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The Science of Pain

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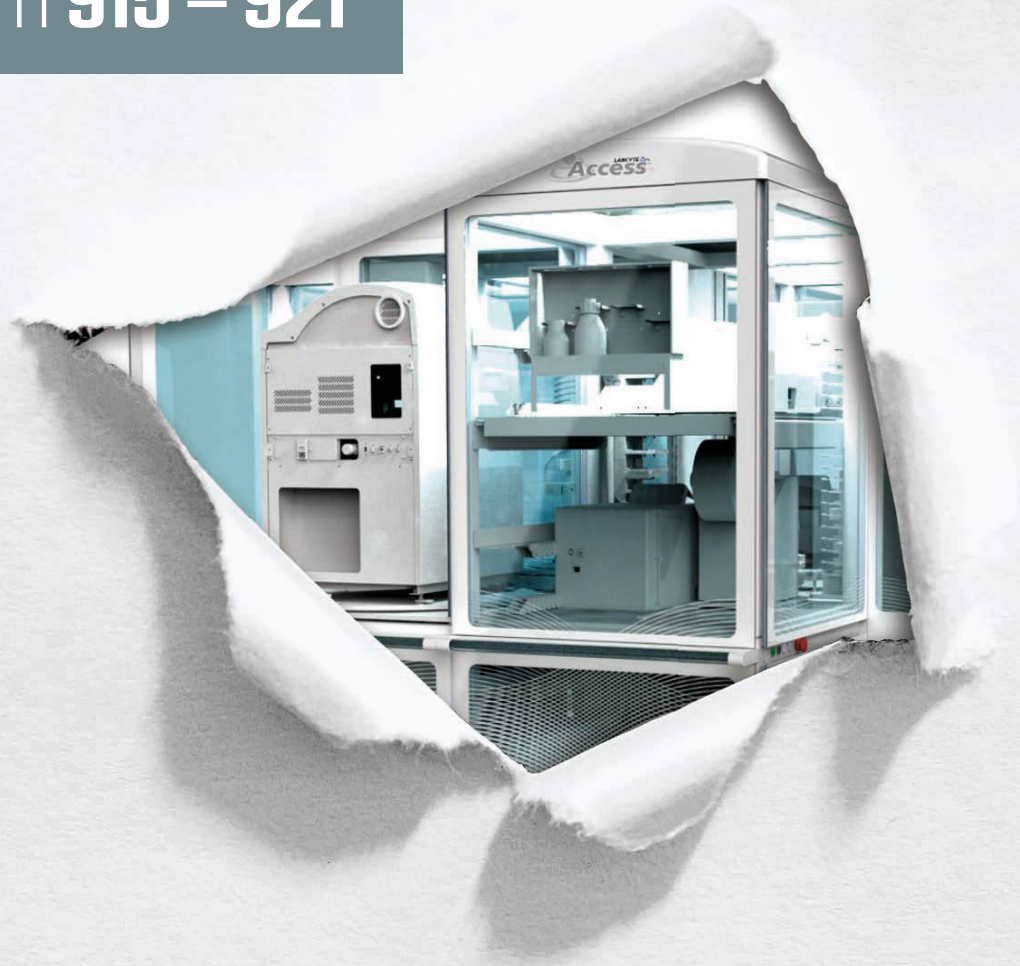

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2017

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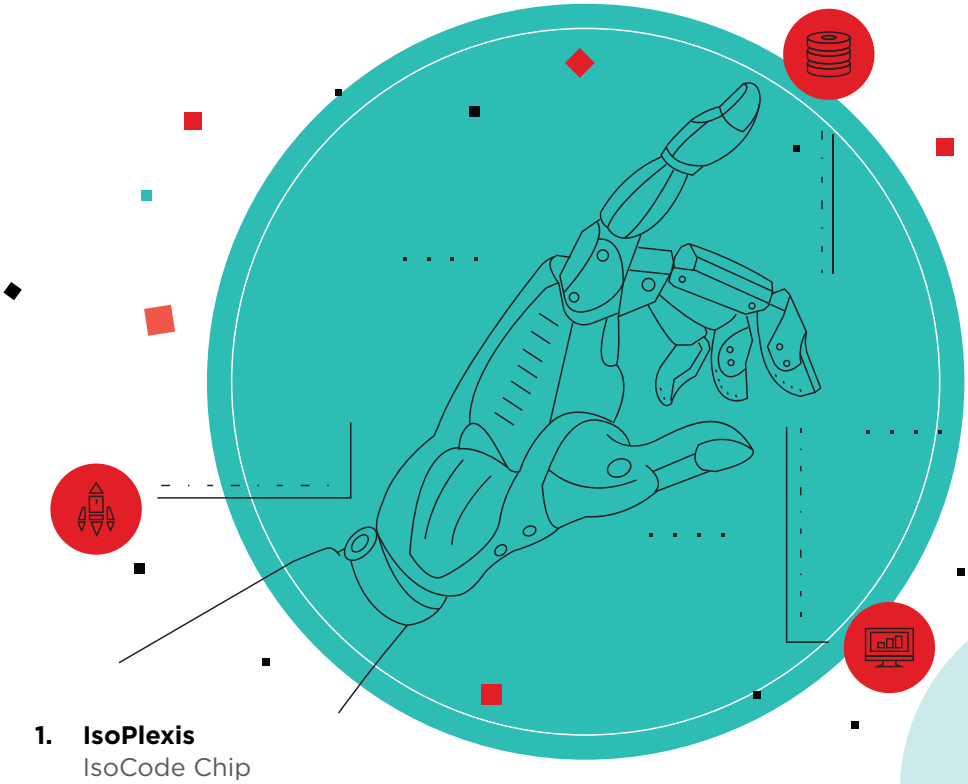

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Contents

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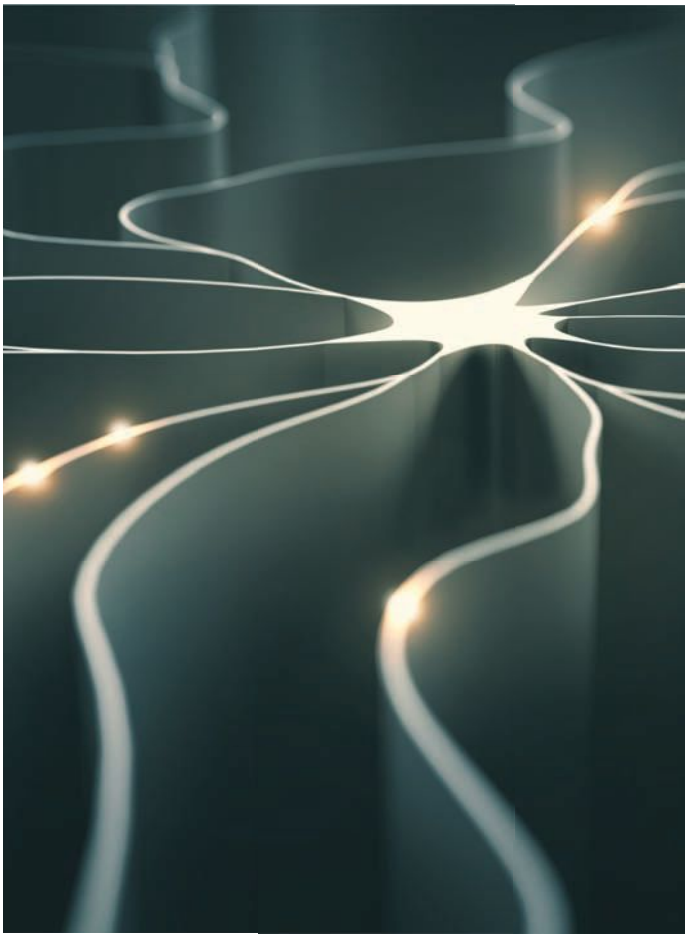


