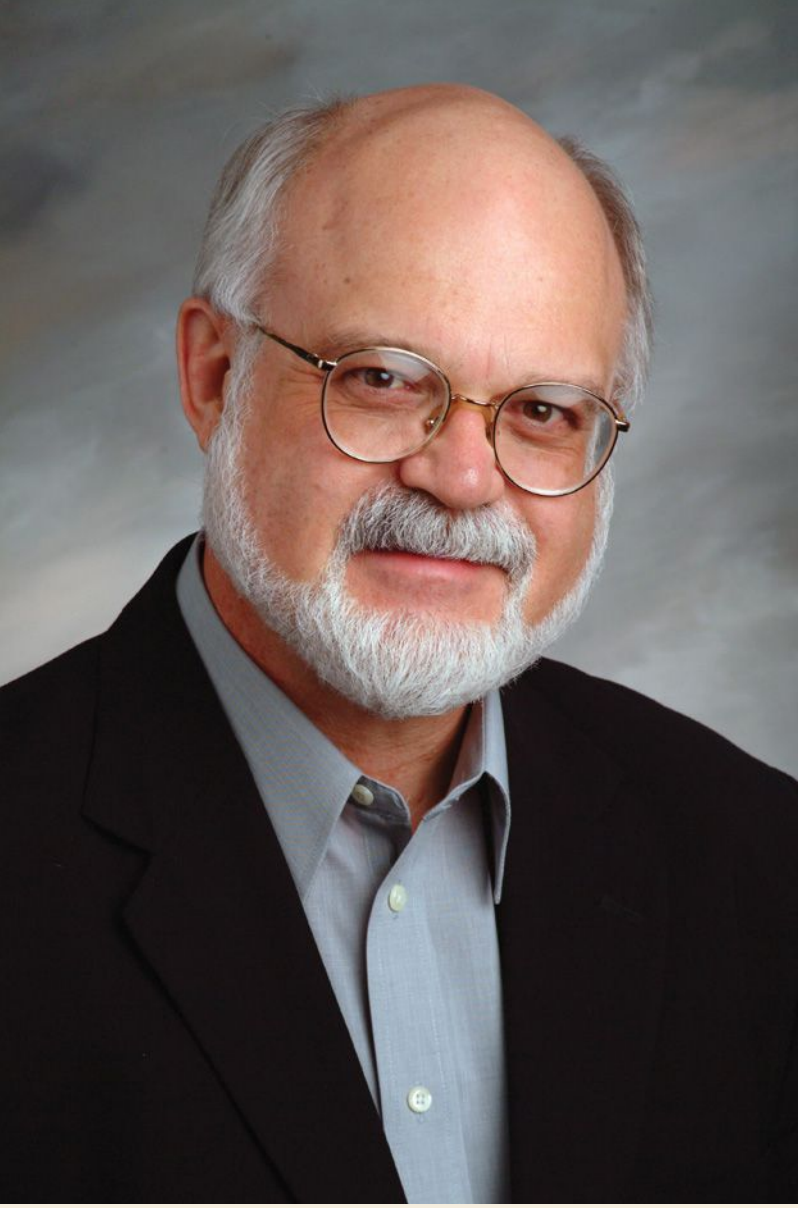
When one door closes . . . "I got to Harvard, intending to be a music major, then realized that I'd made a big mistake. You can study music history, composition, or theory at Harvard, but you can't major in applied music. About the time I started to realize I was in the wrong place, I took a general education science course, called 'The Nature of Living Things,' taught by George Wald (who later won a Nobel Prize). It was supposed to be a biology course, but we started with the physical properties of the universe, and he built the story of where life came from. I thought it was the most fantastic thing ever. By the end of that year, I wasn't going to be a music major anymore. I was co-opted into science."

Learning the game. As a sophomore, Hildebrand began doing research in a lab. "I was lucky I got to do that. That wasn't so common back then in the 1960s. I was secondarily lucky that I stumbled into a wonderful young faculty member, John Law, who welcomed me as a research student in his lab. He was only about 10 years older than I, and he had a wonderful philosophy that young students should get to know the whole game. So I learned all about how to get grants and how to publish." With Law, Hildebrand wrote and published his first paper, on bacterial phospholipids, in *Biochemistry*.

In times of war. Hildebrand went from Harvard straight to graduate school at Rockefeller, but not of his own volition. "If I had my choice, I would have taken a year off, taken a breather and explored the world a little bit. But it was the Vietnam War, and I was subject to the whim of the draft board. They made it clear to me that if I took a day off, *ever*, then I would go to Vietnam. So I went to graduate school literally the day after I graduated from college."

Hands-on lesson. At Rockefeller, Hildebrand joined the research group of Belgian cell biologist Christian de Duve, who would later go on to win the Nobel Prize for his discoveries of lysosomes and peroxisomes. "Although I loved the ideas and the

COURTESY OF JOHN HILDEBRAND



JOHN G. HILDEBRAND

Regents Professor
Department of Neuroscience
University of Arizona
Tucson, Arizona

Greatest Hits

- Described the organization of the insect's antennal olfactory system and its development.
- Showed, for the first time, antennal innervation of the brain has a dramatic effect on sex-specific development and behavior.
- Established the giant sphinx moth as a model for studying olfaction and demonstrated similarities between mammalian and insect olfactory systems.
- Discovered a neural basis for how a complex sensory stimulus activates a behavioral response.

people in the lab, I did not at all like what I was doing. Without going into the gory details, I discovered I didn't like killing animals and ended up quitting his lab in just one year. It was a great life lesson: There is a big difference between the things you find interesting and the things you want to do with your hands and your time." Next, Hildebrand worked with Leonard Spector, in a research group led by Fritz Lipmann, where he wrote a thesis on the mechanism of the succinyl coenzyme A synthetase reaction. "I was back to where I liked to be, between chemistry and biology. All was well, but it was a time of uncertainty, because I knew the kind of biochemistry I was doing was bounded; it was finite. Pretty soon all these biochemical pathways were going to be known, so I didn't know what my future was going to be."

The best of times, the worst of times. Enter the praying mantis. After fatefully reading *Nerve Cells and Insect Behavior*, Hildebrand joined Harvard Medical School's recently launched Department of Neurobiology as a postdoc, once again not taking time off for fear of the draft board. There, he worked for three years with the young biochemist Edward Kravitz, then stayed on at Harvard as an instructor, despite other offers of a faculty position. "The offer [from Harvard] was by far the worst offer I received. Even though it was a tenure-track assistant professorship, there was no salary, just a license to go out and search for grant money to support me. But it was also my best offer, because from an intellectual point of view, I would be part of this amazing department that was in its early golden years. And I was finally free to do exactly what I wanted—to work on bugs."

Bugs in the cupboard. But which bugs? As he prepared his lab, Hildebrand turned to a fellow insect-loving friend at Harvard, Fotis Kafatos, to find an insect that would allow Hildebrand to study the nervous system. "I described what I wanted—a big insect that goes through complete metamorphosis and is easy to rear in the laboratory. Fotis got this wonderful grin on his face and handed me a caterpillar the size of a large cigar. It was the larva of a big moth called *Manduca sexta*, and I started to raise them in a cupboard in my lab in Boston. I've worked on *Manduca* ever since."

HILDEBRAND HITS THE JACKPOT

Smelly start. "Metamorphosis is a great opportunity to look at changes in life cycle, because as this animal goes from a caterpillar to a moth, the whole nervous system gets reorganized, but genetically it's the same animal. With my first graduate student, Joshua Sanes, I

decided to look at the olfactory system." Hildebrand and Sanes studied two physically separate populations of nerve cells in the moth's olfactory system: those in the antennae—the insect's main olfactory organs—and those in the brain receiving signals from the antennae. By manipulating each population of cells, they were able to investigate how much each depended on the other for normal development throughout metamorphosis.

Gender-bending experiment. Following up on those earlier studies, a new graduate student, Anne Schneiderman, accepted the task of testing what influence swapping developing antennal structures had on the development of three male-specific olfactory structures—little knots of neuronal processes called glomeruli—in the *Manduca* brain. "When you take on a new student, you want to give them an orientation project to get them used to the lab, something that it would be okay if it didn't work—a crazy idea or something adventurous. The project I gave her, thinking it would never work, was to transplant the precursors of adult antennae in caterpillars, before they became moths, from a male to a female and from a female to a male. And the bloody thing worked the first time!" The transplant had a dramatic effect on behavior: The female moth, which developed male antennae, flew toward female sex pheromone, while the male moth, which developed female antennae, showed a characteristic female flight pattern. The finding, that sensory input makes an important contribution to brain development and sex-specific behavior, led to two papers published in *Nature* in 1982 and 1986. "In the history of my lab, that was one of the greatest 'wow, gee-whiz' discoveries."

Here comes the sun. In 1980, Hildebrand left Harvard's medically oriented campus to focus on basic biology at Columbia University in New York City. Once settled, Hildebrand never expected to leave New York, but after only five years, he found himself setting up shop at the University of Arizona. "I was sitting in New York on a dreary, wintry day, looking at the sleet and snow, and I got a phone call out of the blue from the University of Arizona's vice president for research. He said that they had decided to develop a research group in the field of invertebrate neurobiology, and would I come there as a consultant to advise them? I said sure, because it was crummy weather, and I thought I'd like to visit Arizona." But the "consulting" gig turned out to be more than promised—Hildebrand met with almost 100 people on campus and was asked to submit a proposal on how he would build a neurobiology research unit. "I was amused, and suspicious," says Hildebrand with a laugh. A year later the university offered him the chance to build his own department and also offered a faculty position to Hildebrand's new wife, Gail Burd, a neuroscientist at Rockefeller University. The couple took the bait and moved to Arizona in 1985. Hildebrand started his department, and Burd went on to become an associate dean and later, vice provost of the University.

Ready, set, fire. In January this year, Hildebrand and coworkers published in *Science* what he describes as the "culmination" of work done in his lab. "Ever since I started focusing on olfaction, we've wanted to understand how naturally occurring, complex olfactory stimuli are encoded in the nervous system." His team discovered that sensory cells in insect antennae deliver odor information to the glomeruli in the brain, whose output neurons then convey patterns of simultaneous neuronal spikes—patterns of action potentials—deeper into the brain to stimulate downstream nerve cells and, through them, elicit a behavioral response. When confronted with a non-natural stimulus, an odor that is not behaviorally significant to the moths, the glomerular output neurons do not fire simultaneously. "Having discovered the phenomenon of coincident firing, we're now searching madly for coincidence detectors. We haven't found them yet, but we have phenomena that look like we're on the right track—cells that are activated under conditions that evoke coincident firing."

> **"People think they have to make choices, but you can have an enriched life with two different passions."**

HILDEBRAND'S HOPES AND FEARS

Berra-isms. "My favorite philosopher of the 20th century is Yogi Berra. One of his greatest statements that has really applied to my life was, 'If you come to a fork in the road, take it.' That's what I did. I kept music going for a very long time, as a professional low brass player for 30 years, freelancing in Boston and New York, while developing a career as a scientist. People think that they have to make choices, but Yogi taught me that you don't. You can follow both paths from the fork and have an enriched life with two different passions."

Life transition. "I'm 71, near the end of my career. I'm not going to keep running a lab forever. But I have already started transitioning to being involved in professional organizations, including the National Academies. I think graybeards like me that have been around awhile need to be involved, not only in education and research but also in trying to shape and influence science policy as we go forward."

Losing the basics. "The federal priorities for science funding are off the rails. Now everything has to be about economic development, national security, or translation. Basic discovery research is imperiled. It's unlikely that anyone could ever start a career today working on what I've done all my career. I think that's a tragedy."

Around the world. "I'm active with the International Brain Research Organization to teach students in South America and Southern Europe. These are intensive, one- to three-week courses for students at different levels, in countries where students are hungry for opportunities for intensive science training. The young people in South America are so passionate. I love to serve students who know why they're there and are motivated and grateful for their opportunities." ■

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Yoav Gilad: Gene Regulator

Professor, Department of Human Genetics, University of Chicago. Age: 38

BY CHRIS PALMER

Growing up in the town of Beersheba in Israel, Yoav Gilad became restless when high school failed to challenge him. So, after testing out of his classes and graduating early, he started taking college courses at age 15, eventually matriculating at Ben-Gurion University his hometown, where he majored in molecular genetics and biochemistry. "I was fascinated by the contrast between the fact that so much is planned and programmed, but there's also so much variability," Gilad recalls.

Mandatory army service sidelined his college career for three years, but Gilad eventually returned to Ben-Gurion, where he got his first taste of laboratory research near the end of his undergraduate tenure. Gilad says he was captivated by the idea that through laboratory science he could potentially become the first person in the world to find the answers to age-old questions. "The charm of that really attracted me," he says.

METHODS: Near the end of his army service, Gilad met geneticist Doron Lancet, who, as part of his reserve duty, occasionally lectured to troops about genetics and the origin of life. Three years later, when Gilad applied to graduate school at the Weizmann Institute of Science, he looked up Lancet, who not only remembered the young graduate, but offered him a lab rotation on the spot. "I accepted him gladly because he is so brilliant," says Lancet. To support himself financially, Gilad worked as a dive instructor and played on the Israeli and German six-man indoor professional volleyball circuits. "It paid better than a graduate school stipend," he says.

In Lancet's lab, Gilad showed that humans have nearly twice as many pseudogenes—genes that have lost the ability to code proteins—for olfactory receptors as do some nonhuman primates, reflecting humans' reduced dependence on olfaction.[1] In subsequent research, he and Lancet speculated that the marginalization of olfaction in some primate species, concomitant with the evolution of color vision, paved the way for humans' predominant reliance on their visual system, which includes three types of color receptors called cones.