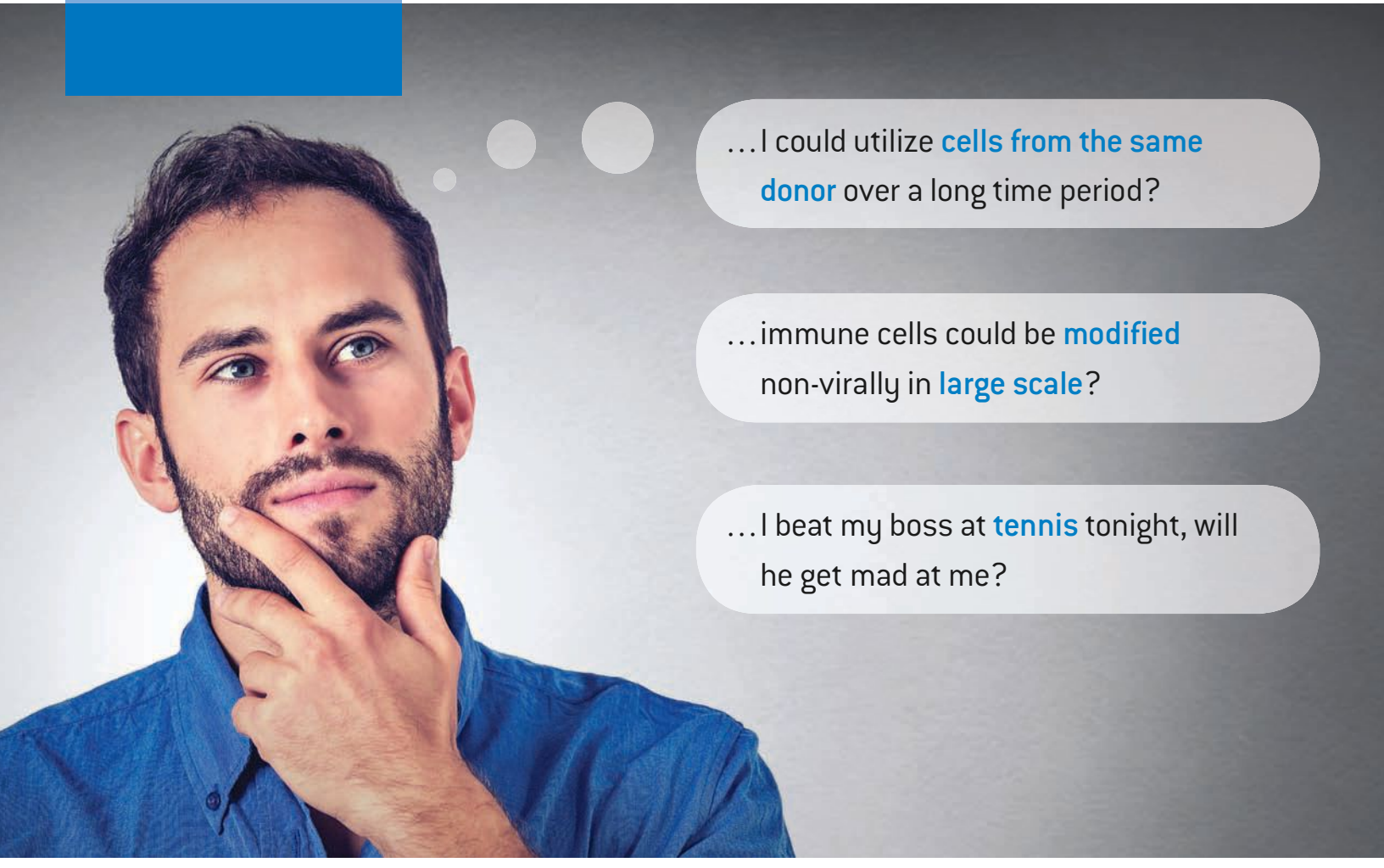
Features

ON THE COVER: © ISTOCK.COM/WUNDERVISUALS

BioResearch

Lonza

What if...



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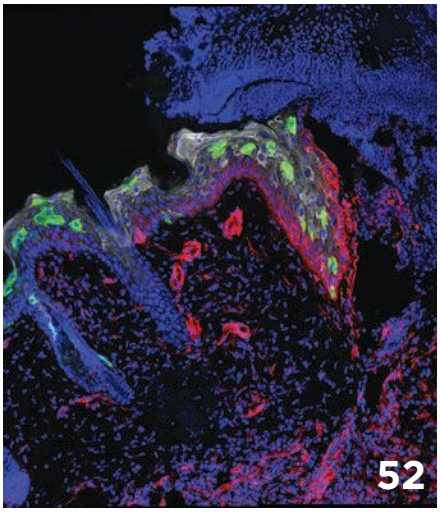

PUZZLE ON PAGE 13

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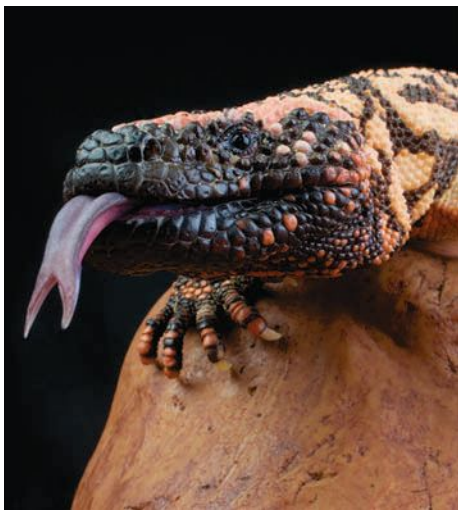


THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Swearing Off Pain
Author Emma Byrne runs down the benefits of cursing, among them an enhanced ability to withstand pain.

VIDEO
Painful Realities
Dave Thomas, a researcher at the National Institute on Drug Abuse, talks about the harsh truths and frustrating complexity of pain and analgesia.

VIDEO
Prospecting for Painkillers
Cone snail venom researcher Mandë Holford discusses the therapeutic potential of toxins found in animals.

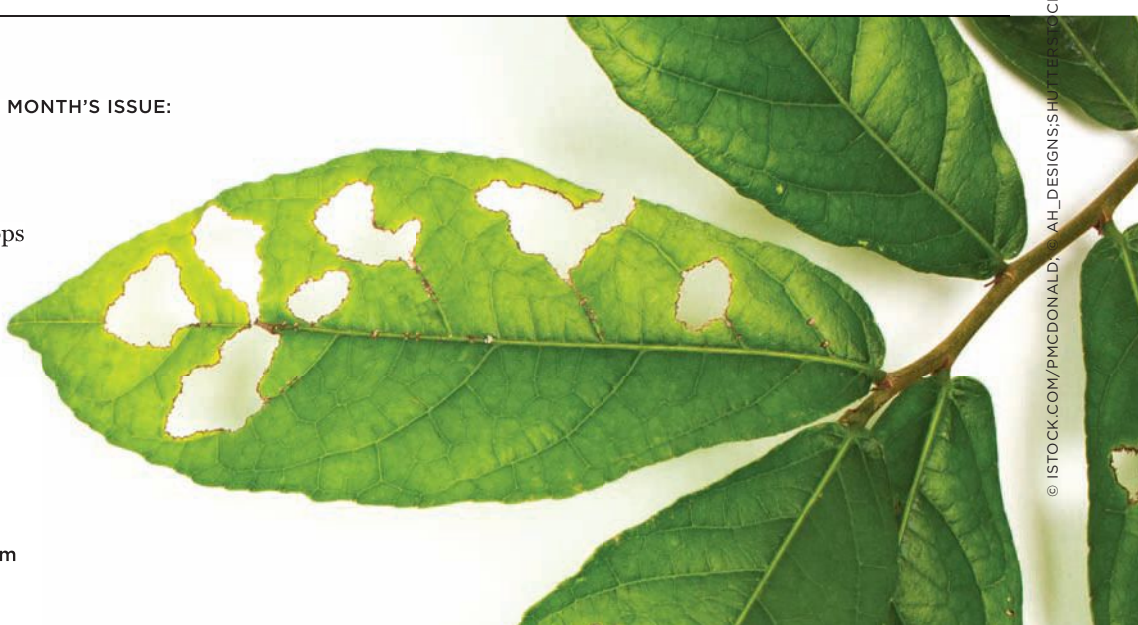
AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

Coming in February

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

- The secret microbial life of plants
- Combatting viral infections in crops
- Next-generation exoskeletons
- Building better peer reviewers

AND MUCH MORE



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INNOVATIONS

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TS WEBINARS

COMING**SOON** | The Precision Medicine Revolution: CRISPR-Based Therapies

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) gene-editing technology has been hailed as a breakthrough and has emerged as the new face of precision medicine. Its potential as a treatment for numerous diseases, stretching from various cancer types to neurological diseases to lethal heritable disorders, has been well documented. But ongoing efforts aim to clarify the complex issues surrounding the legality and ethics of editing human genomes for therapeutic purposes. For a detailed look at the progress made toward CRISPR-mediated correction of human diseases and the continuing ethics debate, *The Scientist* is bringing together a panel of experts who will share their research, summarize the state of the science, and discuss the next steps for those looking to adopt the technique. Attendees will have the opportunity to interact with experts, ask questions, and seek advice on topics related to their research.

DANA CARROLL, PhD
Distinguished Professor, Department of Biochemistry
The University of Utah School of Medicine

JAMES DAHLMAN, PhD
Assistant Professor, Department of Biomedical Engineering
Georgia Institute of Technology and Emory School of Medicine

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www.the-scientist.com/crisprmedicine
The webinar video will also be available at this link.

TOPICS TO BE COVERED:

- CRISPR-generated model systems for in vivo study of a wide range of diseases
- Therapeutic applications of genome editing and their associated societal implications

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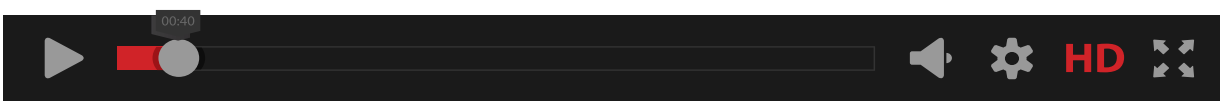
ON**DEMAND** | Microbiome-Centric Human Health: A Call for Systems Biology

With 100 times the number of genes contained in the human genome, and an array of different cell types and functions, one can argue that members of our microbiome constitute an additional human organ system. Research to date has implicated microbial activity in autoimmune disease, cancer, and the obesity epidemic. As a major source of variability across people, understanding and altering an individual's microbiome is both a challenge and novel avenue for personalized medicine and nutrition. For a detailed look at the progress made toward understanding the host-microbiome interplay and the efforts undertaken to achieve a steady state of mutualism for a larger human health benefit, *The Scientist* brings together a panel of experts who share their research, summarize the state of the science, and discuss the next steps in developing personalized microbiome-based therapies.