RESULTS: Although Gilad secured a faculty position at the University of Chicago right out of graduate school, he jumped at the opportunity to first explore a new research direction as a postdoc. "I knew I could gamble, do something a little dangerous," he says. He spent the next two years working in Kevin White's lab at Yale University developing high-throughput multispecies DNA arrays. Using this new tool, Gilad identified, across several primate species, sets of genes whose expression appears to be driven by natural selection, suggesting that gene regulation can be an important driver of evolution.[2] "Yoav is unbelievably good about treating evolution in a quantitative fashion—one of the world's best," says Lancet.

DISCUSSION: Now in his own lab in Chicago, Gilad studies the mechanisms underlying gene regulation variability. In collaboration with Jonathan Pritchard, now at Stanford University, Gilad pegged genetic variants that alter chromatin accessibility and transcription factor binding as key mechanisms by which genetic variation leads to differences in human gene expression.[3] "Yoav's really fearless in terms of pushing new technologies and building up data sets that combine multiple aspects of gene regulation," says Pritchard.

Next up for Gilad: reprogramming induced pluripotent stem cells (iPSCs) in humans, chimpanzees, and rhesus macaques to become cardiomyocytes, hepatocytes, and motor neurons, thereby expanding his search for variation in gene regulation to whole new classes of cells to which he previously has not had access. "Now we're not limited by cells we can get from a blood draw." he says, "Rather, we are limited to cells that we can differentiate from the iPSCs."

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3. J.F. Degner et al., "DNase I sensitivity QTLs are a major determinant of human expression variation," *Nature,* 482:390-94, 2012. (Cited 52 times)

Scents in a Flash

The modern technique of optogenetics stimulates the complex act of smelling with a simple flash of light.

BY AMBER DANCE

To study the sense of smell is to sign on for a challenge. The olfactory bulbs comprise an elaborate network of cell types and connections, and olfaction begins with input from odor receptors expressed by sensory neurons in the nose: about 400 different receptor types in humans, 1,000 in mice. Each receptor can typically respond to several different odorants. Compared to the visual system, which has just three color receptors—for red, green, and blue—olfaction is complex.

Furthermore, the stimuli themselves are complex. A seemingly simple aroma like fresh strawberry may comprise multiple odorants, each activating different receptors. And should you waft the enticing scent under the nose of a mouse, how do you control precisely when it inhales those odor molecules, and when the neural receptors are activated? "It's a notorious problem to control stimulus for the olfactory system," says Rainer Friedrich, a professor at the Friedrich Miescher Institute for Biomedical Research in Basel, Switzerland.

Enter the modern technique of optogenetics, in which researchers genetically outfit cells of interest with light-sensitive molecules. In neuroscience, channelrhodopsin—an ion channel from a green alga—is a popular choice. When hit with blue light, the channel opens up. This depolarizes the neural membrane, creating an action potential. In essence, the light stimulates the neuron to fire.

The technique finally gives scientists some of the control they need to scrutinize the smell system, using light to activate olfactory receptor neurons, or cells further on in the smell-processing pipeline, at an exact time. They have also developed ways to precisely dictate where the stimulatory light lands in the olfactory bulb, isolating the neurons of interest while leaving others unstimulated.

In some of the first studies uniting optogenetics and olfaction, scientists have begun to probe how the brain recognizes smells; how it distinguishes odors based on the time they hit the nose or the synchronicity of the nerves firing; and how animals make decisions based on odors they sense. Here, *The Scientist* profiles three experimental systems where optogenetics has lit the way.

SNIFF CYCLE

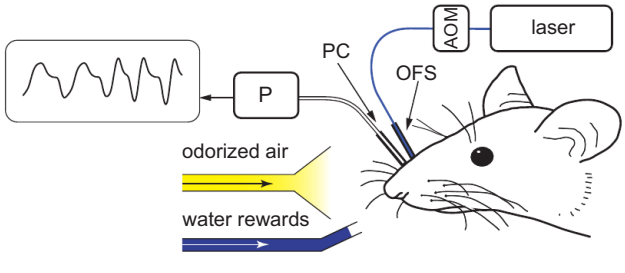
USER: Dmitry Rinberg, Associate Professor, New York University Neuroscience Institute

QUESTION: The timing of a mouse's inhalations and exhalations affects the precise moment when a new odor reaches its olfactory receptor neurons. That is, the animal will notice a novel smell presented at the start of an inhalation faster than one presented at the start of exhalation. But can a mouse distinguish when, in its inhale/exhale cycle, an odor first hits those receptors?

LIGHT SENSOR: Collaborator Thomas Bozza, at Northwestern University, engineered mice to express channelrhodopsin in every olfactory receptor neuron. Exposure to blue light will activate all the receptors. Rinberg has no idea what sensation this overall receptor activation produces in the mice, though he jokes, "Probably it stinks."

METHOD: To activate a mouse's odor receptors, the researchers placed an optical fiber into one nostril. They fed the stimulatory blue light—laser or LED will work—into that fiber to activate the neurons. Into the other nostril, the team placed a pressure cannula to follow the animal's inhale/exhale cycle. The intranasal air pressure dropped as the mouse inhaled, and rose as it exhaled.

Then, the team needed a way to tell when the mouse noticed a smell. They trained each animal to lick for a drop of water in front of its nose when an odorant wafted its way. The mouse's tongue would break a beam of light set up across the opening of the water tube, allowing the scientists to record just when the mouse licked, indicating it had smelled something. The team then substituted blue light, via the optical fiber entering the nose, for an odor and turned on the light at different times in the inhale/exhale cycle, as determined by the pressure cannula. Then, they trained the mice to only expect water for a blue light



OLFACTION IN ACTION: In this experimental setup pressure changes (P) during sniffing are measured via a pressure cannula (PC) inserted into one nostril. An optical fiber activated by laser light inserted through a separate cannula (OFC) into the other nostril stimulates the mouse's optogenetically modified olfactory sensory neurons. The mouse is trained to expect a water reward at specific times during inhalation after light stimulation.

COURTESY OF DMITRY RINBERG, *NATURE*, 479:397–400, 2011

provided during inhalation, and thus only lick under those conditions, ignoring blue light input during exhalation.

RESULT: Not only did the mice readily differentiate stimulatory light provided during inhale versus exhale, they could be trained to distinguish signals provided just 10 milliseconds earlier or later during the cycle, which averages 300 ms total (*Nature*, 479:397-400, 2011). This means any experimenter who provides smells at a specific time should be aware that the mouse's inhale/exhale cycle might affect that timing. In nature, noticing the timing of an odor's appearance might help animals to sense if it's in high or low concentration, or to localize the source, though this hypothesis is unproven.

PROS:
- Mice easily learn the task.
- Since light travels at the eponymous speed, optogenetics allowed Rinberg to provide a "smell" with high temporal precision, something he could never do with chemical odorants.

CON: Critics note that because it uses light instead of real odors, the system is highly artificial and may not activate odor processing in the same manner.

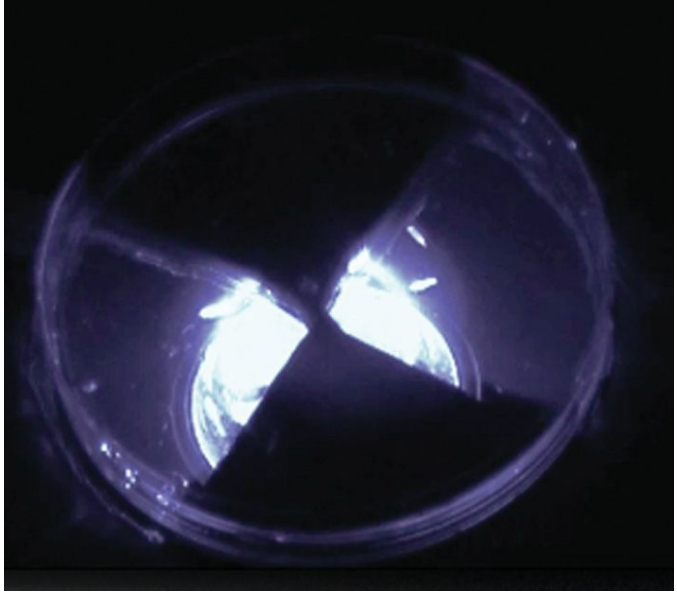
EQUIPMENT: The lab group built the setup themselves, with an apparatus to fix the mouse's head near the pressure cannula, fiber optic, and water bottle. It wasn't difficult, says Rinberg, but he was unable to estimate how much the system cost to create.

ATTRACTANTS AND REPELLENTS

USER: Klemens Störtkuhl, Professor, Ruhr-University of Bochum, Germany

QUESTION: Störtkuhl studies smell in *Drosophila*. The larvae are attracted to many odors, such as the marzipan smell of benzaldehyde present in the rotting fruit they eat. They are also repelled by a few scents, such as the glue-like smell of octyl acetate. How does the fly larva brain decide whether to crawl toward or away from a particular smell? Störtkuhl and colleagues suspected the response would be hardwired into the individual neurons that sense each odor, rather than learned by the brain. If this hypothesis is correct, then the decision to go forward or back is essentially made as soon as an odorant hits its receptor.

LIGHT SENSOR: *Drosophila* larvae have 28 neurons on each side of their olfactory dome organ, located right at the front tip of the body, and each one expresses a different odor receptor. The researchers engineered several lines of flies to express light sensors in only one receptor neuron at a time. They first used channelrhodopsin. It worked, but the signaling requires a cofactor, retinal. *Drosophila* do not make retinal on their own, as many mammals do, and the larvae were disinclined to eat the unpleasant-tasting stuff. So the team switched to a photoactivated adenylyl cyclase, PACα,



COURTESY OF KLEMENS STÖRTKUHL

SMELLING THE LIGHT: *Drosophila* larvae move toward blue light when olfactory sensory neurons that perceive an odor as pleasant are optogenetically stimulated. This noninvasive assay shows that activating the sensory receptor neuron was enough to cause a behavioral response.

another blue light-sensitive protein from a simple eukaryote. When stimulated, it activates the production of cyclic AMP, a messenger molecule that opens membrane ion channels and activates neurons.

METHOD: The researchers tested whether each fly line was attracted to or repelled by a blue light that stimulated the receptor neuron containing the transgene. They placed flies in the center of petri dishes positioned over a blue light. A paper mask blocked the light from two of the four quadrants of the dish. Then, the team simply counted how many larvae crawled into the blue sections, compared to the dark ones (*Front Neurosci*, 5:72, 2011).

RESULT: Despite being averse to light under normal circumstances, most of the fly lines were attracted to the blue light. But those expressing light-activated sensors in two particular odor neurons, which presumably respond to unappealing odors, avoided it (*Front Behav Neurosci*, 4:27, 2010). This indicated to Störtkuhl that activating the receptor neuron was enough to cause the behavioral response. The same hardwiring may occur in people, he suggested. For example, the scent of lemons is known to enhance calm and concentration, and that response might be hard to override or unlearn, he posits.

PROS:
- Noninvasive experiments can be performed on live, normally behaving animals.
- Fruit fly larvae have simple neural wiring, making it easy to activate any neuron desired.
- Movement away from or toward something is an easy output assay.

CONS:
- Fly larvae naturally avoid light, which could confound results. Störtkuhl solved the problem by checking his results in genetically blinded lines.

- For organisms that are not transparent, such as adult flies, higher light intensity may be required to reach the neurons, which could warm the animals and interfere with the experiment.

EQUIPMENT: The behavioral assay is simple to set up, requiring easily obtained items such as a blue LED light, petri dishes, and paper to make the light-blocking mask. Störtkuhl estimates a cost of $300–$500 to get up and running.

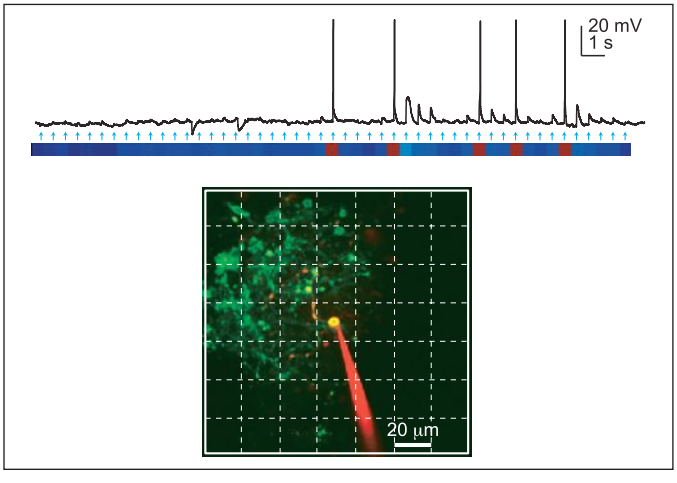
SIGNAL SYNCHRONY

USER: Rainer Friedrich, Professor, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

QUESTION: Friedrich is interested in how zebrafish brains decode incoming odor signals. The input from the olfactory bulb to the dorsal telencephalon, the fish's olfactory cortex, consists of nerve impulses that are either synchronous—with all mitral cells firing at the same time—or asynchronous. Synchronized spikes tend to convey the general category of an odor, like fruity or fishy. The asynchronous patterns communicate more specific odor identities, like lemon or lime. Does the dorsal telencephalon distinguish synchronous from asynchronous inputs and decode them?

LIGHT SENSOR: The researchers expressed channelrhodopsin in all the fish's olfactory sensory neurons. Zebrafish make retinal, so the cofactor was not an issue. By aiming light at the sensory neuron's axons, which project toward the glomeruli, the researchers were able to indirectly activate the glomeruli, and downstream mitral cells, in different patterns.