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

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

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

TOPICS COVERED:

- Mechanisms by which human microbiota influence health and disease
- How multidimensional data are being employed to develop personalized therapies

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TS WEBINARS

ONDEMAND | Spheroid Cell Culture: New Dimensions in 3-D Assays

The last decade has seen a large variety of models developed to mimic cells organized into tissues and even organs. These have collectively been termed 3-D cell culture models. Three-dimensional methods are deemed superior to culturing cells in a monolayer on lab plasticware because such 3-D set ups increase extracellular matrix (ECM) formation, cell-to-cell, and cell-to-matrix interactions, which are all important for differentiation, proliferation, and cellular functions in vivo. Perhaps the most popular method of 3-D cell culture is aggregating cells into spheroids. *The Scientist* brings together a panel of experts to discuss the value of spheroid culture systems, and to explore the technical benefits and challenges of making the switch from 2-D to 3-D culture.

WATCH NOW! www.the-scientist.com/3Dassays

ESMAIEL JABBARI, PhD
Professor, Departments of Chemical and Biological Engineering
College of Engineering and Computing
University of South Carolina

MARGARET MAGDESIAN, PhD
Founder and CEO
Ananda Devices

TOPICS COVERED:

- Using 3-D culture to turn individual cells into organoids and organs
- Novel options for 3-D culture scaffolding

WEBINAR SPONSORED BY:

BioTek

ONDEMAND | Cancer Stem Cells: Getting to the Root of Cancer

The stem cell theory of cancer implies that anticancer therapies must target and destroy all resident cancer stem cells in order to produce a durable response. Scientists are testing therapies that target cancer stem cells to confirm their safety and efficacy, while research into the weaknesses of cancer stem cells continues. To explore the knowns and unknowns in the field of cancer stem cell research, *The Scientist* brings together a panel of experts to share their results, as well as the lessons they've learned from studying the root cause of cancer.

WATCH NOW! www.the-scientist.com/rootofcancer

IRVING WEISSMAN, MD
Director, Institute for Stem Cell Biology and Regenerative Medicine
Stanford University School of Medicine

ALKA MANSUKHANI, PhD
Associate Professor, Department of Microbiology
New York University School of Medicine

TOPICS COVERED:

- How stem cells become cancer stem cells
- Methods for constraining cancer stem cell proliferation

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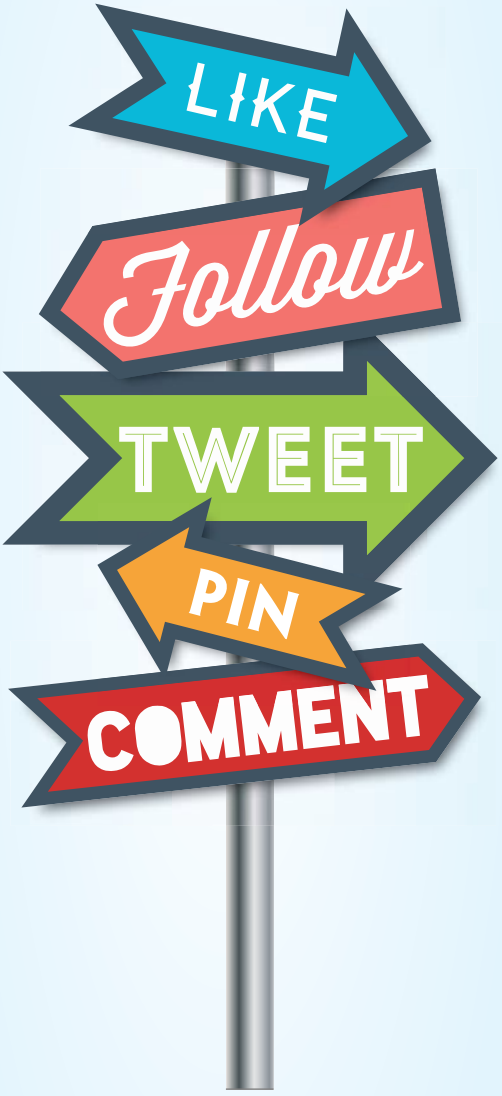

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Contributors

Mark Hutchinson was not a stellar student during his high school days in Adelaide, Australia. He says he found studying difficult and was often described as one of those students who "needs to apply himself more to reach his full potential." But things would soon turn around for the young Hutchinson, who is now a biologist. "It wasn't until I was at university, when I was presented with the big questions, that all the small stuff fit into place," he says. His newfound fascination with biology led him to pursue a PhD in medicine at the University of Adelaide, where he studied clinical pharmacology. Toward the end of his doctoral research, he was working on a clinical trial with patients who were receiving analgesics for their pain. "I got more interested in understanding how we could optimize pain treatment because I was seeing that they weren't getting as much pain relief as perhaps they should be getting," Hutchinson says. In the early 2000s, he went to the University of Colorado Boulder lab of neuroscientist Linda Watkins to explore the neuroscientific and immune bases of pain. He then returned to the University of Adelaide, where he is now a professor of medicine and director of the Australian Research Council Center of Excellence for Nanoscale BioPhotonics. On page 34, Hutchinson writes about the role of glial cells in persistent pain, which has proven particularly difficult to treat. His aim is to eventually create a blood test for pain, which will be useful for those who can't communicate their suffering, such as young children, the elderly, or animals. "Because we cannot ask them," he says. "We're not Dr. Dolittle."

Susan Calvin, the "robopsychologist" from Isaac Asimov's science fiction series Robot, was an early inspiration for author and researcher **Emma Byrne**. "She's basically a computational neuroscientist," Byrne says. As she pursued her bachelor's in business and languages at Aston University in the U.K., Byrne was more interested in her flatmate's coursework in computational programming than her own curriculum. After graduation, she worked for a few years as a software engineer and persuaded a researcher at University College London to take her on as a PhD student in artificial intelligence (AI). Since then, Byrne has worked on a number of AI projects, including developing algorithms to automate tedious genetic experiments in a yeast lab at the University of Wales in Aberystwyth. Now a researcher at 10x Future Technologies, an AI-powered financial services startup, Byrne writes about science and robotics on the side. Her first book, *Swearing is Good for You: The Amazing Science of Bad Language*, explores the neuropsychology behind swearing and hits shelves January 23. Since researching the book, Byrne says, she has curtailed her use of swearwords to maintain their potency. "I actually swear less now I've written the book than I did before," she says. Read Byrne's essay about the relationship between swearing and pain on page 65.

London native **Katarina Zimmer** is a long-time animal lover. "I think the only gift I ever enjoyed was getting stuffed animal toys, and they had to look like real animals; [they] couldn't have big googly eyes of Disney animals," she recalls. "That drove me toward becoming really interested in animals and their biology." After living in Germany for a decade with her family, Zimmer returned to the U.K. to attend University College London, where she worked in a lab exploring the genetics of regeneration in brittle stars. "We cut their arms off [to] see what genes they needed to regrow them, which they do really quickly," she explains. But for her master's project, Zimmer switched gears to computational biology, working in Kate Jones's lab to build a tool to detect species of bat based on audio recordings of their vocalizations. Although she considered continuing on the research track in a PhD program, Zimmer transitioned to science writing. "I just realized that there is quite a big gap between public understanding of science and what scientists actually do." Zimmer attended Columbia University's one-year journalism program, graduating in May 2017. She then tested the waters as a freelancer, writing about illegal fishing in the Pacific Ocean for *Quartz* and *The New Food Economy*, before becoming *The Scientist*'s intern this fall. "I think it's fantastic preparation for learning how journalism works in a real-world setting," Zimmer says of her current role. "At journalism school you learn a lot of things, but if what you're doing isn't getting published, it's just not the same."

AUSTRALIAN RESEARCH COUNCIL CENTRE OF EXCELLENCE FOR NANOSCALE BIOPHOTONICS; VANITY STUDIOS; KIMBERLY FLORES GUZMÁN

Prizes and Penalties

Life is filled with pleasure and pain. Science and society are struggling mightily with both.

BY BOB GRANT

A couple of months ago, I was reading a graphic novel with my children, and one of the characters voiced this well-constructed line: "Food is the prize of living." The simplicity of the thought appealed to me—that life comes with a handful of pleasures that compel us to march onward and, in some instances, serve as fuel for that march. But as we at *The Scientist* assembled this issue on the science of pain, my mind eventually wandered to the other side of that coin. If pleasures such as food, laughter, or dreams are the prizes of living, I thought, then surely pain is the penalty.

For all the mystery that shrouds pain, science is making strides in explaining it.

Pain is a penalty we all pay, to some degree. The lucky among us experience it only intermittently: the searing singe of a hot pan, the twang of a stubbed toe, the stab of a bee sting. But a growing cohort—some 50 million people in the U.S. alone—suffers pain that is far more intractable and insidious. Chronic, or persistent, pain engenders great suffering and lies at the base of the opioid epidemic consuming America. The Centers for Disease Control and Prevention reported last year that, from 1999 to 2015, more than 183,000 people in the U.S. died from overdoses related to prescription opioids. Pain begets more pain.

For a sensory experience so familiar to humanity, pain remains a stubborn mystery; science is still struggling to understand its full complexity. Doctors don't have an objective way to measure pain, researchers don't have a complete grip on the biology of chronic pain, and drug developers haven't yet arrived at the most effective and safest strategies to alleviate pain.

Yet for all the mystery that shrouds pain, science is making strides in explaining it. On page 34, researcher Mark Hutchinson describes progress in characterizing the role of glial cells in chronic pain. These immune-like cells of the central nervous system aren't mere support for neurons but can modulate neurons' likelihood of firing, sometimes encouraging them to send sensations of pain to the brain even in the absence of an immediate bodily insult. Meanwhile, other researchers are looking to sodium channels on the surface of neurons as targets for pain relief. Editor Catherine Offord reports on page 26 that researchers have learned that loss-of-function mutations in the gene for a particular sodium channel result in a syndrome characterized by the complete absence of pain sensation, while gain-of-function mutations in the same gene cause excruciating pain. These findings make the channel an attractive drug target, but realizing the therapeutic potential has proven tricky. "In principle, it may be a good target," one researcher tells Offord. "However, from what we have seen in recent years, [exploiting] it seems to be really complex and difficult."