METHOD: The researchers dissected out the brain and nose of the fish to produce an ex vivo preparation that is viable for 8 hours at room temperature. To project odor-mimicking patterns onto the glomeruli, they used a digital micromirror device (DMD). This chip, with an array of 768 x 1,024 or more mirrors, works the same way as the chip in a digital projector. When a micromirror is slanted toward the fish brain, it sends light to the corresponding spot. When the mirror is slanted away, that part of the brain remains darkened. Using a series of images of light and dark squares as their input, the researchers could activate different regions of the glomeruli in synchronized or asynchronized patterns (*Nat Protocols*, 7:1410-25, 2012). These patterns do not exactly reflect any particular smell, but they are similar to the patterns of real smells. Then, the team used patch clamping to measure the signaling going on downstream in the dorsal telencephalon.



DECODING ODORS: Whole-cell patch clamp recording of the membrane potential in a single zebrafish olfactory bulb mitral cell during optical stimulation (top) generated by a digital micromirror device (DMD). In the bottom image, channelrhodopsin-expressing neurons (green) are visible in the field of view and the recorded neuron was filled with a red fluorescent dye through the patch pipette (red).

COURTESY OF RAINER FRIEDRICH, *NAT PROTOCOLS* 7:1410-1425, 2012

RESULT: Friedrich hypothesized that synchronous signals would result in more action potentials in the cortex, because neurons tend to have a stronger response to synchronized input. To his surprise, the dorsal telencephalon did not differentiate between synchronous and asynchronous patterns, suggesting that the cortex is deciphering specific smells, not general categories of odors (*Nature*, 479:493-98, 2011). That does not mean that the synchrony of signals goes unnoticed by the entire brain, however. Friedrich's team found preliminary evidence that there is another area, which they have not yet characterized, that does distinguish synchrony.

PROS:

- Zebrafish have small brains, and the olfactory bulb contains 25,000–30,000 neurons in an area 400–500 microns across. That means Friedrich can access nearly a third of those neurons with the DMD, a much larger percentage than he could in a larger mouse brain.
- Fish are vertebrates, so the neurobiology is likely more similar to humans' than that of *Drosophila*.

CON: At the moment, there are not good behavioral assays for fish, as there are for other organisms, Friedrich says.

EQUIPMENT: Texas Instruments of Dallas makes DMDs, which are marketed by authorized distributors. The most inexpensive way to get one is to buy a digital projector, but these chips may not have the high frame rate (1 kiloHertz or greater) needed to precisely manipulate neurons at the natural speed, Friedrich says. You can get a more advanced version in a developer's kit from companies such as Wintech Digital Systems Technology of Anaheim, California (www.wintechdigital.com; LightCrafter 4500 costs $1,299), Digital Light Innovations of Austin, Texas (www.dlinnovations.com; DLi4100 entry-level package costs $7,999), or Vialux Messtechnik of Chemnitz, Germany (www.vialux.de; DLP 4100 V-module costs $9,550). You'll also need an LED or laser light source, and mirrors and lenses to mount the DMD to your microscope. ■

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Data Drive

Solutions for sharing, storing, and analyzing big data

BY KELLY RAE CHI

As next-generation sequencing gets ever cheaper and higher-throughput, data file size continues to surge, creating some new, pressing needs for scientists. It's not enough to be able to acquire big data using their own machines; researchers have to be able to store it, move it, and analyze it, and they often want to share it. Large collaborations complicate these steps. As a result, many researchers have resorted to planning their workflows around having a single site for analyses—it's that, or physically shipping hard drives.

Not only are data files growing in size and number, especially those amassing sequence data, but data handling in genomics, epidemiology, and other fields has become unwieldy in other ways. Copying thousands of files, or sharing them with others, has become a laborious process, and as analysis options proliferate, choosing the right tools for the job can take some guesswork. Figuring out how to make data easy to handle and process is a big challenge for life scientists, according to Stan Ahalt, director of the Renaissance Computing Institute and a professor of computer science at the University of North Carolina at Chapel Hill. "The other challenge is learning how to utilize other people's data to accelerate their own lab's science," he says.

Data demands in genomics and other omics projects have helped drive development of new and improved platforms for data analysis, sharing and pooling, and transfer. Profiled by *The Scientist*, four such advances can help with sequencing projects and more.

ViPAR

bioinformatics.childhealthresearch.org.au/software

Pooled data analysis

PROBLEM: A multisite international project, aimed at investigating the epidemiology of autism through population-based health records, needed a method for pooling harmonized data sets. The concern was avoiding the legal and ethical challenges associated with sharing patient data across national borders.

SOLUTION: Using open-source tools, Kim Carter and Richard Francis of the Telethon Institute for Child Health Research in Perth, Australia, created a platform for so-called data federation, in which individual researchers keep control of their own data but can also—through a web-based interface—analyze data pooled among collaborators as if it were their own.

In reality, the researchers using the new tool—dubbed ViPAR for virtual pooling and analysis of research data—are pulling smaller bits of information from each data set for analysis. When they're finished with an analysis run, the underlying data are wiped from the system's memory, which helps address privacy issues. Data fed into the system can also be stripped of participant identifiers. Carter says ViPAR is applicable to projects in traditional and genetic epidemiology as well as targeted studies in genetics and genomics.

GETTING STARTED: The duo launched a more user-friendly version of the site this fall. It will include detailed instructions and cases to help scientists try out the platform or customize it for their particular problem. But the more complex the project, the more likely users are going to need help from bioinformaticians and their local information technology staff in customizing the program, Carter says.

TIP: Data analysis through ViPAR does still involve some data transfer because users must route the relevant bits of data to

a central server for a given analysis run. Although the group is working to expand the system's capacity for the amount of data transferred, the upcoming version can't handle early-stage next-generation sequencing analyses, such as alignment. "[But] once you've nailed down your set of variables, or come up with putative SNPs you're interested in, it's at that point when the system could be really powerful," Carter says.

COST: The software platform itself is free but relies on all sites that share data having their own physical or virtual server. (It's possible to use ViPAR if you don't have a server, but you'll have to have another site host your data.) A central site needs an analysis server, which should have enough memory and processing cores to handle the amount of data generated in a single analysis run of pooled data. About $5–$10K is the range for a project similar in scale to the autism project, called iCARE, says Carter.

iPLANT

www.iplantcollaborative.org

Data storage, sharing, transfer

PROBLEM: Several years ago, the free cyberinfrastructure available through the National Science Foundation (NSF), which gave scientists a way to store, manage, and share project data, was geared more for astrophysicists than it was for biologists. Using these resources required intense training to meet even simple needs such as data storage, says Nirav Merchant, director of information technology at the University of Arizona's BioComputing Facility in Tucson.

SOLUTION: Funded by the NSF, Merchant's team created the iPlant Collaborative, which offers a more intuitive package of platforms for researchers—initially plant scientists, but now the broader life sciences community—to manage, analyze, and share data.

The iPlant Data Store, a cloud-based platform, provides up to 100 GB of storage space through which researchers can share data. On top of the Data Store, Merchant's team built Discovery Environment, an analytical platform that packages commonly used sequencing analysis tools into user-friendly apps. A third platform, Atmosphere, is for cloud-based analysis. Connected to the Data Store, Atmosphere gives users multiple configurations of CPUs and memory for computationally intense analysis—and the ability to share. "If you and I are analyzing data together [from different locations] we can see the same screen and share the same mouse regardless of the platform—and all of this is running inside our cloud infrastructure," he says.

The Data Store, the centerpiece of iPlant, is powered by iRODS, open source "middleware" that helps researchers store, manage, and share their data. Focused on bulk data handling and metadata (data about your data), iRODS has numerous capabilities but requires IT know-how to implement for specific projects. The iPlant Collaborative is one example of how experts have made iRODS more accessible for end users, but iRODS developers are also working to make the tool easier for life scientists.

GETTING STARTED: iPlant is scalable. Single labs or small collaborations can import their data into iPlant and use its tools immediately; institutions or large consortia that already have started projects using their own cyberinfrastructure can keep their data local and work with it using iPlant's resources through the Powered By iPlant project, Merchant adds.

TIP: Check out iPlant's hands-on workshops, online video introductions for each platform, and written tutorials for apps in its Discovery Environment, such as those designed for analyzing ChIPseq and RNAseq data. Users can create a single login that will work for all platforms, says Merchant.

COSTS: Free for all. Users who need larger data storage or processing can request an additional allocation using an online form on iPlant's site. Requests for particularly large allocations are evaluated by a committee, and so far, no user or group has had to pay, Merchant says.

GALAXY

wiki.galaxyproject.org/FrontPage

Shared data analysis and analysis tools

PROBLEM: There's a lot of guesswork involved in choosing the right computational analysis tools for big data projects—not to mention the headaches associated with implementing new software, which can prove challenging for biologists with no informatics training.

SOLUTION: Computational biologist James Taylor of Emory University in Atlanta, Georgia, and his colleague Anton Nekrutenko at Pennsylvania State University created Galaxy, a platform that now has thousands of data-analysis tools for biomedical research. Most are geared for genomics, though more tools specific for proteomics and imaging analysis are also becoming available on the platform. A community of Galaxy users helps vet the software tools for specific applications.

The ideal user is a bit larger than a single lab, but the platform scales up to the institution level. "[Galaxy] definitely has lots of collaborative features, so if you have a larger group of people using the

same Galaxy instance"—that is, a single installation of Galaxy set up on the same server or commercial cloud—"then you get benefits from that," Taylor says. People using different instances of Galaxy cannot perform shared analyses, but Taylor is working on a way to make that possible. For now, users across different instances can at least share analysis tools through the platform's Tool Shed.

GETTING STARTED: You'll first need to access Galaxy through the platform's publicly available server, commercial clouds (ideal if data acquisition is sporadic), or another institution's public server. Or, with your own server, you can perform your own private installation of Galaxy. You import your data into the platform, and use the web-based interface to deploy individual tools or build a workflow. "The hardest part is bringing your data in in the beginning, where your data is in its most raw form," Taylor says. "Getting data out in the form of aggregate results or visualizations is not difficult."

TIP: Take advantage of Galaxy's online video and written training materials. In addition, free in-person training sessions are available at some conferences; the American Society of Human Genetics 2013 meeting this month (Oct. 22–26) is one example.

COST: Galaxy is free, but if you want to download it and use it with your own infrastructure—the best option if you're working with sensitive data, have large computing demands, or want to customize the software—you'll need to have a server. Costs of cloud computing, through Amazon or other commercial vendors, can also add up, Taylor says.

ASPERA

asperasoft.com

Data transfer

PROBLEM: Especially in genomics, data file sizes have become too large to send over Internet hubs using traditional mechanisms such as file transfer protocol. Moving data in and out of cloud storage can be even tougher because it sometimes requires users to break files into smaller chunks, adding to the slowness. Software available from cloud vendors or open source can make it "unusably slow to post large file data to the cloud or download it back out, especially if you're at any distance from the cloud environment," says Michelle Munson, CEO of Aspera.

SOLUTION: Aspera's commercial software relies on technology called "fasp" to replace other file transfer mechanisms. "Our protocol is designed in a radically different way, such that it allows extremely large file sizes to be transferred over long distances," Munson says. If Internet bandwidth allows, this could mean transport speeds of up to 10 gigabits/second.

Aspera's Connect Web Browser Plug-in, which installs on your web browser, is free to end users, but it does require a central site to have an Aspera Connect Server. Alternatively, the plug-in is available on major cloud platforms. The server software necessary for those regular, large transfers is available for purchase as a perpetual license or on cloud platforms as a subscription service.

GETTING STARTED: For more modest users who transfer data only occasionally, Aspera's pay-as-you-go service through Amazon Cloud allows users to move as little as 100 gigabytes per month. Those looking to host their own server software can take it for a trial run before they buy. "We spend a lot of time with customers allowing them to evaluate the software and get to know what it can do, and determine how to use it for their own workflow," Munson says.

COSTS: The cost of server software is tiered based on the bandwidth of the connection you're using it with, but ranges from $4K–$100K. Through cloud vendors such as Amazon, subscription plans are based on the amount of bytes transferred in a year, ranging from a penny to $1 for each gigabyte transferred. ■

TIPS:

» **DEVELOP A PLAN.** Who is involved in your project? What data are you collecting? What platforms are you using? What questions are you asking from the data? How would you like to analyze the data set, who is analyzing it, and what are your expected outcomes? From the answers to these questions should come a basic workflow that will help guide the steps of data collection, management, and analysis.

» **RECRUIT HELP.** Take your plan to a bioinformatician and a biostatistician. The biostatistician will make sure that you have adequate sample sizes and analysis methods before you start the study. The bioinformatician will help you select the right tools and methods for managing your data, says Kim Carter of ViPAR.

» **DON'T BUILD YOUR OWN PLATFORM.** Even though it might seem easier at the outset, most scientists would be wise to avoid building their own software tools from scratch, says Stan Ahalt of the Renaissance Computing Institute. "Scientists will benefit in the long run if they invest time in both identifying and learning key data management tools that are available in the open source."

» **MANAGE YOUR TIME.** There are new software packages coming out all the time. "The hardest thing is getting those to work for you in the same way that has been described in a paper," says Carter. In addition, "there's a tension between learning new tools and getting your science done, and that has to be carefully managed," Ahalt says. You can't learn them all.

Bonding in the Lab

How to make your lab less like a factory and more like a family

BY KATE YANDELL

When Mehmet Berkmen was accepted to Jon Beckwith's bacterial genetics lab at Harvard as a postdoctoral scholar in 2000, others joked that he was about to join a mafia. Berkmen, who was getting his PhD from the University of Vienna but doing most of his research at the University of Houston, was alarmed. He knew that Beckwith had been part of the team that, in 1969, was the first to isolate a bacterial gene, *lacZ,* from an intact chromosome, and that his lab had continued to turn out seminal research on topics including gene expression regulation, protein secretion, and disulfide bonds. He imagined Beckwith marshaling regimented phalanxes of postdocs as they crisply turned out results and dominated the field.

But Beckwith turned out to be humble and shy. And despite its famous productivity, the lab was such a warm, friendly place that, according to Berkmen, now a staff scientist at New England Biolabs, members cry when they have to leave, and they get together every three years for reunions that draw people who weren't even members of the lab. The term "mafia," it turned out, was a term of endearment used among lab members. Far from being a pejorative, it just "means that we are very, very connected," says Jennifer Leeds, who was a postdoc in the lab between 1996 and 2001 and is now head of antibacterial discovery at Novartis. "We are a family."

The strong ties and shared values fostered in a tight-knit lab like Beckwith's can help make the difference between a highly successful career and a lackluster one—both for the lab's principal investigator and for its members. Loyal, nurtured young scientists will be productive and will recommend the lab to other high achievers. They, in turn, leave the lab with an instant clan of researchers who can help them with anything, from solving experimental problems to finding new collaborators.

LAB FUN: Jon Beckwith is pictured with his camera in the middle of a collage of his lab members enjoying hikes, beach days, and more.

COURTESY OF MEHMET BERKMEN

But not every principal investigator succeeds in forming this type of influential, inclusive community. *The Scientist* spoke to members of two tight-knit labs to try to pinpoint the factors that led to their becoming such congenial, nurturing places.

THE BECKWITH LAB

Harvard Medical School, Department of Microbiology and Immunology

Around one hundred postdoctoral scholars and graduate students have passed through his doors since Beckwith came to Harvard as a young professor in 1965. They have repeatedly turned out field-defining research on basic bacterial biology. Beckwith began with his work on the *lac* operon, followed by discoveries on how to identify amino acid sequences that destine proteins for secretion and on how to predict the arrangement of proteins within a membrane. By the 1980s he had become fascinated with the disulfide bonds in bacterial periplasmic proteins, which have been pursued as antibiotic targets, and in 1991 his lab discovered an enzyme responsible for forming those bonds.

Perhaps more extraordinary than the lab's contributions to microbial biology is the strength of the relationships between lab members. Beckwith's legendary reunions, which have been going on since 1996, are mini-conferences for bacterial geneticists, and the lab's e-mail list, which includes both past and present lab members, buzzes with questions

and advice. All this sharing happens even though many of the lab's former members are now competing with each other professionally. Leeds and another alumna are running parallel clinical trials at different companies, and they still have meals together and talk (while steering clear of confidential information).

Despite his lab's present-day cohesion, when he first got to Harvard Beckwith was terribly shy and wasn't certain he had chosen the right path. Although he's not sure how his lab became so tight-knit, he thinks the dance parties he started throwing at his house in the late 1960s may have helped. Beckwith also became passionate about activism during this time, voicing his concerns about scientists' and doctors' potential misuse of genetic manipulation and testing, among other topics. "I think I progressively shared more over the years with people," he says. The atmosphere in the late '60s and early '70s "really loosened me up."

Graduates of his lab, however, say it was more than that. "He constantly questions himself," says Leeds. This self-critical nature seems to relax those around him and spur them to think critically about themselves and their own work. And the rigorous but respectful intellectual atmosphere fostered by Beckwith, more than anything, is what allows lab members to have stimulating conversations wherever they meet.

MARK Q. MARTINDALE LAB

The Whitney Laboratory for Marine Bioscience, University of Florida; formerly of Kewalo Marine Laboratory, University of Hawaii at Manoa

In Mark Q. Martindale's evolution and developmental biology lab, members are always looking over each other's shoulders and into each other's microscopes at developing embryos. "That's not [the case] in every lab," says David Q. Matus, a graduate student and then a postdoctoral scholar in the lab between 2000 and 2007. He's now a postdoc at Duke University and soon to be an assistant professor at Stony Brook University.

COURTESY OF JESSICA LONG

STORY TIME: On the lawn of the University of Florida's Whitney Lab, Mark Q. Martindale reads to his lab members about embryonic development.

Martindale traces the warmth, fun, and focus of his lab back to his undergraduate years and his own academic lineage. When he went to his first scientific conference as a sophomore, one of his friends put Qs, Xs, and Zs as their group's middle initials on the sign-up sheet as a joke. The Qs stuck, and now Martindale and several of his friends publish using Qs as middle initials and are passing the tradition on to their own students—anyone who comes to the lab is allowed to adopt the new letter, although not all choose to do so.

One of Martindale's current postdoctoral scholars, Michael Layden, who did not take the Q, credits the lab's nonhierarchical structure, diverse research topics, and Martindale's enthusiasm for the lab's openness. "Lab meetings can take you from characterizing these weird species to a hard-core functional molecular approach, to looking at extracellular matrices," he says. The diversity of projects takes away some of the pressure of competition.

Because of the unique shared middle initial, even the lab members who do not "take the Q" see their lineage as a wider community. "I think it inadvertently has sort of created this immediate bond," says Layden, who recalls running into a Q at a conference and immediately being able to strike up a conversation.

TIPS ON BUILDING YOUR OWN LAB FAMILY:

CATCH THEM WHILE THEY'RE RELAXED. PIs are told to interact with their lab members, but limiting contact to formal meetings can be intimidating. Jon Beckwith's office was, until a recent relocation, connected to his lab, and he only closed the door when he was having a meeting or taking a nap. "I feel like the kind of science we do is puzzle-solving," he says, "and coming up with good ideas is a constant need. I have to shoot the breeze for a while to really talk about the ideas." Martindale, meanwhile, has a knack for injecting embryos, so he likes to sometimes have these chats at the lab bench while helping to guide the process. "We sit in a dark room injecting embryos, we put on some music, we talk about their work, how they're interacting, what their priorities are," he says.

BE RESPECTFULLY CRITICAL. Science is based on criticism and revision of ideas, and yet, in a competitive setting, questioning a lab member can be perceived as an attack. The Beckwith Lab meetings famously cultivate an atmosphere of respectful questioning. "It was very much an open and transparent and relatively unstructured scientific debate," former

postdoc Leeds says. "The only rule was that you be respectful." Beckwith's students think that he is able to be so critical in part because he questions himself so constantly that it is impossible to feel singled out.

CREATE RITUALS. Beckwith loves to throw dance parties at his home, so some of his current and former lab members presented him with a disco ball for his 65th birthday. Annual celebrations include Beckwith Beach Day, a lab hike, and his famous Christmas party. Beckwith's lab refrigerator is adorned with photographs from the annual hike over the years. "You see yourself there in a photo with all those other people," some of them very well-known in the field, and you know you've arrived, says Berkmen. Martindale's former graduate student Matus says that the bar across the street from the Kewalo Lab in Hawaii was a major hub of lab life, and all victories were celebrated and failures shared over drinks. "I've tried to foster that in my postdoc lab I'm in now, and I think these things are recipes for making things more tight-knit," he says.

BE FLEXIBLE. Beckwith has always been laissez-faire about the nonscientific contents of his lab. Members credit video games and ample sporting equipment with helping them relax and possibly work longer and harder in the end. And the lab welcomes members' families. Mere hours after Leeds gave birth to her son at nearby Brigham and Women's Hospital, Beckwith and at least a dozen others crowded into her hospital room. Later, lab members would play with the child on the Harvard Medical School lawn while Leeds did experiments. "When he was born it was like, he's already part of the lab," she says.

HOW TO PICK LAB MEMBERS:

GET TO KNOW CANDIDATES. The key to a fun, productive lab is choosing the right lab members—and yet, according to Beckwith, it's not as simple as selecting the candidate with the most published papers or the best grades. Instead, he likes to pick students he can enjoy conversing with and who show curiosity. "I don't judge people on how committed they are, how hard they work," says Beckwith. An ability to ask questions and be self-critical and a desire to collaborate are valued more highly.

CANVASS THE LOWEST-RANKING LAB MEMBERS. Ask undergraduates, not just grad students and postdocs, what they think of candidates, Martindale says. You want someone who will get along with and respect everyone, regardless of status or seniority. A candidate who has an unpleasant side is more likely to show it to people perceived to be lowest in the hierarchy.

HOW TO PICK A LAB:

LOOK FOR THE QUIRKY. Unorthodox approaches can be a sign of a creative lab with a PI who is unafraid to take risks. Leeds was in part inspired to apply to the Beckwith lab after going to a meeting at Cold Spring Harbor Laboratory, where she came upon one of Beckwith's lab members doing an odd poster presentation. "He had taken blank pieces of paper and thumbtacked them onto [his poster board], and he started drawing while I was talking," she says. "I was like, wow, this is science in the moment. This is not prebaked and rehearsed and made to look pretty and digested and spit out in a way that was all for show."

FURTHER CONSIDERATIONS. Does the PI micromanage students? Does the PI try to compete with his or her students? Does the PI pit students against each other, making them compete for attention? Do the lab members like to spend time together? These are all questions that current students and postdocs can answer. For Leeds it comes down to this question: "Do they want you in the lab because they have a grant they need data for? Or is it because they see you as an integral part of their existence?" ■

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Dr. Darwin at the Bedside

It's time for evolutionary medicine to fully inform clinical research and patient care.

BY ROBERT PERLMAN

Shakespeare memorably described the human life course, from "the infant,/ Mewling and puking in the nurse's arms" to the "mere oblivion" of the aged, "Sans teeth, sans eyes, sans taste, sans everything." Scientists now appreciate that human life histories have been shaped by natural selection. Evolutionary life history theory provides a valuable, if less poetic, framework for understanding our life cycle and the diseases that accompany aging.

Natural selection adjusted how humans use energy and other resources throughout our life cycles in ways that optimized the reproductive fitness of our evolutionary ancestors. Optimizing fitness has meant devoting energy to growth and development and to reproduction, at the expense of maintaining and repairing our bodies. Our evolved mechanisms of bodily maintenance and repair are sufficient to keep us alive and healthy long enough to have and raise our children, and perhaps contribute to the development of our grandchildren. But these mechanisms are not perfect. Over time, we accumulate unrepaired damage that leads to the diseases of aging and, ultimately, to death.

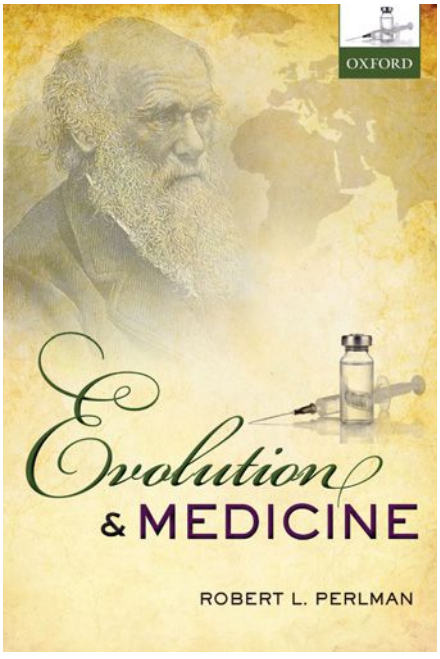
In my new book, *Evolution and Medicine*, I discuss the emerging field of evolutionary medicine. I show how integrating life history theory and other evolutionary concepts into medicine has the potential to improve our understanding of disease and, most importantly, clinical practice.

Perhaps the most dramatic contribution of evolutionary medicine to patient care has been the development of highly active antiretroviral therapy (HAART) for HIV-infected patients. HAART typically includes two reverse transcriptase inhibitors to block the activity of the enzyme that retroviruses use to replicate. The development of HAART was based on the recognition that mutant virus strains that were resistant to both drugs would have two or more mutations in the reverse transcriptase gene, and so were likely to have decreased fitness. HAART has revolutionized the treatment of HIV. The use of combination therapy for patients with hepatitis C infections and other diseases—and more broadly, our increased concern with resistance management to prolong the useful lives of new antibiotics—shows the reach of evolutionary medicine into the clinic.

Until the 20th century, most if not all humans were chronically infected by parasitic worms, or helminths. Helminths used to be so common in the environment that our ancestors evolved traits that optimized fitness in their wormy world. The effect of living in relatively worm-free environments is thought to underlie the increasing incidence of allergic and autoimmune diseases in economically developed, modern countries. These considerations have led to novel clinical trials using helminth extracts or eggs to treat patients with multiple sclerosis or inflammatory bowel disease. Although it is too early to know if these trials will be successful or will lead to new therapies for patients, they illustrate another way in which an evolutionary perspective can inform clinical research.

Evolutionary life history theory has great but as yet largely untapped potential to improve medical practice. We now know that although aging is inevitable, its time course is not fixed. Life expectancy in the United States has increased dramatically over the last century, from about 47 years in 1900 to almost 80 years today. Because of better nutrition and a decline in infectious diseases, we are born with greater amounts of physiological reserves, we experience lower rates of bodily damage, and we live longer than our grandparents and great-grandparents.