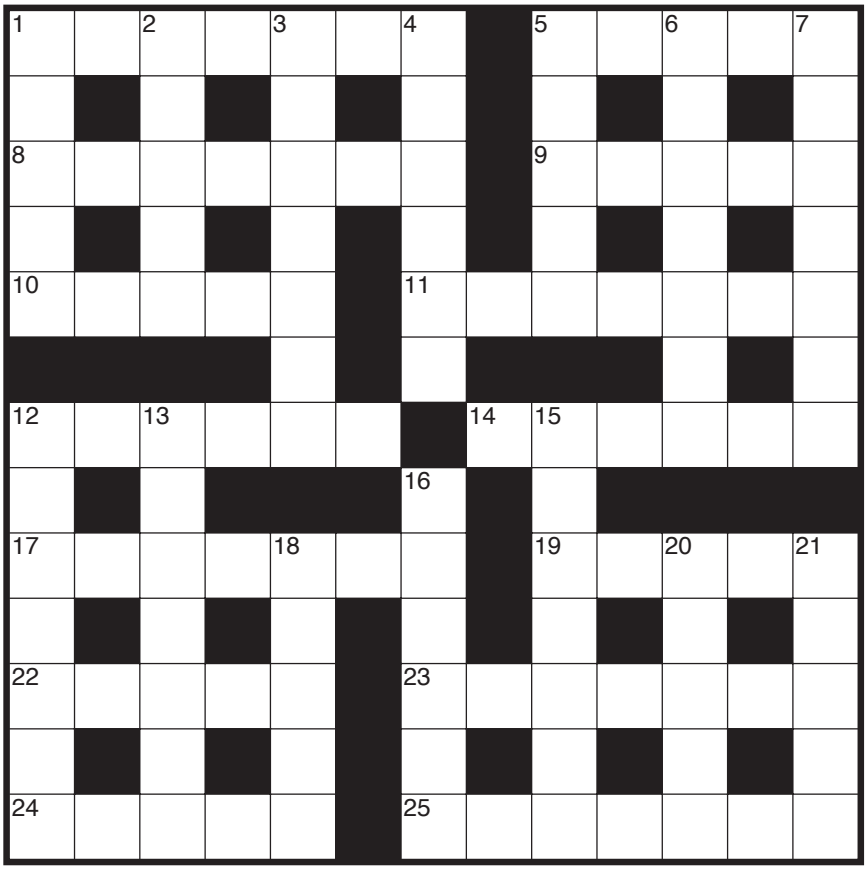
This special issue covers other strategies for treating pain as well. For example, researchers have spent decades prospecting for novel analgesics in the animal toxins that activate or inhibit mammalian pain pathways, and now, they're using modern technologies and methods such as genomics and proteomics to aid their search (pg. 42). Meanwhile, scientists working to develop safe and nonaddictive painkillers are ever closer to accomplishing that goal, though for now, traditional opioids remain the go-to treatment for the vast majority of pain patients (pg. 61).

As we enter 2018 and bid 2017 goodbye, we at *The Scientist* wish you a New Year filled with life's prizes. And as we all encounter the penalties that come with our intricate biology, may we face them with the knowledge that smart people are working every day to better understand and more effectively combat our pain. ■

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

ANDRZEJ KRAUZE

Speaking of Science



Note: The answer grid will include every letter of the alphabet.

BY EMILY COX AND HENRY RATHVON

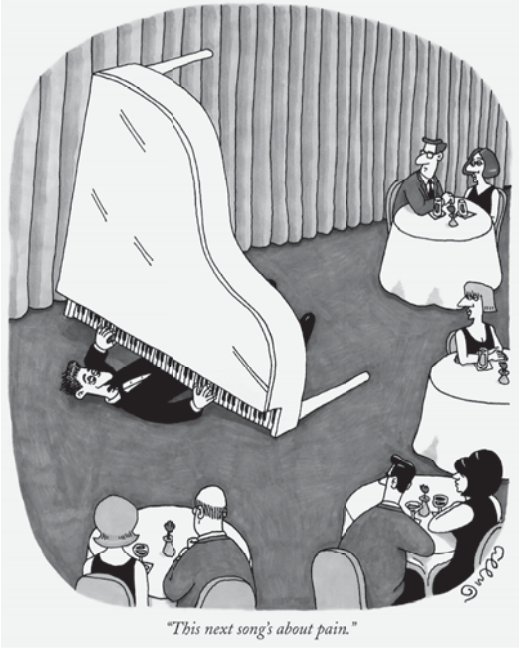
ACROSS

1. Big moment in the life of a cell
5. Located in the region of the kidneys
8. Having no right angles
9. Masked man who took his name from a fox
10. Down under canine
11. Use for ambergris, musk, and civet
12. Experimental runner of mazes (2 wds.)
14. What diazepam treats
17. Root crop of the species *Manihot esculenta*
19. Guy who put the Howe in invention
22. Oxymoron maker when applied to shrimp
23. Niels Bohr: "How wonderful that we have met with a ___. Now we have some hope of making progress."
24. Avian features missing in moas
25. Marsupial burrowers who avoid the 10-Across

DOWN

1. Spoke in the language of Angus or Brangus?
2. Primary tool for a raptor
3. Giant in the Sierra Nevada
4. What antihistamines may make you
5. Type of long, narrow, saltwater clam
6. Nature's opposite, in some contexts
7. Female in family Felidae
12. Sustained spasm of the masseter muscle; trismus
13. One of southern Africa's San people
15. Pythagorean proposition
16. Fruit tree with a handy name?
18. Units posited by Democritus
20. Home country of astrophysicist Subrahmanyan Chandrasekhar
21. What X and Y chromosomes determine

Answer key on page 5



"This next song's about pain."

Pain is a symphony—a complex response that includes not just a distinct sensation but also motor activity, a change in emotion, a focusing of attention, a brand-new memory.

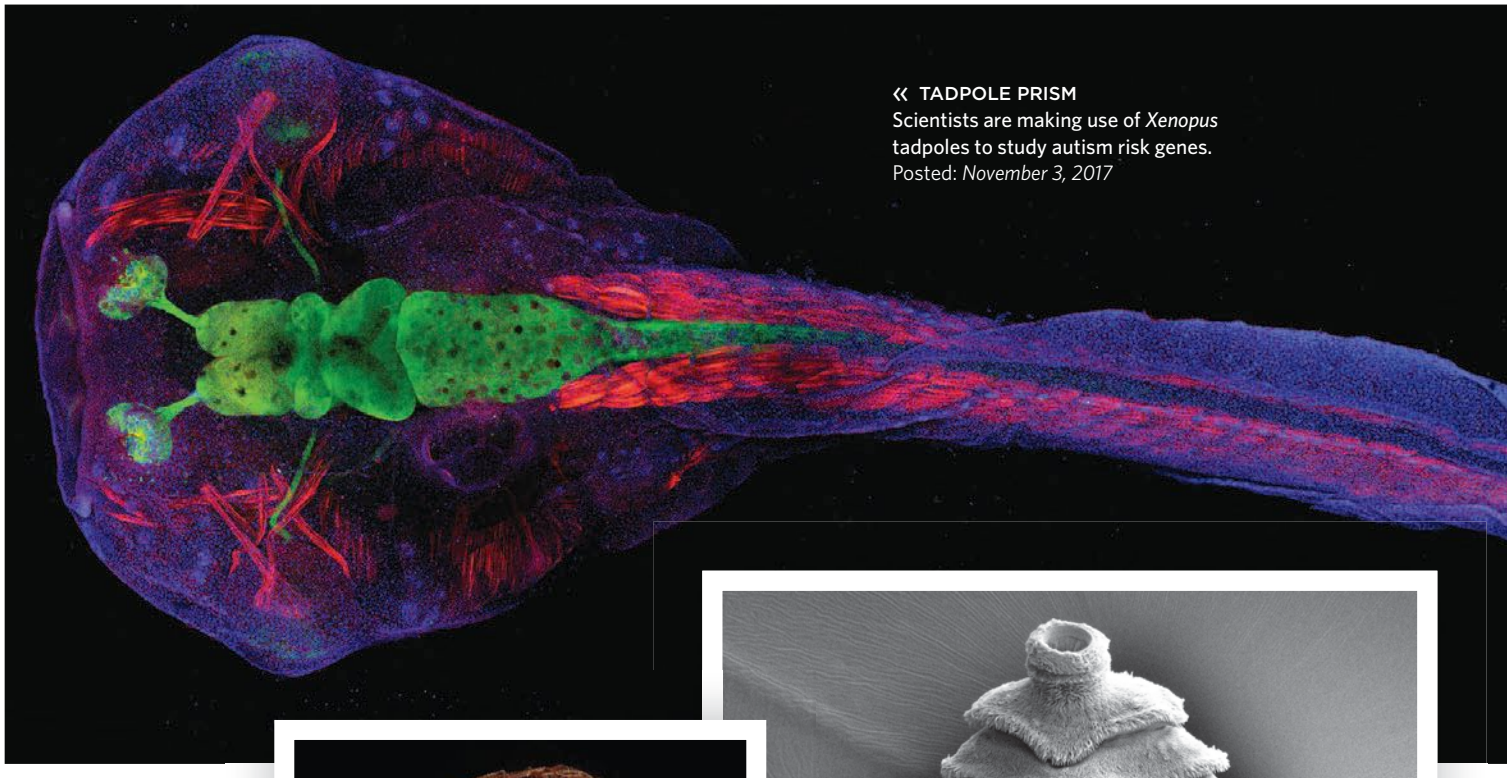
—Surgeon and author **Atul Gawande**, in his 2002 book, *Complications: A Surgeon's Notes on an Imperfect Science*

Of pain you could wish only one thing: that it should stop. Nothing in the world was so bad as physical pain. In the face of pain there are no heroes.