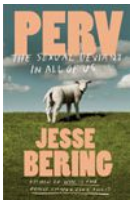
Many hormones regulate energy utilization and so play important roles in our life histories. Physical and psychosocial stresses, acting through neuroendocrine regulatory mechanisms, appear to accelerate the aging process. Better understanding these mechanisms may help us modulate the rate of aging and extend life. The integration of evolutionary medicine with biomedical research offers untold and exciting new opportunities for improving human health and well-being. ■



Oxford University Press, July 2013

Robert Perlman is Professor Emeritus in the Departments of Pediatrics and Pharmacological and Physiological Sciences at the University of Chicago. *Read an excerpt of* Evolution and Medicine *at www.the-scientist.com.*

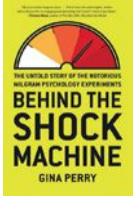
Perv: The Sexual Deviant in All of Us
Scientific American/Farrar, Straus and Giroux, October 2013



Science writer extraordinaire and erstwhile psychologist Jesse Bering has again plumbed the depths of human sexuality in his latest offering, *Perv*. After last year's *Why is the Penis Shaped Like That?,* Bering again demonstrates that he feels right at home exploring the more salacious aspects of the human condition. In *Perv*, Bering deconstructs scores of "paraphilias," which he defines as "sexual orientations toward people or things that most of us wouldn't consider to be particularly erotic." He injects a fair amount of historical and societal perspective into his treatment of both well-known and rarer ones: acrotomophilia (arousal to amputees), lithophilia (arousal to stone and gravel), and psychrophilia (arousal to being cold and watching others who are cold), to name a few.

Against a colorful backdrop of science, history, and psychology, Bering calls on human society to stop judging people's sexual preferences based on a personal belief about what's normal or natural, instead asking what is harmful. The author throws a bucket of ice-cold water on topics that often become overheated by the fires of morality, religion, and politics.

Behind the Shock Machine: The Untold Story of the Notorious Milgram Psychology Experiments
By Gina Perry
The New Press, September 2013

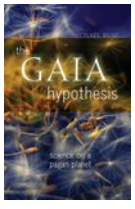


It's classic psychology-textbook fodder: in 1961 Yale professor Stanley Milgram conducted experiments in which paid subjects were made to believe that they were delivering electric shocks to another person for failing at memory tasks. The work showed that a surprising proportion of test subjects were willing, with prodding from Milgram, to inflict the maximum 450-volt shock (though it wasn't actually delivered) to fellow test subjects (actors pretending to get shocked).

Though Milgram's controversial experiments have been dissected in psychological and ethical circles for decades, no one had ventured to take a good, hard look at the data and lives behind the study until now. Australian psychologist Gina Perry tracked down some of Milgram's test subjects and dug through his unpublished data to uncover startling truths, which she divulges in *Behind the Shock Machine*. Among them: Milgram's oft-cited statistic that 65 percent of test subjects were obedient and willing to administer painful shocks is somewhat off. In reality, only 56 percent of the test subjects fully believed the shocks were real, and of those, two-thirds proved disobedient, refusing to deliver the most painful shocks. Perry also determined that some of those subjects who believed they were really shocking people weren't properly debriefed and continued believing the ruse for years after the experiments concluded.

Will Perry's exposé of the infamous Milgram experiments rewrite the textbooks? Probably not, but this book certainly deserves the attention of serious students of psychology.

The Gaia Hypothesis: Science on a Pagan Planet
By Michael Ruse
The University of Chicago Press, September 2013

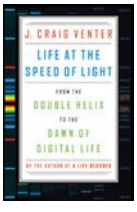


The entire Earth functions as a living, self-regulating organism, each of its individual life-forms unconsciously striving for the overall betterment of the planet. Philosopher of science Michael Ruse makes that idea, called the Gaia Hypothesis, the title and focus of his latest book.

While countless tomes have explored the intricacies of the hypothesis—most notably James Lovelock's own *Gaia: A New Look at Life on Earth* (1979)—Ruse's latest effort digs into the societal and philosophical impacts of the concept. The author considers the viewpoints of Gaia's supporters, such as Lovelock's frequent collaborator, the equally controversial biologist Lynn Margulis, and vociferous detractors, like Stephen J. Gould and Richard Dawkins, who have dismissed the hypothesis as teleological pseudoscience.

In the end, Ruse comes down more on the side of Gaia fan than Gaia foe, commending Lovelock and Margulis for their injection of philosophy into the scientific discourse at a time when the environmental movement had little idea of what hurdles lay ahead. "Lovelock and Margulis were big people with a big vision," Ruse writes. "Whether science likes it or not, the vision lives on."

Life at the Speed of Light: From the Double Helix to the Dawn of Digital Life
By J. Craig Venter
Viking, October 2013



Synthetic genomics gets the J. Craig Venter treatment in this latest book from the maverick scientist who was instrumental in creating the private-versus-government tension that marked the race to sequence the human genome. In *Life at the Speed of Light*, Venter recounts his team's effort in 2008 to synthesize the first complete man-made genome, a 582,970 base-pair stretch of *Mycoplasma genitalium* DNA, and the subsequent advances, such as transferring the synthetic genome into a living cell that then "booted up" the genetic program, that occurred at the J. Craig Venter Institute.

When he's not tooting his own horn in *Life*, Venter discusses the ethical implications and societal ramifications of synthetic genomics, stressing that debate surrounding these emerging technologies is healthy and necessary. Venter ventures into some pretty futuristic territory in the latter part of the book, hitting on the science behind "biological teleportation," a digitization process that could potentially shoot the DNA to build synthetic phages around the globe to battle drug-resistant microbes. —Bob Grant

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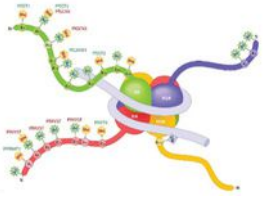

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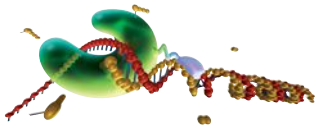

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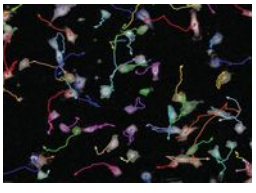

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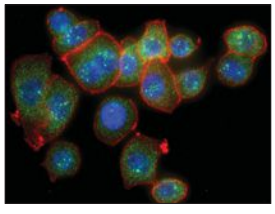

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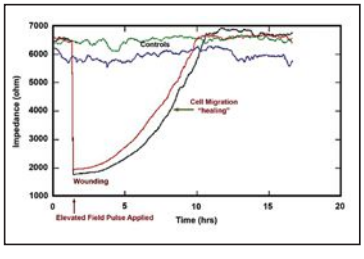

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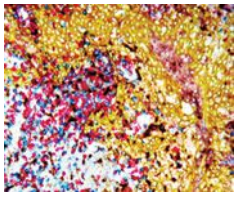

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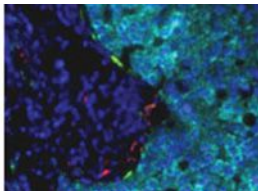

PanCK, uPA and PAI in FFPE breast cancer tissue

The RNAscope® Multiplex Assay uses a novel and proprietary method of in situ hybridization (ISH) to visualize single RNA molecules within individual cells in cultured cells, fresh frozen or FFPE tissue samples. RNAscope® Assays do not require an RNA-free environment as in traditional ISH. The exceptional sensitivity and background suppression of RNAscope provides single-molecule detection of multiple RNA targets simultaneously and is ideal for co-localization studies of any expressed gene set in nearly any tissue type.

ADVANCED CELL DIAGNOSTICS
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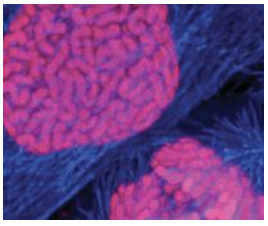
Breast Cancer Mouse Model Cell Line Panel



ATCC now offers the Breast Cancer Mouse Model Cell Line Panel (ATCC® TCP-1005™). This panel includes cell lines that stably overexpress the MEK1 activated mutant (MEKDD), EGFR2/neu, Myc, or Ha-Ras. These cell lines have been successfully used to generate mouse models of breast cancer for studying metastasis and EGFR-MEK signaling pathways, oncogenes in cell transformation, and for testing anti-cancer compounds.

ATCC
800-638-6597
www.atcc.org

TPP Cultureware Enhances Cell Growth

The TPP cultureware line includes standard, peel-off lid and reclosable flasks; standard, bioreactor and centrifuge tubes; multiwell plates; and dishes, racks and cryoboxes. Distributed in North America by MIDSCI™, the products promote and support the highest levels of growth for both adherent and suspension cells, reduce media and labware costs, and enable consistent and reproducible experimental results.

MIDSCI INC.
1-800-227-9997
www.midsci.com/TPP

RNAi Screening in Primary Cells

Lonza's HT Nucleofector™ System is a 384-well platform for efficient high-throughput transfection of primary cells, e.g. human T cells. With its fast plate processing time and easy integration into liquid handling systems it is a convenient tool for siRNA, shRNA or miRNA screens in biologically relevant cell types. Nucleofection™ is a proven non-viral transfection method for hard-to-transfect cells.

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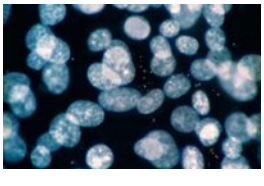
Detect, eliminate and prevent cell culture contamination

PromoKine offers a range of excellent and cost-effective products for the detection and elimination of cell culture contaminating mycoplasma and bacteria. This includes highly sensitive and specific mycoplasma and bacteria detection kits based on conventional or Real-Time PCR, as well as antibiotic and non-antibiotic reagents for the complete and reliable elimination of mycoplasma contamination in cell culture. In addition, several solutions and sprays are provided that can be used to clean and disinfect laboratory surfaces and prevent microbial growth in water baths and incubators.

See also our current promotion at www.promokine.info/promotion!



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info@promokine.info
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Neural Cell Isolation Kit

Worthington's Papain Neural Cell Isolation System is a complete kit for the convenient, consistent isolation of morphologically intact neural single cells for culture, flow cytometry or other applications. The method is based upon published techniques using papain to dissociate CNS tissues providing higher yields and viability than trypsin procedures. Each kit includes five single-use Enzyme Vials, EBSS, Inhibitor and is use-tested to assure performance.

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Fax: 800.368.3108 • 732.942.9270
www.worthington-biochem.com

Imaging Chemiluminescence by Scanning

The C-DiGit™ Chemiluminescent Western Blot Scanner is a digital replacement for film, combining the sensitivity of film with the convenience and flexibility of a CCD imager. To achieve this breakthrough at an affordable price, LI-COR implemented a new imaging approach that was previously considered impossible: line-read scanning of chemiluminescent signals.1 Film-quality Western images are digitally captured in as little as 6 minutes, and no changes are required in detection protocols or reagents. The low-noise sensor array and short optical working distance maximize the efficiency of light collection and enable rapid scanning. Multiple, inverted scan passes correct for any time-dependent change in signal intensity. The sensitivity, dynamic range, and affordability of the C-DiGit Scanner make it a true digital replacement for film in Western blotting.

Outline:

Introduction to chemiluminescence

- Conventional imaging methods for chemiluminescent Westerns

The C-DiGit approach

- Maximizing light collection – short working distance and high numerical aperture
- Affordable low-noise sensors

Imaging chemiluminescence by scanning

- Very short scan times
- Multiple, inverted scan passes for maximum accuracy
- Multi-scan, multi-exposure imaging and wide dynamic range

Summary and References

1. Introduction to chemiluminescence

Enhanced chemiluminescence (ECL) is widely used for detection of target proteins on Western blots. For detection, horseradish peroxidase (HRP) enzyme is typically conjugated to a secondary antibody. The enzyme causes oxidation of the luminol-based chemiluminescent substrate, creating an excited state product. As this product decays to a lower energy state, it transiently produces light (Fig. 1).2 Unlike fluorescent detection, chemiluminescence does not require excitation light. Light emission only occurs in areas where the chemical reaction occurs, enabling low optical background and high detection sensitivity.

LI-COR Biosciences
4647 Superior Street
Lincoln, NE 68504, USA
Tel: 402-467-3576

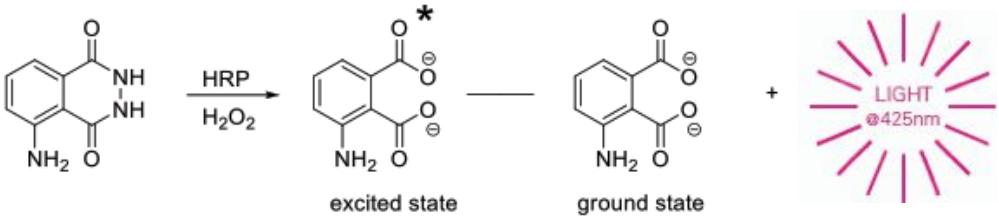


Figure 1. Chemiluminescent reaction. Luminol is a widely used chemiluminescent reagent. Oxidation of luminol by peroxide creates an excited-state product, 3-aminophthalate. Photons of light are released when this product decays to a lower energy state.

A note on Cell Line Authentication from ATCC

Imagine a dimly lit auditorium; it is 1966, and the founding fathers of cell culture fill the audience. We are in a packed room of men in grey suits with thin black ties. One great man of science lounges confidently in a seat towards the back - cleaning his horn-rimmed glasses on his jacket – while another great man of science dozes unashamedly in the front row. Into our scene walks the geneticist Stanley Gartler. He makes his way to the podium and announces that the myriad new cell lines they have gathered there to discuss - the cell lines that represent not only an array of cell types and tissues, but the brilliance of the men seated before him . . . are mostly just HeLa cells grown in new media under new names.

I like to think of those stoic, staid scientists booing and throwing their slide-rules at the podium (although in reality they just sat there in stunned silence). Nevertheless, the intensity of the scene led one scientist to later remark "He [Gartler] showed up at that meeting with no background or anything else in cell culture and proceeded to drop a turd in the punch bowl."*

How could this happen? To answer this question, we need to go back to the beginning. Sterile techniques were in their infancy when many "new" cell lines were "immortalized." Couple that with an understandable ignorance of how hearty HeLa cells are, and no reliable method to verify the molecular identity of cells, and it isn't difficult to understand how this situation arose.

It is more difficult, on the other hand, to understand how this problem could persist to the present day - and yet it does. Estimates suggest that up to a third of the cell lines in use are contaminated (most often with HeLa cells) or misidentified. In fact, data obtained through the use of known misidentified or contaminated cell lines have been used to support clinical trials, grant applications, U.S. patents and publications. So, the potential costs in lost research time, money and success are enormous.

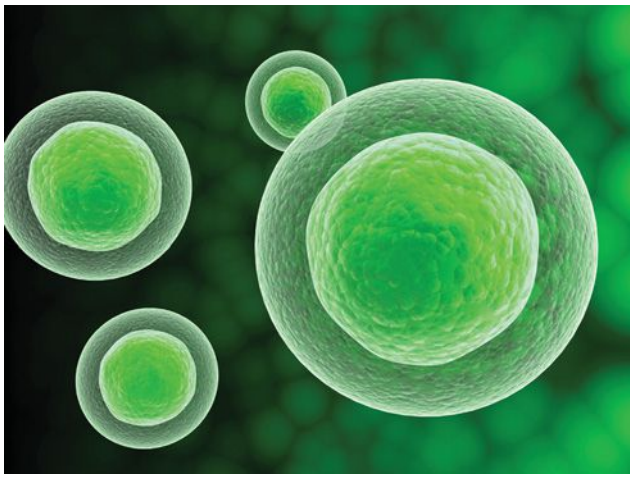
Once again, we find ourselves asking, "how could this happen?" Here are a few likely explanations:

- Getting cells from a neighboring lab may seem like an easy and cost-effective method of setting up your experiments, but there is inherent risk to starting with unauthenticated material.
- Using multiple cell lines in the lab or growing cells on a non-human cell feeder layer increases the likelihood of cross-contamination.
- Simple human error accounts for many of the contaminated and misidentified cell cultures in use today. Importantly, these errors can be propagated if the contaminated cell line is shared between investigators.

The best way to ensure your cell lines are authentic is to start with cells from a reliable source. Cell repositories, such as ATCC, hold the lines in their collections to rigorous standards to make sure they are properly identified and free from contamination. To maintain the integrity of your materials, it is important to stop periodically and replace them with a fresh vial or to re-authenticate them.

Nobody wants to go back to that dimly lit auditorium of 1966, but it will be difficult to make solid progress forward without addressing cell line contamination and misidentification head-on.

Happy Culturing,
ATCC Cell Biology



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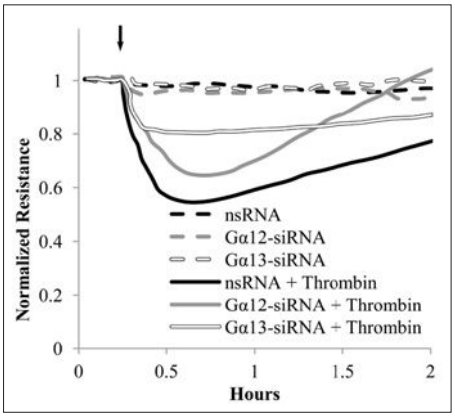
ECIS® for Label-free Cell Research (Electric Cell-substrate Impedance Sensing)

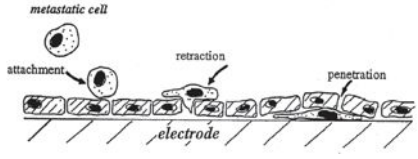
Barrier Function

Epithelial cells and endothelial cells regulate the passage of molecules across cell layers. Diseases, especially vascular disease, occur when regulation is impaired. Passage of molecules across an endothelial or epithelial cell layer occurs in two ways; actively by transport through the cell or passively by diffusion in the para-cellular space. ECIS® measurements at frequencies below 5kHz are highly sensitive to changes in the barrier function. ECIS® has been used to demonstrate the effects of many regulating molecules including VEGF, thrombin, TNFalpha, and sphingosine-1-phosphate.

Data derived from Birukova, A. et at., 2004 FASEB J. 18:1879

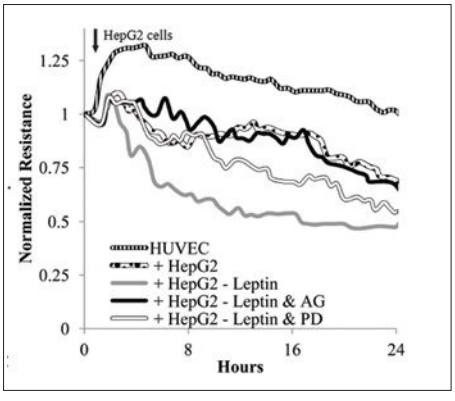


Cell Invasion

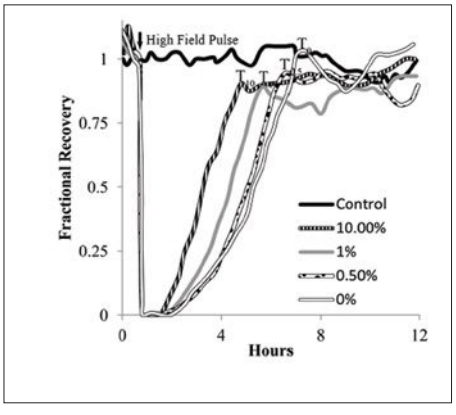


By quantifying cell behavior, ECIS® can give new insight into how invasive cells and pathogens cross endothelial and epithelial monolayers. By simultaneously monitoring both barrier function and cell viability, ECIS® can distinguish between transmigration mechanisms that leave the monolayer intact from those that disrupt the cell layer. Published examples include metastatic cell and leukocyte trans-endothelial migration as well as the migration of pathogens such as yeast, anthrax, streptococcus, plasmodium, trypanosomes, and spirochetes.

Data from Saxena, N.K. et al., 2007, Cancer Res. 67:2497.

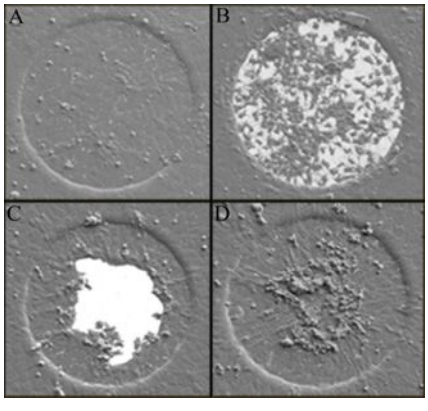


Cell Migration



Operating in elevated field mode used for electroporation, ECIS® instruments apply a high electric field for several seconds causing cell death. The ECIS® wound is precisely defined, as it includes only those cells on the electrode. Additionally, the ECM protein coating is not scraped off and is unaffected by the current.

Datafrom Keese, C.R., et al., 2004 PNAS 101:1554.



Scanning electron micrographs of the ECIS® electrode at time points just prior to (A), just after (B), 4 hours after (C), and 8 hours after (D) the application of a high field pulse across the ECIS® electrodes.

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nCounter® Panel-Plus - A New Level of Flexibility for Gene Expression

nCounter® Panel-Plus enables any off-the-shelf panel kit to be customized with up to 30 additional genes of interest. Add your favorite genes related or unrelated to the panel focus or include your unique set of controls to quickly enable a customized project.

- For use with nCounter Gene Expression and CNV products, including Single Cell applications
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To learn more about the advantages of nCounter and our Panel-Plus protocol, visit www.nanostring.com/panel-plus.

The nCounter® Analysis System offers a simple, cost-effective way to profile hundreds of mRNAs, microRNAs, or DNA targets simultaneously with high sensitivity and precision. The digital detection of target molecules and high levels of multiplexing eliminate the compromise between data quality and data quantity, producing high sensitivity and reproducibility for studies of hundreds of targets. The system uses molecular "barcodes" and single molecule imaging to detect and count hundreds of unique transcripts in a single reaction.

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Cultureware Performance Enhances Overall Value to the Researcher

Culturing cells and tissues is an increasingly important and delicate process in many biotech, pharma, government and academic research labs. Hurdles in *in vitro* cell culture translate to expensive re-use of cells, reagents and, most of all, time. It is imperative that all media, reagents, labware and equipment not only be designed for the process of cell culture but also perform at a consistently high level.

Every step taken in the culture process is important, as is every component used. While cultureware – the containers and devices used to store and contain cells – may seem like a rather innocuous tool, the various products that make up these lines can be very sophisticated in design and construction. TPP cultureware, distributed in North America by MIDSCI™ of St. Louis, Missouri, offers a range of features that promote consistent growth, optical clarity and maximum harvesting of cells and tissues. These products are provided sterile and free of potentially contaminating substances, are manufactured to the strictest standards in an ISO 9001 certified facility, and offer an array of features that facilitate consistent reproducibility of cell culture treatments.

As an example, flasks in the TPP line feature an absolutely flat growth surface that promotes even and consistent cell growth. Vented caps close with an audible click and include a visual indicator to prevent over or under tightening. Filtered caps are also available. Flask bottom edges are raised to facilitate airflow and uniformity of cell growth throughout the stack. Flasks in 25, 75, 150 and 300 cm^2 capacities have no dead corners, allowing 100-percent retrieval of cells.

Flasks are also offered with Peel-Off foil lids that provide easy access to cells post culture. Flasks with recloseable lids permit multiple openings under sterile conditions. Peel-Off and Recloseable flasks, in 115 and 150 cm^2 capacities, are ideal for transgenic cell construction involving the selection of individual candidate clones post-transfection, or applications such as skin graft cultures encompassing the removal of an entire layer of cells or intact tissue. Tiny Flasks (10 cm^2) complete the offering, allow cells to be cultured, viewed and spun in the same vessel and fit in a standard 50 ml tube rotor for centrifugation at rcf of up to 1200 xg.

The TPP Reclosable Tissue Culture Flask from MIDSCI.

The TPP line of cultureware includes TubeSpin Bioreactors, which are ideal for the high throughput screening and optimization of suspension cell line propagation; tissue culture plates and dishes; centrifuge tubes; serological pipettes; scrapers and spatulas; bottle filters; and Polar cryo tubes. Common features include clear markings and writing areas and consistent, quality manufacturing to ensure performance.

TPP cultureware from MIDSCI provides a level of performance that ultimately delivers value to the researcher in the form of healthy cultures that are readily viewed, measured and accessed. Working with the labware is easy, viewing and harvesting of cells is enhanced, and TPP products from MIDSCI provide a level of confidence and surety from experiment to experiment that is invaluable.

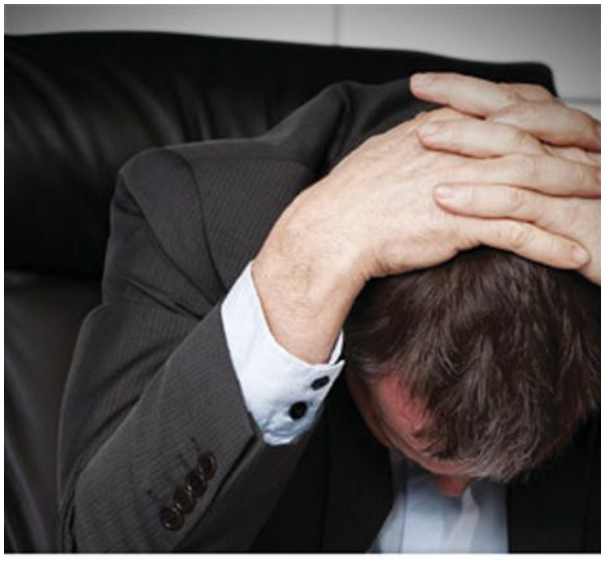
MIDSCI
280 Vance Rd.
St. Louis, MO 63088
1-800-227-9997
custserv@midsci.com
www.MIDSCI.com/TPP

How to Avoid the Top 5 LIMS Nightmares

Imagine - no custom coding, automatic workflow validation and flexible and fast deployment! The new process and execution-centric Accelrys LIMS solves the problems that legacy LIMS have for too long failed to address.

Because current traditional LIMS have not delivered on their promise, many organizations are still searching for solutions to optimize their laboratory operations. For those engaged in deploying traditional LIMS, frequent sleep-disturbing issues include poor flexibility and configurability, expensive and time-consuming customization, difficulties extending and upgrading systems, poor usability, lack of modular functionality, poor service/support, problems integrating with existing instrumentation/IT systems and extra time and resources required to meet critical qualification/compliance requirements.

Learn how you can avoid the top 5 LIMS nightmares and rest easier with today's next-generation process and execution-centric LIMS.
www.accelrys.com/thescientist



The Accelrys LIMS Difference

Accelrys LIMS is purpose-built to manage 21st-century product and process informatics requirements with a specific focus on scale-up, manufacturing and compliance. Accelrys' process- and execution-driven approach to LIMS deployments is fundamentally different from the sample-driven approach of traditional LIMS.

By requiring no custom coding, providing automatic workflow validation and enabling flexible and fast deployment, Accelrys LIMS solves the problems that sample-centric legacy LIMS have for too long failed to address. By focusing on process and execution, rather than samples, Accelrys LIMS takes a flexible approach tailored to the business requirements of downstream operations allowing for fast and easy deployments offering substantially lower total cost of ownership and rapid time to value.