—**George Orwell**, in his dystopian classic, *1984*

Caught on Camera

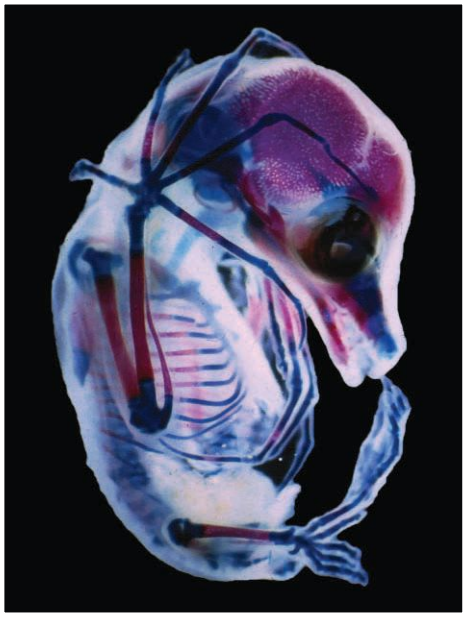
Selected Images of the Day from the-scientist.com



« TADPOLE PRISM
Scientists are making use of *Xenopus* tadpoles to study autism risk genes.
Posted: *November 3, 2017*

DEEP SEA FANGS »
Scientists are taking a close look at the skeletons of deep-sea fish, such as this common fangtooth (*Anoplogaster cornuta*), for inspiration to solve engineering problems.
Posted: *December 1, 2017*

GUT SWEET HOME »
Researchers describe more than 200 species of tapeworm—such as this *Litobothrium nickoli*, which scientists pulled from the gut of a pelagic thresher shark—collected from the digestive systems of animals around the world as part of a decade-long project.
Posted: *November 22, 2017*



« FETAL FRUIT BAT, UNVEILED
This stereomicrograph reveals the delicate inner structures of a third-trimester fetal fruit bat (Megachiroptera).
Posted: *October 23, 2017*

» PLASTIC FEAST
New research suggests that plastic might "taste good" to hard corals, such as this northern cup coral (*Astrangia poculata*) polyp.
Posted: *October 30, 2017*

« OVERKILL
The Sakishima habu *(Protobothrops elegans)* can compensate for the weakness of its venom by overdosing its prey.
Posted: *October 6, 2017*

Tadpole Prism: Helen Willsey, University of California, San Francisco; **Fetal Fruit Bat, Unveiled**: Rick Adams, University of Northern Colorado, Nikon Small World Photomicrography Competition; **Plastic Feast**: Alex Seymour, Duke University; **Overkill**: Steven Aird, Okinawa Institute of Science and Technology; **Gut Sweet Home**: Kirsten Jensen/Kansas University; **Deep Sea Fangs**: Adam Summers, Friday Harbor Laboratories, University Of Washington

Notebook

JANUARY 2018

JENS RYDELL

BATTY HEAR, BATTY DO: The vocalizations of Egyptian fruit bats (*Rousettus aegyptiacus*) and other species of the winged mammals may be shaped by the sounds of their colony mates.

Bat-ccents

Scientists can trace the evolutionary histories of bats and humans back to a common ancestor that lived some tens of millions of years ago. And on the surface, those years of evolutionary divergence have separated us from the winged mammals in every way possible. But look on a sociobehavioral level, as some bat researchers are doing, and the two animal groups share much more than meets the eye.

Like humans, bats form huge congregations of up to millions of individuals at a time. On a smaller scale, they form intimate social bonds with one another. And recently, scientists have suggested that bats are capable of vocal learning—the ability to modify vocalizations after hearing sounds. Researchers long considered this skill to be practiced only by humans, songbirds, and cetaceans, but have more recently identified examples of vocal learning in seals, sea lions, elephants—and now, bats.

In humans, vocal learning can take the form of adopting styles of speech—for example, if a Brit were to pick up an Australian accent after moving down under. Yossi Yovel, a physicist turned bat biologist at Tel Aviv University who has long been fascinated by animal behavior, recently demonstrated that bat pups can acquire "dialects" in a similar way.

Egyptian fruit bats (*Rousettus aegyptiacus*), like most bats, are very vocal creatures. If you walk through the streets of Tel Aviv at night, Yovel says, you will often encounter the mammals eating in fruit trees found throughout the city. In contrast to many of the sounds bats generate that are outside the frequency range of human hearing, the social vocalizations of Egyptian fruit bats are clearly audible. Their daytime roosts—mostly in caves, containing hundreds or thousands of individuals—are often tightly packed and are usually quite loud with what sound like arguments. Many of Yovel's videos show young bat pups jabbing colony mates with a wing, a provocation that tends to elicit an irritable chirp in response. Yovel says an accurate translation would be: "Get out of my way!"

To study vocal learning in bats, Yovel and his team went to nearby caves and caught 15 pregnant mothers. He separated them into three captive colonies, where they each gave birth to one pup. After about two and a half months, when the bat pups were independent and no longer clinging to their parent, he released the mothers. From the pups' very first day of life until the end of their first year, Yovel played them recordings of the "get out of my way" sounds they would usually hear around them in colonial roosts. Yovel played a distinct dialect to each colony. Although the pups retained a hint of their mothers' dialect, their vocalizations eventually became more similar to the ones they were exposed to in the lab (*PLOS Biol*, 15:e2002556, 2017).

Researchers long considered vocal learning to be practiced only by humans, songbirds, and cetaceans, but have more recently identified examples of the behavior in seals, sea lions, elephants—and now, bats.

Brock Fenton, a bat biologist at Canada's Western University, can't explain why scientists hadn't looked for vocal learning in bats before. "If you were looking for an ideal study animal where vocalizations are really important, you'd have to really search hard to find something better than bats."

But what exactly is a bat dialect? Yovel distinguished the three dialects mainly based on the differing fundamental frequencies—that is, the pitch—of the bats' quarrelling sounds. One group, for instance, had a low base pitch at around 250 Hertz, and one group had a higher pitch at 1,315 Hertz. This is the same as the difference between a B3 piano key—the note sung by Lady Gaga throughout the verses of her song "Poker Face"—and an E6, the highest note Kesha reaches in her chorus of "Praying." The third dialect had an intermediate fundamental frequency.

Bat dialects don't just differ by frequency. Other features, such as the duration of a call or the frequency at which a particular bout is loudest, also vary. Using computational methods to extract these features, Yovel identified distinct dialects that can be acoustically characterized in *R. aegyptiacus*.

Remarkably, bats are not just learning their dialect from their mothers (which is what many species of songbird do). They are listening and learning from the whole colony that surrounds them. Yovel calls this "crowd vocal learning," and speculates that it likely evolved to make it easier for bats to recognize colony mates. A young, inexperienced fruit bat pup, for example, might arrive at a tree it hasn't visited before. Being able to recognize its own colony in the same tree will help it decide whether it's safe to eat the fruit or not.

Yovel's research is not only useful for understanding how bats communicate with one another, but also has broader applications for conservation science. Many bat populations are suffering serious declines due to diseases, habitat loss, or wind turbines, and Fenton says it's vital to get a handle on where they are, and in what abundances. Fortunately, many species continually leak information about their whereabouts through high-frequency pulses they emit while echolocating, as well as through social sounds. The first global bat monitoring project, staffed by NGOs and volunteers around the world, aims to count bats by recording these vocalizations. "If you let the bats tell you where they are, then you know what to protect," explains Fenton.

But researchers are still far from transforming bat noises into population data, Fenton cautions. Bats represent a whopping fifth of all mammals and comprise about 1,600 species, he says, and the noises they make can be highly variable, so it's difficult to know which species you are eavesdropping on. But findings like Yovel's that parse the diversity of bat acoustic signals could aid in this challenge, Fenton says.

As for Yovel's 14 bat pups (one of the original 15 died), which are now adults with heavy accents, they've been returned to their original roosts. Now, Yovel is using GPS devices to track a colony that roosts on the Tel Aviv University campus to learn more about bat behavior and how they use vocal learning.

Fenton is excited for the future: "It's almost revolution time," he says. "We're really on the edge of a bunch of interesting discoveries." —Katarina Zimmer

His and Hers Analgesia

Graduate student Anne Murphy had run out of rats. Or rather, she'd run out of male rats, the animals she was using to study brain regions involved in pain modulation for her PhD at the University of Cincinnati in the early 1990s. At a time when neuroscientists almost exclusively used male animals for research, what Murphy did next was unusual: she used a female rat instead.

"I had the hardest time to get the female to go under the anesthesia; she wasn't acting right," Murphy says. Her advisor's explanation? "'Well, you know those females, they have hormones, and those hormones are always fluctuating and they're so variable,'" Murphy recalls. The comments struck a nerve. "It really got to me," she says. "I'm a female. I have hormones that fluctuate. . . . It made me determined to investigate the differences between males and females in terms of pain processing and alleviation."