This entirely new approach to LIMS implementations eliminates the complexities, excessive customization and lengthy associated validation requirements inherent with legacy LIMS—offering fast, "out-of-the-box" deployment capabilities, no custom coding, easy integration into existing software platforms and enterprise-wide data management capabilities. The result is streamlined deployments, a substantially lower total cost of ownership and rapid time to value.

Want to know more about Accelrys LIMS? Learn more with our datasheet by visiting **www.accelrys.com/lims-ds**

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Fast and versatile high content imaging with ImageXpress Micro XLS System

Introduction

High content imaging utilizes automated, high-resolution microscopy systems to assay and visualize phenotypic responses in cells. In biological research or drug development, high content imaging drives rapid characterization of how small molecules, such as RNAi and drug compounds, as well as antibody therapeutics, affect cellular processes and morphology in a quantitative, high throughput manner.

Molecular Devices' high content imaging portfolio delivers ultimate flexibility and performance to enable complex assays and to reduce time to result. The ImageXpress® Micro XLS System is a widefield high content microscope capable of imaging slides and microplate wells in fluorescent, transmitted light, and phase-contrast modes for fixed- or live-cell assays.

Key features

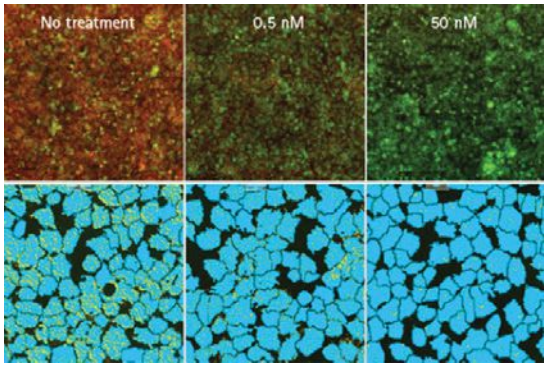
- **High throughput:** image at speed up to 2400 wells per hour using a large field-of-view camera, further powered by analysis that is faster than acquisition
- **Superior versatility:** unlimited hardware configurations with numerous options including light sources, objectives, filters, environmental control, fluidics and transmitted light
- **Powerful analysis:** turnkey application modules and custom analyses can be configured easily to address common image analysis challenges or unique applications

Toxicity Application

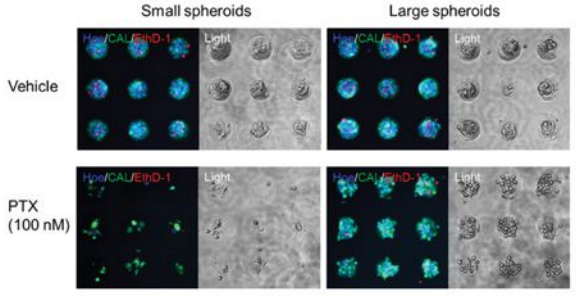
Highly predictive assays for safety and efficacy testing are crucial for improving drug development and reducing drug toxicity. Multi-parametric image analysis using iPSC-derived cells significantly enhances assay sensitivity while providing rich information about compound-induced cellular responses. Assay analyses have been developed to quantitate a broad array of relevant readouts including cell size or shape, total number of viable cells, plus mechanistic toxicity endpoints such as apoptosis markers and loss of mitochondria membrane potential.

3D Spheroid Application

Cancer cell line-derived spheroids grown on a three dimensional matrix are believed to reflect tumor physiology more closely than cells plated to grow on a flat surface. With spheroids grown uniformly in a microplate format, anti-cancer drugs can be screened for efficacy in a high throughput manner. Image analysis software can then be used to generate relevant measurements on imaged spheroids using standard application modules for quantitation of proliferation, live/dead cell analysis or scoring cells labeled with different stains at multiple wavelengths.



iPSC-derived iCell® hepatocytes from Cellular Dynamics International were treated with Valinomycin for 60 minutes. Live cells were stained with JC-10 (mitochondrial integrity indicator) and imaged with a 10X objective. Top: Healthy mitochondria retain the red staining whereas dye leaking out of the mitochondria fluoresces green in the cytoplasm (overlay). Bottom: Resulting mask (zoomed) after image analysis shows cell bodies in aqua with mitochondria identified in yellow in a dose response to the compound.



Images of diminished cell viability with fluorescent Live/Dead Cell viability assay after paclitaxel (PTX) treatment of DU145 human prostate cancer cells plated at 10K cells (left) or 30K cells per well (right), on Cell-able™ Oncology microplates fromToyo Gosei Co., Ltd. Hoechst stains all nuclei blue, live cells are green (Calcein AM), dead cells are red (ethidium homodimer). The transmitted light image shows disintegration of spheroids at high dose of PTX. Larger spheroids were found to be more resistant to PTX treatment.

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Ingenuity® Powers Insights

Ingenuity® Systems, a QIAGEN company, is the global leader in applications to quickly analyze and interpret genomic data. Founded in 1998 by Stanford graduate students, Ingenuity is a leading provider of biomedical information and analysis solutions for the exploration, interpretation and analysis of complex biological systems showcased by its innovative software solutions: Variant Analysis, IPA and iReport. Ingenuity has invested years in the innovation of semantic search, ontology, manual literature curation, and software development to create ground breaking technologies that help researchers more effectively search, explore, visualize, analyze and interpret biological and chemical findings related to genes, proteins and small molecules. In April 2013, Ingenuity Systems became part of the QIAGEN family, and together are developing a combined NGS sample to insight workflow.

Ingenuity® Variant Analysis™ identifies causal variants from human sequencing data in just hours. Variant Analysis combines analytical tools and integrated content to help you rapidly identify the most compelling disease variants, in real time, using selection criteria based both upon published biological evidence and your own knowledge of disease biology from one to hundreds of samples. For researchers who need to identify causal variants from human sequencing data. Variant Analysis allows researchers to drill down on biologically relevant variants based on information in the Ingenuity® Knowledge Base, including primary literature on human mutations in patients with particular diseases or abnormal phenotypes.

IPA® is an all-in-one, web-based software application that enables you to analyze, integrate, and understand data derived from gene expression, microRNA, and SNP microarrays; metabolomics, proteomics, and RNA-Seq experiments; and small-scale experiments that generate gene and chemical lists. With IPA you can search for targeted information on genes, proteins, chemicals, and drugs, and build interactive models of your experimental systems. IPA's data analysis and search capabilities help you understand the significance of your data, specific target, or candidate biomarker in the context of larger biological or chemical systems, backed by the Ingenuity Knowledge Base of highly structured, detail rich biological and chemical Findings.

Ingenuity® iReport™ is the fastest way to get biological meaning from your expression data. iReport is an interactive web-based report, optimized for gene expression experiments from RNA-Seq, microarray, and real-time PCR platforms. Each iReport is optimized for the experimental objective of providing fast and accurate biological and statistical interpretation of expression data. Ingenuity has invested over 10 years in the innovation of semantic search, ontology, and software development to create ground breaking technologies that help researchers more effectively search, explore, visualize, analyze and interpret biological and chemical findings related to genes, proteins and small molecules.

Ingenuity's solutions utilized the unparalleled depth and breadth of the information in the Ingenuity Knowledge Base to better understand complex biological systems, answer questions, analyze and interpret data. To date, Ingenuity solutions have been cited in over 9000 articles in the peer-reviewed scientific literature. Ingenuity Systems is recognized as a technology leader, providing complete solutions for thousands of researchers and clinicians at hundreds of leading pharmaceutical, biotechnology, academic and clinical institutions worldwide to better understand the complex systems foundational to human health and disease. With such a strong focus on our customers, Ingenuity has developed a loyal and passionate user base that is the core of the company's success in this dynamic and evolving market.

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Lonza's Integrated Product Offering for Microarray Applications

The use of primary cell culture is known to provide more biologically relevant results than cell lines. When performing *in vitro* research, you need to replicate the *in vivo* environment as closely as possible. Research has shown that primary cells, which are non-transformed, non-immortalized cells isolated directly from tissue, provide conditions that closely simulate a living model and yield more physiologically significant results.

Primary cells provide the biological relevance and the specificity required in microarray applications. Primary cell cultures have extensive applications in reverse-phase protein array (RPPA) and antibody microarrays for genome wide proteomic/antibody screening. Cell lines are generally utilized but require pre-characterization because they typically go through multiple passages, resulting in modifications/mutations in the process. Primary cells generally retain the post-translational modifications that actual human proteins possess since they are non-transformed and isolated directly from tissues. For this reason, lysates obtained from primary cells are better candidates for antibody characterization, disease-state proteomic profiling, monitoring protein-expression levels, drug screening and drug development studies.

In an RPPA, lysates from primary cells are immobilized on an array with the proteins most commonly found in the actual *in vivo* system. The array is probed with antibodies against the target protein which helps determine antibody specificity, presence of altered protein states or other agents, and detection of post-translational modifications specific to diseased states.

In an antibody-array, a library of antibodies is plated and primary cell lysates are utilized as substrates to detect specific antibodies. This microarray allows for determination of protein expression levels or identification of diseased states.

Streamline your microarray research by choosing from our convenient and innovative selection of tools that have been designed and tested for more relevant results in your research.

Clonetics™ Human Primary Cells and Media

Lonza offers extensively characterized Clonetics™ Primary Cells from human, rat and mouse origin with optimized media systems for each cell type. We strive to ensure that all of our products improve the biological relevance of your research. Our cells and media are tested together to guarantee optimal performance. We use strict industry standards of quality control to ensure consistent performance on every lot manufactured. All tissue utilized for our human cell products is ethically obtained with documented, informed donor consent.

Choose from a variety of normal and diseased primary, non-transformed cells from Lonza. Our cells have been sourced from a variety of donors, including those diagnosed with asthma, COPD, cystic fibrosis, diabetes type I and diabetes type II.

Improve your research by:
- Comparing diseased cells to normal cells for a better understanding of effects
- Obtaining more information about donor characteristics via our scientific support team
- Using clean cells that test negative for bacterial, fungal, and mycoplasma contamination

Normal and Diabetic Human Cells:
- Fresh human pancreatic islets
- Adipose-derived stem cells
- Aortic endothelial cells
- Aortic smooth muscle cells
- Cardiac microvascular endothelial cells
- Coronary artery endothelial cells
- Coronary artery smooth muscle cells
- Dermal microvascular endothelial cells
- Epidermal keratinocytes
- Pre-adipocytes, subcutaneous and visceral
- Pulmonary artery endothelial cells
- Pulmonary artery smooth muscle cells
- Renal proximal tubule epithelial cells
- Skeletal muscle myoblasts

For best results, use these cells with our optimized Clonetics™ Media BulletKits™.

Normal and Diseased Human Airway (Asthma, COPD, Cystic Fibrosis) Cells:
- Bronchial epithelial cells
- Bronchial smooth muscle cells
- Lung fibroblasts
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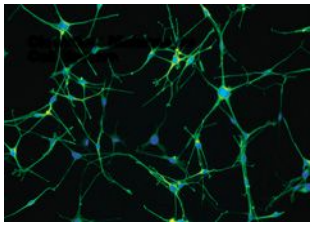

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The 58 Keystone Symposia Meetings of 2013–2014

Advancing Vaccines in the Genomics Era
October 31-November 4, 2013 | Windsor Barra Hotel | Rio de Janeiro | Brazil

Sensing and Signaling of Hypoxia: Interfaces with Biology and Medicine
January 7–12, 2014 | Beaver Run Resort | Breckenridge, Colorado | USA

The Ubiquitin System: From Basic Science to Drug Discovery
January 7–12, 2014 | Big Sky Resort | Big Sky, Montana | USA

Nuclear Receptors: Biological Networks, Genome Dynamics and Disease
January 10–15, 2014 | Sagebrush Inn and Conference Center | Taos, New Mexico | USA

Tissue-Resident Memory T Cells
January 12–16, 2014 | Snowbird Resort | Snowbird, Utah | USA

Aging – Pushing the Limits of Cellular Quality Control
January 12–17, 2014 | Sheraton Steamboat Resort | Steamboat Springs, Colorado | USA

Challenges and Opportunities in Diabetes Research and Treatment
joint with Obesity: A Multisystems Perspective
January 12–17, 2014 | Fairmont Hotel Vancouver | Vancouver, British Columbia | Canada

Emerging Cytokine Networks
joint with Inflammatory Diseases: Recent Advances in Basic and Translational Research and Therapeutic Treatments
January 17–22, 2014 | Fairmont Hotel Vancouver | Vancouver, British Columbia | Canada

Pathogenesis of Respiratory Viruses
joint with Innate Immunity to Viral Infections
January 19–24, 2014 | Keystone Resort | Keystone, Colorado | USA

New Frontiers in the Discovery and Treatment of Thrombosis
January 26–30, 2014 | Keystone Resort | Keystone, Colorado | USA

Mechanisms and Consequences of Invertebrate-Microbe Interactions
January 26–30, 2014 | Granlibakken Resort | Tahoe City, California | USA

Growth and Wasting in Heart and Skeletal Muscle
January 26–31, 2014 | Eldorado Hotel & Spa | Santa Fe, New Mexico | USA

RNA Silencing
January 31–February 5, 2014 | Sheraton Seattle Hotel | Seattle, Washington | USA

The Science of Malaria Eradication
February 2–7, 2014 | Fiesta Americana | Mérida, Yucatán | Mexico

Developmental Pathways and Cancer: Wnt, Notch and Hedgehog
joint with Stem Cells and Cancer
February 2–7, 2014 | Fairmont Banff Springs | Banff, Alberta | Canada