Her decision was timely. Since the '90s, evidence has been accumulating to suggest that not only do women experience a higher incidence of chronic pain syndromes than men do—fibromyalgia and interstitial cystitis, for example—females also generally report higher pain intensities. Additionally, Murphy notes, a handful of clinical studies has suggested that women require higher doses of opioid pain medications such as morphine for comparable analgesia; plus, they experience worse side effects and a higher risk of addiction.

Although the explanations for sex differences in pain are still a topic of considerable scientific uncertainty, Murphy, now a professor at Georgia State University, has

been working with her lab to clarify the picture by teasing out some of the neurological mechanisms underlying pain's alleviation. As part of this work, one of Murphy's graduate students, Hillary Doyle, recently carried out a project to look for predictors of morphine's effectiveness in healthy rats in a brain region called the periaqueductal gray (PAG)—a key pain-response control center. In the PAG, as in many other parts of the body, morphine binds to μ opioid receptors and triggers signaling pathways to kill pain. Studies have shown that female rodents receiving the drug via direct PAG injection need at least 10 times more morphine than males do in order to achieve comparable levels of analgesia.

But previous work by Murphy's lab and others suggested that morphine can also act through another mechanism in the PAG by binding to protein receptors expressed on microglia—specialized immune cells implicated in neuropathic pain (see Glia and Pain, pg. 34). This binding triggers inflammatory pathways that, paradoxically, work against the drug's analgesic effects. If females possessed higher microglia densities, Murphy and Doyle hypothesized, then their brains might be more susceptible to the inflammatory effects of morphine and less responsive to the drug's intended analgesia.

When the researchers looked, however, they found that male and female rats showed similar densities of microglia in the PAG. But studying the cells themselves, Doyle identified a different sort of variation. Microglia in different states of activation look different under the microscope, she tells *The Scientist*. Resting microglia have what's called a "ramified" structure, where arm-like processes extend from the cell body; activated microglia, by contrast, have a round, amoeboid structure. "We see a much greater percentage of active-type microglia in females than in males," says Doyle, now a medical associate at the Scienomics Group, a health and science communications consulting company. "These were changes specific to regions involved in pain, like the PAG."

Sex differences in morphine responsiveness, then, might result partly from fundamental differences in baseline microglial activity, not density. Sure enough, Murphy's team found that the percentage of microglia in an active state in a rat's PAG correlated with the amount of morphine needed to render the animal indifferent to a thermally painful light beam aimed at a hind paw. What's more, when the researchers administered drugs to block the inflammatory pathways triggered by microglia, they found that sex differences in these responses disappeared: females no longer needed substantially higher doses for the same analgesic effect (*J Neurosci*, 37:3202-14, 2017).

Ultimately, these studies could point the way to developing more-specific and targeted pharmacotherapies.

—Alan Gintzler
State University of New York
Downstate Medical Center

"It's a beautiful example of mechanisms differing in males and females," says Alan Gintzler, a biochemist and neurobiologist at the State University of New York Downstate Medical Center who was not involved with the work. His own lab has also been documenting sex differences in pain, in particular, in the spinal cord. For example, the group has found that female rats require combined activation of both μ and K opioid receptors in their spinal cords for morphine analgesia, and often express the proteins as a single complex; males, by contrast, require only μ activation and show much lower levels of the heterodimer (*PNAS*, 107:20115-19, 2010).

The hope, Murphy notes, is that one day, with enhanced understanding of the biology of pain in both sexes, researchers might design better drugs to specifically target pathways that induce analgesia. Her group is cur-

ANDRZEJ KRAUZE

rently working on compounds that could effectively block microglia activation, for example. "Ultimately, [these studies] could point the way to developing more-specific and targeted pharmacotherapies," Gintzler says. Such therapies would "target certain pathways, or certain cells, that are more active in males or females—rather than bathing the entire nervous system in a narcotic."

But Gintzler also notes that the backdrop to such work is highly complicated. For one thing, researchers are divided on whether women really do need more morphine than men do—some studies suggest they require less, or about the same amount—clouding the human relevance of the Georgia State team's most recent findings in rats. For another, researchers have proposed alternative explanations for sex differences in human pain processing and alleviation that have no obvious links with the PAG. One famous study, published in the early 2000s, found that after taking into account people's "gender role expectations"—beliefs about whether men should express feeling pain, for example—once-significant sex differences in pain threshold disappeared (*Pain*, 96:335-42, 2002).

Although there are important differences in the way such studies are carried out—in rodents or humans, in models of chronic or acute pain, in studies of pain sensitivity or pain alleviation—this tangle of vying explanations for sex differences in pain research points to a larger confusion in the field, says Jeffrey Mogil, a neuroscientist at McGill University. "You would imagine that [each of these theories] would take a little chunk out of the sex difference and reduce it somewhat," he says. "But that's not what all these studies show. . . . They're all 'complete' explanations."

Of course, it's conceivable, Mogil adds, that multiple factors—spanning the range from molecular biology to culture—act in series, in which case blocking any one factor could substantially reduce sex differences in humans' pain experiences. For now, though, he suggests that the most important finding from studies like Murphy's is simply that qualitative differences exist and warrant further study.

"It doesn't matter what direction the sex differences go in and how they're resolved," Mogil says. "This is a wonky scientific question that scientists will figure out eventually. The bigger picture is: there are sex differences here, and no one would have seen them if they weren't [conducting research with] both sexes."

—Catherine Offord

Metastatic Data

Last October, after a hectic couple of days campaigning for metastatic breast cancer awareness and research funding in Washington, D.C., Lisa Quinn boarded an RV owned by the advocacy group METAvivor for a road trip up the East Coast. The stay-at-home mom from northwest Arkansas was diagnosed with stage IV breast cancer in July 2015 at age 36, and in 2016, she had donated saliva and blood samples, along with her clinical data and information about her disease, to the Metastatic Breast Cancer Project (MBCproject).

Launched by the Broad Institute of MIT and Harvard University in October 2015, the MBCproject aimed to collect patient-donated samples from which to extract molecular and genomic information on metastatic breast cancer, pairing those data with clinical records and patient-reported information. Now, Quinn was going to tour the Broad, where the data were generated and compiled.

"It was very exciting to meet the researchers that started all of this, that are doing everything they can to help us to live longer," says Quinn.

Quinn had learned about the project through Facebook. From the beginning, MBCproject organizers knew that Facebook would be a major driver of recruitment. "In the era of social media, the Internet, online groups, organized patient and organized advocacy groups, we thought there would be an opportunity to engage patients directly," says project director Nikhil Wagle, a medical oncologist at Harvard Medical School and Dana-Farber Cancer Institute and a Broad researcher.

The ultimate goal, he says, is to collate as much data as possible and make it publicly accessible to accelerate research into the disease, which has a median life expectancy of just three years following diagnosis, and to speed the development of treatments. Progress, Wagle adds, depends largely on scientists' ability to capture participants and samples that had previously slipped through the cracks.

"The numbers of samples with clinical annotation that were being used for research seemed much smaller than what should be possible," he says. Only a small fraction of cancer patients are treated at large academic centers that also conduct research, Wagle says—the vast majority goes to community hospitals, where their samples and records sit on a shelf. "No one has ever asked those patients if they'd be willing to contribute their samples and information."

PATRICK QUINN

LYING DOWN FOR A CAUSE: Cancer patients and research advocates, among them Lisa Quinn, stage a "Die In" at the Stage IV Stampede in Washington, D.C., on October 13, 2017.

To Wagle's and his colleagues' delight, the MBCproject collected data from more than 2,000 women and men from across the U.S. in its first seven months. Now that number has doubled, with more than 2,400 of those patients consenting to share their records and tumor samples and more than 1,500 providing saliva samples. The participants come from more than 1,000 different institutions, mostly small community hospitals and treatment centers, Wagle notes. "This is a study that would be completely inefficient, or actually impossible to do, to get them to sign on to a multicenter collaboration," he says. "Because we're going directly to patients, it doesn't matter."

Last October, the project released a preliminary data set, comprising de-identified information from 78 patients. The data include whole exome sequences from 103 tumor samples as well as detailed pathology, diagnostic, and treatment history reports from medical records and patient-reported information on treatment responses and experiences. "Those two types of data—the clinical data and the genomic data from samples—independent databases exist of each of those," Wagle says, "but to my knowledge, there isn't a large database that has both of those pieces of information from the same patients."

The MBCproject team is continuing to work through the thousands of samples it has collected and plans to release a new set of data every six months. "Our hope is that six months from now we're going to double the size of the database, and within the next couple of years, more than 500 patients will be in the database with full genomic, clinical, and pathologic data," Wagle says.

"The commitment of people with metastatic breast cancer to contribute their own health data to enhance progress in research [and] discovery for all who experience this condition is laudable," Joe Selby, executive director of Patient-Centered Outcomes Research Institute, which aims to partner patients with research projects, writes to *The Scientist* in an email. "We are thrilled to see others supporting initiatives that will improve patient-centered outcomes research."

"It's really exciting for us to see things like the MBCproject happening," agrees epidemiologist Carolyn Hutter, acting director for the division of genome sciences at the National Human Genome Research Institute (NHGRI) and former NHGRI team lead for The Cancer Genome Atlas. "The movement toward participant-centered research and participant involvement in the projects . . . has been gaining momentum in recent years . . . not just in cancer, but broadly in biomedical research," she adds.

The commitment of people with metastatic breast cancer to contribute their own health data to enhance progress in research and discovery for all who experience this condition is laudable.