Cancer Epigenetics
joint with Transcriptional Regulation
February 4–9, 2014 | Santa Fe Community Convention Center | Santa Fe, New Mexico | USA

Plant Signaling: Dynamic Properties
February 5–10, 2014 | Beaver Run Resort | Breckenridge, Colorado | USA

Molecular Cell Biology of Macrophages in Human Diseases
February 9–14, 2014 | Hilton Santa Fe Historic Plaza Hotel | Santa Fe, New Mexico | USA

Prophylactic and Therapeutic Antibodies
joint with Biology of B Cell Responses
February 9–14, 2014 | Keystone Resort | Keystone, Colorado | USA

Omics Meets Cell Biology: Applications to Human Health and Disease
February 18–23, 2014 | Sagebrush Inn and Conference Center | Taos, New Mexico | USA

Mitochondrial Dynamics and Physiology
joint with The Chemistry and Biology of Cell Death
February 18–23, 2014 | Santa Fe Community Convention Center | Santa Fe, New Mexico | USA

The NF-κB System in Health and Disease
February 23–28, 2014 | Keystone Resort | Keystone, Colorado | USA

Long Noncoding RNAs: Marching toward Mechanism
February 27–March 4, 2014 | Eldorado Hotel & Spa | Santa Fe, New Mexico | USA

Cilia, Development and Human Disease
March 2–7, 2014 | Granlibakken Resort | Tahoe City, California | USA

Parkinson's Disease: Genetics, Mechanisms and Therapeutics
joint with Alzheimer's Disease – From Fundamental Insights to Light at the End of the Translational Tunnel
March 2–7, 2014 | Keystone Resort | Keystone, Colorado | USA

Mobile Genetic Elements and Genome Evolution
March 9–14, 2014 | Hilton Santa Fe Historic Plaza Hotel | Santa Fe, New Mexico | USA

Inflammation, Infection and Cancer
joint with Immune Evolution in Cancer
March 9–14, 2014 | Fairmont Chateau Whistler | Whistler, British Columbia | Canada

HIV Vaccines: Adaptive Immunity and Beyond
joint with HIV Pathogenesis – Virus vs. Host
March 9–14, 2014 | Fairmont Banff Springs | Banff, Alberta | Canada

Metabolism and Angiogenesis
joint with Tumor Metabolism
March 16–21, 2014 | Whistler Conference Centre | Whistler, British Columbia | Canada

Lipid Pathways in Biology and Disease
March 19–24, 2014 | Royal Dublin Society | Dublin | Ireland

Big Data in Biology
March 23–25, 2014 | Fairmont San Francisco | San Francisco, California | USA

Fibrosis: From Bench to Bedside
March 23–28, 2014 | Keystone Resort | Keystone, Colorado | USA

Chromatin Mechanisms and Cell Physiology
March 23–28, 2014 | Oberstdorf Haus | Oberstdorf | Germany

Complications of Diabetes
joint with Innate Immunity, Metabolism and Vascular Injury
March 23–28, 2014 | Whistler Conference Centre | Whistler, British Columbia | Canada

The Ins and Outs of Viral Infection: Entry, Assembly, Exit and Spread
March 30–April 4, 2014 | Beaver Run Resort | Breckenridge, Colorado | USA

Novel Therapeutic Approaches to Tuberculosis
March 30–April 4, 2014 | Keystone Resort | Keystone, Colorado | USA

GPCRs: Structural Dynamics and Functional Implications
joint with Frontiers of Structural Biology
March 30–April 4, 2014 | Snowbird Resort | Snowbird, Utah | USA

Exploiting and Understanding Chemical Biotransformations in the Human Microbiome
April 1–6, 2014 | Big Sky Resort | Big Sky, Montana | USA

Epigenetic Programming and Inheritance
April 6–10, 2014 | Boston Park Plaza | Boston, Massachusetts | USA

Emerging Concepts and Targets in Islet Biology
April 6–11, 2014 | Keystone Resort | Keystone, Colorado | USA

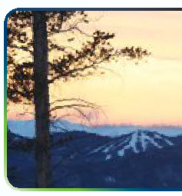
Engineering Cell Fate and Function
joint with Stem Cells and Reprogramming
April 6–11, 2014 | Resort at Squaw Creek | Olympic Valley, California | USA

Adult Neurogenesis
May 12–17, 2014 | Clarion Hotel Sign | Stockholm | Sweden

Autophagy: Fundamentals to Disease
May 23–28, 2014 | Hyatt Regency Austin | Austin, Texas | USA

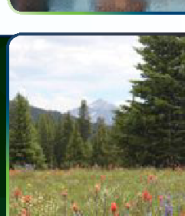
The Brain: Adaptation and Maladaptation in Chronic Pain
June 15–20, 2014 | Keystone Resort | Keystone, Colorado | USA









Submitting by the discounted abstract deadline (four months before meetings) and registering by the discounted registration deadline (two months prior) provides discounts of $50 and $150, respectively, on later fees. Information shown is subject to possible change. Visit www.keystonesymposia.org/2014meetings for full program information.

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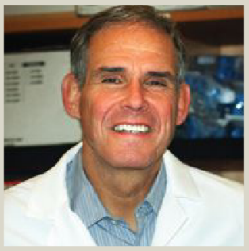

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The Leprosy Bacillus, circa 1873

BY KATE YANDELL

Norwegian physician Gerhard Armauer Hansen first saw rod-shaped microbes in samples harvested from leprosy patients in 1873. Seven years later, Hansen, who worked in the leprosy hospital in the coastal town of Bergen, was on trial for attempting to infect a patient with bacteria without permission, using a cataract knife to inoculate a woman's eye with material from leprous lesions.

Hansen resorted to such an extreme measure because he was having trouble proving his conviction that the microbes caused leprosy—which results in peripheral nerve damage and skin lesions—and that the disease was infectious. He had tried in vain to infect rabbits and to cultivate the microbe in vitro—evidence considered necessary to prove contagiousness. "Leprosy was afterwards called the least contagious of contagious diseases," says Tony Gould, author of *A Disease Apart: Leprosy in the Modern World*, which might explain why Hansen had struggled to come up with the necessary proof.

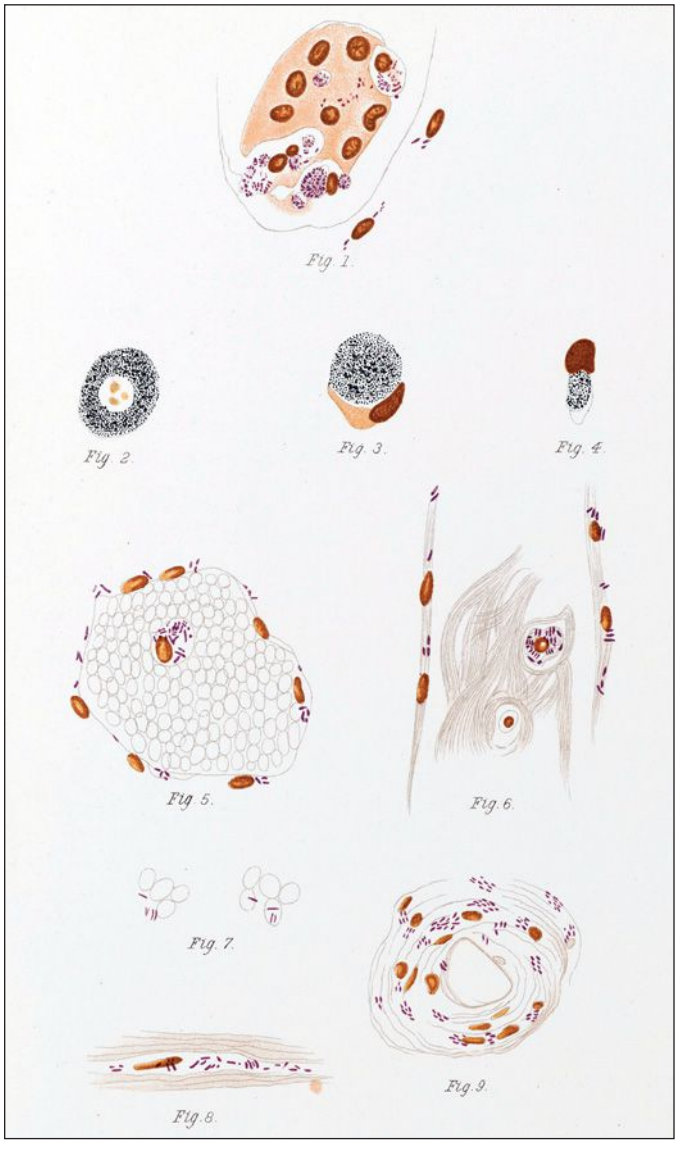
Hansen's unfortunate patient, a 33-year-old woman named Kari Nielsdatter, already had tuberculoid leprosy, one form of the disease, but Hansen hoped to infect her with a second form, called lepromatous leprosy. The infection did not take hold, but Hansen was punished for conducting the experiment. He was stripped of his position at the leprosy hospital but allowed to keep his position as Norway's chief medical officer for leprosy, which he used to push through measures that kept leprosy patients in partial isolation.

Despite his misdeeds, Hansen was later honored as the discoverer of *Mycobacterium leprae*, which was officially accepted as the cause of the disease at the first International Leprosy Conference, held in Berlin in 1897. Today, leprosy is often called Hansen's disease.

Some of the early skepticism about the contagiousness of the disease came from Daniel Cornelius Danielssen, Hansen's mentor and a preeminent leprosy expert of the day. Danielssen was convinced that leprosy could not be transmissible and instead thought it ran in families, or arose from poor living conditions. He had even inoculated himself and others with material from leprosy patients without causing illness, which bolstered his conviction.

While Hansen's assertion that leprosy is infectious was ultimately vindicated, "there appears to be a very strong genetic predisposition to leprosy," according to Richard Truman, acting chief of the laboratory research branch at the National Hansen's Disease Program in Baton Rouge, Louisiana. Only up to 5 percent of people are susceptible to leprosy, and susceptibility appears to run in families, but is additionally enhanced by malnutrition and conditions that compromise the immune system.

INFECTIOUS AGENTS: Gerhard Armauer Hansen observed *Mycobacterium leprae* for the first time in infected nodules excised from leprosy patients. Barely distinct, rod-shaped bacteria (purple) became apparent under Hansen's microscope. However, it took German bacteriologist Albert Neisser's stain for the bacterium, developed after visiting Hansen in 1879, to make *M. leprae* clearly visible. Pictured are illustrations of *M. leprae*-infected cells from a testicle, taken from Hansen's 1895 book *Leprosy: In its Clinical and Pathological Aspects*.



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In the end, then, perhaps Hansen and Danielssen were both partly right: Hansen's mysterious rods cause leprosy, but only in those with the poor luck to be genetically and environmentally susceptible. ■

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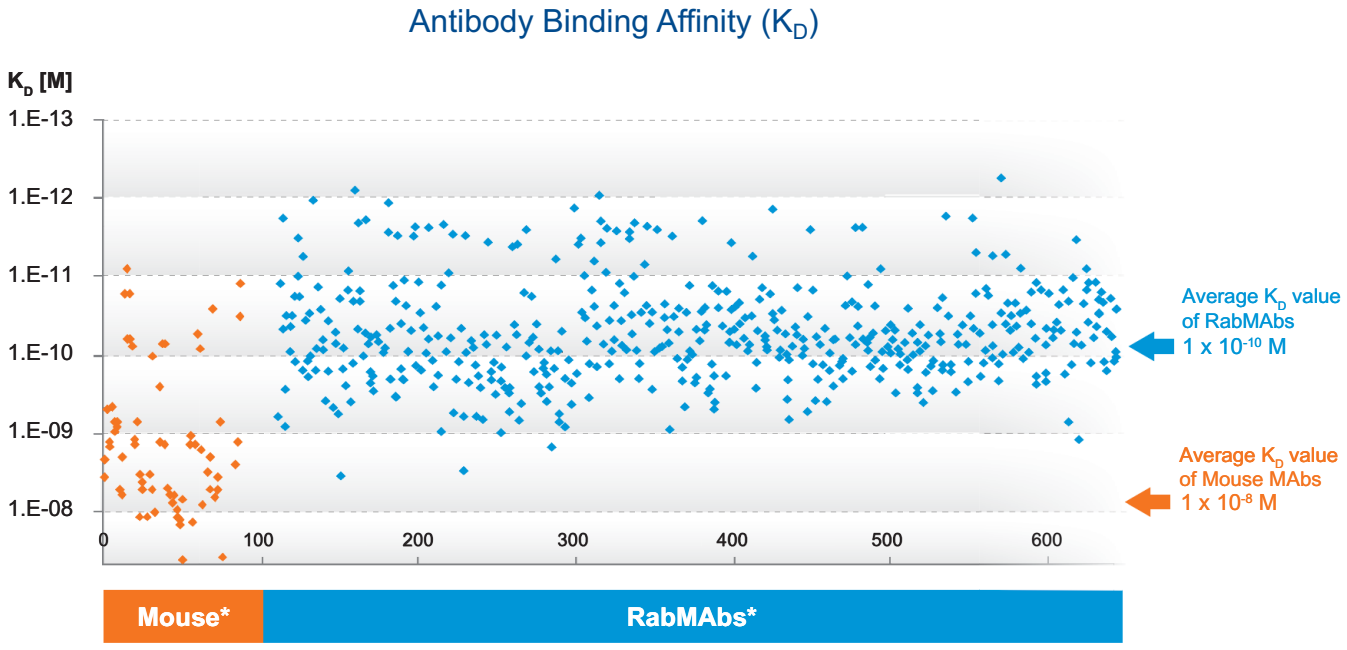

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