—Joe Selby, Patient-Centered Outcomes Research Institute

"[It's] a recognition of the information and the value that they bring into a project."

Wagle notes that patients' roles in the MBCproject go beyond donating their samples and medical history. Right from the beginning, he says, patients and advocates have helped design and implement the whole thing, from building the project's website to deciding on the names of data categories in the recent release. "It's a real partnership and a project that's truly co-owned by all the patients who participate."

With the MBCproject demonstrating the practicality of this sort of patient-driven data generation, the Broad Institute is now applying the strategy to other cancers: the Angiosarcoma Project launched in March 2017, and the Metastatic Prostate Cancer Project is set to launch soon. And, thanks to additional philanthropic funding following the MBCproject's success, "we anticipate launching many more projects in more cancers hopefully in [2018]," Wagle says.

In the meantime, Wagle and his colleagues also plan to dive into the data they've just released. "If I put my researcher hat on, this data is incredibly exciting to answer important questions into the biology of breast cancer," he says. For example, "Can we figure out features of the tumor that might predict response to certain therapies or resistance to other therapies? If we can do that, that helps us develop diagnostic tests to figure out which patients should get certain therapies."

Now, says Quinn, it's important to spread the word. "It's a great resource that's there, but now it's got to be used," she says. And not just by researchers, but by patients, too, she adds. "If there's a place we can go and see what treatments people have done [and] had successes, that's so important," she says. "I think it's very important that patients go in and look at that information. We have to be our own advocate." —Jef Akst

MOMMY!

When the Voyager I spacecraft left Earth in 1977, it carried with it a "Golden Record" containing audio recordings of messages meant for any intelligent life that might cross its path. It bore sounds from around the world, including greetings in 55 languages, Chuck Berry's "Johnny B. Goode," and a fussy baby being soothed by its mother. According to Marc Bornstein, a developmental psychologist at Eunice Kennedy Shriver National Institute of Child Health and Human Development, Carl Sagan and other members of the committee who decided what to include on the record were spot on in picking the latter track. "Infant cry is . . . the very first communication between an infant and a caregiver," Bornstein says.

Crying is infants' best tool for ensuring they get the care they need, but Bornstein and his research collaborators wondered about the caregivers' responses: to what extent were those innate versus learned? To investigate, they enrolled 684 new mothers and their babies from 11 countries around the world and put cameras in their homes. Each time a baby began crying, the researchers recorded what the mother did in the next five seconds. Did she pick the baby up? Kiss or stroke it? Talk to it? Try to distract it with a toy?

"Within five seconds, the predominant kinds of responses are picking up and holding and talking to the baby," says Bornstein (*PNAS*, 114:E9465-73, 2017). The degree of uniformity surprised him. "People in Kenya and Cameroon . . . the mothers are growing up and have been reared in wildly different circumstances than mothers in Brazil and Argentina or the United States, or certainly than Japan or South Korea."

Bornstein and his colleagues next looked at regions that became more active in the brains of mothers in response to their infants' cries to see whether they matched across cultures, too. They put women in the U.S., Italy, and China into fMRI scanners and imaged their brains as they listened to their babies' cries, and then compared those results with brain scans made when the mothers heard other sounds, such as infant babble or an adult crying.

The results were consistent: hearing her own baby's cry tended to increase activity in a mother's supplementary motor area, which is associated with the intention to move or speak, and in the inferior frontal regions, signaling the intention to speak. "The really interesting aspect is the common brain regions that are sensitive to baby cry across cultures," says Pilyoung Kim, a developmental psychologist at the University of Denver who was not involved in the study but has collaborated with some of its authors. "I thought they did provide strong evidence that there are some common responses to the baby cry, both in behaviors and the brain activation."

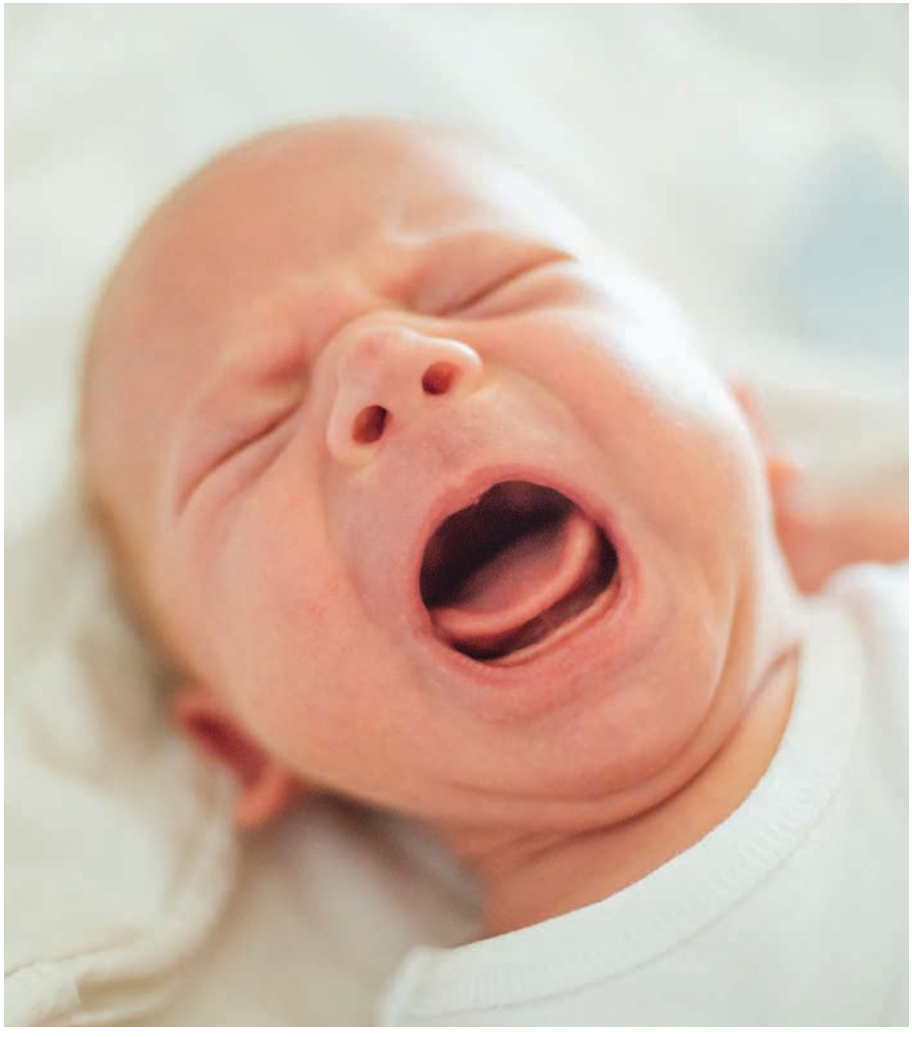
Devanand Manoli, who studies the neural basis of social behavior in prairie voles at the University of California, San Francisco, says that this uniformity extends, to some extent, to our evolutionary kin. While there is a considerable amount of species-specific variation, he says, researchers have turned up reproducible patterns in the behavior of primates, rodents, and even some bird species in response to vocalizations from their offspring.

The behavioral findings reported by Bornstein's team "really speak to the fact that, like in other mammals, there are pretty hard-wired responses" in humans, Manoli says. The specific brain regions that respond to infant cry, versus other types of sounds, also support the idea of hard-wiring, he adds. "The behaviors that are elicited in a more invariant way tap into the parts of the motor system that are closer to behavior and closer to actual action, as opposed to other types of vocal gestures, or vocalizations, that involve parts of planning and parts of interpretation, which is where you would see the cultural variation."

For Bornstein, it's not a surprise that responses to infant cries appear to be largely innate. "In order for the next generation to survive, it's the caregiver's job to respond to [infant] cry and meet the needs of that cry," he says. "The notion is that evolutionarily, these two systems, the infant cry and the caregiver response, have developed together to ensure the survival of the species."

One omission from the study is, of course, dads. Bornstein says he plans to include men in similar studies in the future, building on established differences in male responses to infants. For instance, "if there's an infant cry, women's brains shift in attentiveness faster than men's brains do," he says (*NeuroReport*, 24:142–46, 2013). This pattern dovetails with "a broad tendency across more than 100 societies for babies to be taken care of by either mothers or grandmothers or older sisters relative to fathers or brothers." One question he'd like to explore, Bornstein says, is, "How early does this pattern of brain responses appear in women versus men?"

—Shawna Williams



PICK ME UP! There are remarkable consistencies across cultures in mothers' behaviors and brain activities when responding to their babies' cries.

Child Receives Transgenic Skin

A combination gene-and-cell therapy has given a boy with a grievous skin disease a new lease on life, and resolved a dermatology debate to boot.

BY RUTH WILLIAMS

Thanks to an international team of scientists and doctors, a young Syrian refugee who lost most of his outer skin to a life-threatening genetic disease now has a transgenic replacement, derived from his own cells, covering approximately 80 percent of his body. And, as the team documented November 8 in *Nature*, he's doing well.

"The work provides in-depth, novel information on skin stem cells and demonstrates the great potential of these cells for treating a devastating disorder," says Alessandro Aiuti, a professor of pediatrics at the San Raffaele Scientific Institute in Italy who was not involved in the study.

"[It] establishes a landmark in the field of stem cell therapy," Elaine Fuchs, a skin scientist at the Rockefeller University who also did not participate in the research, writes in an email to *The Scientist*. "In addition, it makes considerable headway in resolving a brewing controversy in the epidermal stem cell field." Specifically, the study has clarified the way skin cells regenerate, which has long been a subject of intense discussion among skin biologists.

Epidermolysis bullosa is a genetic disease in which mutated connective proteins prevent the epidermal layer of the skin from attaching properly to the underlying dermis. The result is skin that readily blisters, causing large, chronic wounds and immense pain to the patient. Sufferers are also at an increased risk of infections and skin cancer; in severe cases the disease can be lethal.

In the summer of 2015, regenerative medicine specialist Michele De Luca of the University of Modena and Reggio Emilia in Italy was contacted by doctors in Germany regarding a young Syrian boy with a very severe form of epidermolysis bullosa. The cause was a mutation in the gene encoding laminin b3, an extracellular matrix protein that controls, among other things, the anchoring of epidermal cells.

NEW SKIN: A boy was dying of a genetic skin disease until researchers transplanted skin grafts that had been genetically modified to correct the mutation.

After arriving in Germany with his family, the boy's condition had significantly deteriorated—De Luca suspected because of the family's distressing relocation, the resulting lack of consistent clinical care, and the family's language difficulties. Suffering a double bacterial infection, the boy had been admitted to the Children's Hospital of Ruhr-University in Bochum where the infections ultimately led to the loss of approximately 80 percent of his epidermis. His chances of survival were so slim that palliative care was considered the only option.

De Luca had previously performed two proof-of-principle transgenic cell therapies on patients with epidermolysis bullosa, both of which had been limited to small patches of replacement epidermis—nothing close to the scale the boy would require. But because of his poor prognosis, his parents gave their consent for expanding the experimental treatment on their son, and the authorities quickly granted approval on compassionate grounds.

A biopsy was taken from what remained of the boy's undamaged epidermis and, from this, keratinocytes were extracted, expanded in culture, and then transduced with a retroviral vector carrying the full-length, healthy version of the laminin b3 coding sequence. The cells, which grow in sheets, were then further expanded until enough sheets

were produced to cover the boy's limbs and torso. They were transplanted in two operations performed in October and November of 2015. A third, smaller operation in January of 2016 filled in most of the remaining gaps.

In the weeks that followed the operations, the transplanted cells proliferated to close the wounds. In the months that followed, skin biopsies from the boy showed that his new skin adhered firmly to the underlying dermis, and had normal morphology and levels of laminin b3. His skin also appeared to have normal elasticity and wound-healing behavior. Now, two years later, the boy's skin still functions as it should without blistering or itching.

Put simply, "it's a beautiful piece of work," stem cell and regenerative medicine researcher Fiona Watt of King's College London writes to *The Scientist* about the team's accomplishment.

Resolving a skin regeneration debate

The boy's experience revealed novel insights into the way keratinocytes regenerate. Each month, human skin is entirely replaced with new cells, but whether this renewal is the result of a large population of equally potent progenitor cells or of a smaller number of individual stem cells that dominate regeneration has been hotly debated.

If the former situation were true, the authors argue, then genomic sequencing of the boy's skin biopsies would have revealed that each contained thousands of different genotypes—the result of random integrations of the viral vector into each cell. As it was, they contained only a few hundred, strongly supporting the latter theory.

"Both theories were valid, but what had been lacking was real proof in vivo, in patients," says Aiuti. "Now we have it."

Can skin cell therapy reduce cancer risk?

The presence of hundreds of genotypes in each biopsy was also a good sign that a variety of cells were present in the boy's transgenic skin and that no particular genotype dominated (a sign of a potentially cancerous growth advantage).

Because of the random integration of retroviral vectors and the possibility of a resulting cancer-causing mutation, there has been concern about their use for gene therapy. But, says De Luca, the cells of patients with epidermolysis bullosa already "are extremely prone to cancer," so by replacing their diseased cells with functional transgenic ones, "we might even decrease the risk." Futhermore, should a cancerous event occur, he says, "this would be easily identified because it is in the skin."

Even with the theoretical chance of cancer, "this is a disease that is so devastating to children that it is worth the risk," says skin biologist Valerie Horsley of Yale University who was not involved with the study.

To date, there have been no indications of cancerous events in the now nine-year-old patient, nor in the two patients who previously received the smaller-scale therapies—in one case, "even after 12 years," says De Luca.

The boy will receive regular checkups to assess for any problems, but for now, "he's back to school, he's exercising, he's started to play soccer . . . it's quite amazing," De Luca says. (T. Hirsch et al., "Regeneration of the entire human epidermis using transgenic stem cells," *Nature*, doi:10.1038/nature24487, 2017.) ■

A version of this story was published at the-scientist.com on November 8, 2017.

He's back to school, he's exercising, he's started to play soccer . . . it's quite amazing.

—Michele De Luca, University of Modena and Reggio Emilia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Economical Ethomics

Low-cost, versatile ethoscopes lower barriers to high-throughput studies of fly behavior.

BY RUTH WILLIAMS

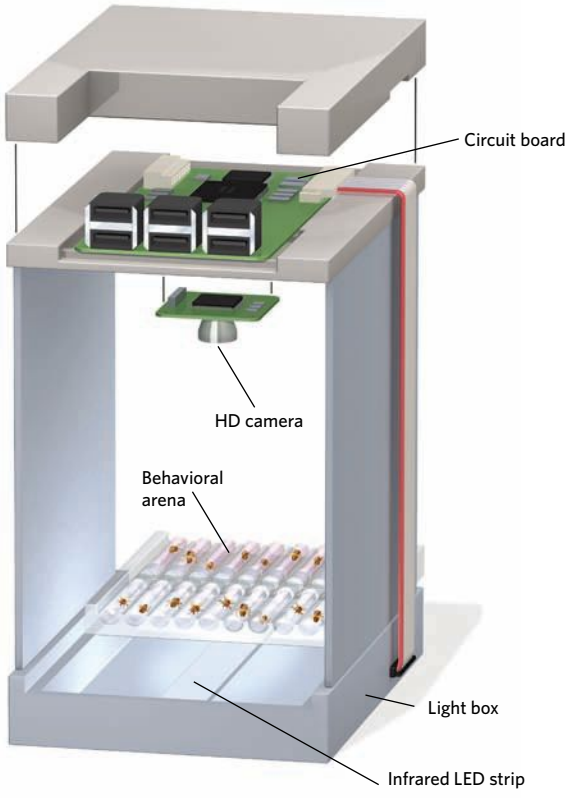
Understanding how the brain controls behavior is a holy grail for many neuroscientists. Model organisms such as the fruit fly offer a wide array of genetic tools for investigating behavioral questions, but for scientists wishing to do such experiments on a large scale—a discipline called ethomics—there are few technological options.

Drosophila activity monitoring (DAM) systems (from TriKinetics in Massachusetts)—which use one or more infrared light beams to detect the movements of flies in assay chambers—are among the most commonly used ethomic devices. But they lack experimental versatility, and, because the only data they provide are the number of times a fly breaks a light beam, behavioral information is limited.

Systems that use an HD camera mounted above a fly-containing chamber, on the other hand, provide detailed behavioral data, but have typically been costly, says Gerry Rubin of the Howard Hughes Medical Institute's Janelia Research Campus in Virginia. So using a large number of such devices, as would be necessary for ethomic studies, would be prohibitively expensive.

Taking advantage of recent cost reductions in computer parts, HD cameras, and 3-D printing, Giorgio Gilestro of Imperial College London and colleagues have created affordable and adaptable ethoscopes. They're so cheap, in fact, that unlike other camera-based systems, they make running many devices at once eminently doable.

Each ethoscope consists of a single circuit board linked to a small HD camera, supported in a 3-D–printed housing above one of eight possible 3-D–printed behavior arenas. The arenas are designed for different behavioral assays, and three optional modules—enabling light stimulation, chamber agitation, or gas infusion—increase the system's versatility further. For example, Gilestro's team employs the agitation module to arouse individual insects that have fallen asleep during studies of sleep deprivation in flies.



PRINT AND BUILD: The basic setup of the Gilestro-designed, research-grade ethoscope consists primarily of a circuit board, a camera, an infrared LED light strip, and the 3-D–printed housing components. The housing is assembled with regular hardware-store nuts and bolts and the device is powered via a USB cable. Between 1 and 20 flies can be placed in any of eight possible 3-D–printed behavior arenas, which slide into place above the housing's light box.

"Collecting all these different assays and integrating them into an apparatus that you can make by 3-D printing . . . has made the cost of entry [for behavioral scientists] much lower," says Rubin, who was not involved with the project. "It's an important contribution to the field."

The 3-D printing plans, parts list, and software are all free to download at gilestrolab.github.io/ethoscope/. The team even provides plans for Lego or cardboard ethoscopes for educational purposes, but recommends the 3-D–printed version for research. (*PLOS Biol*, 15: e2003026, 2017) ■

AT A GLANCE

ETHOMIC DEVICE	HOW IT WORKS	PRICE	NUMBER OF FLIES ASSAYED PER DEVICE	NUMBER OF DEVICES THAT CAN BE USED SIMULTANEOUSLY
DAMS (TriKinetics)	Fly movement is detected when an insect disturbs an infrared beam.	Approximately $500 to $800	32—each fly in an individual glass tube	Limited mainly by space
Ethoscope	An HD camera continuously monitors fly behavior. Using the additional modules, certain fly behaviors (e.g., sleep) can be detected by the computer and used to trigger a computer-controlled stimulus (e.g., shaking that fly's chamber).	Less than $100	Up to 20—either in individual tubes, individual decision-making mazes, or together in social arenas	Limited by space within a temperature-controlled environment (multiple ethoscopes generate considerable heat and thus require cooling)

CHANNELING THE PAIN

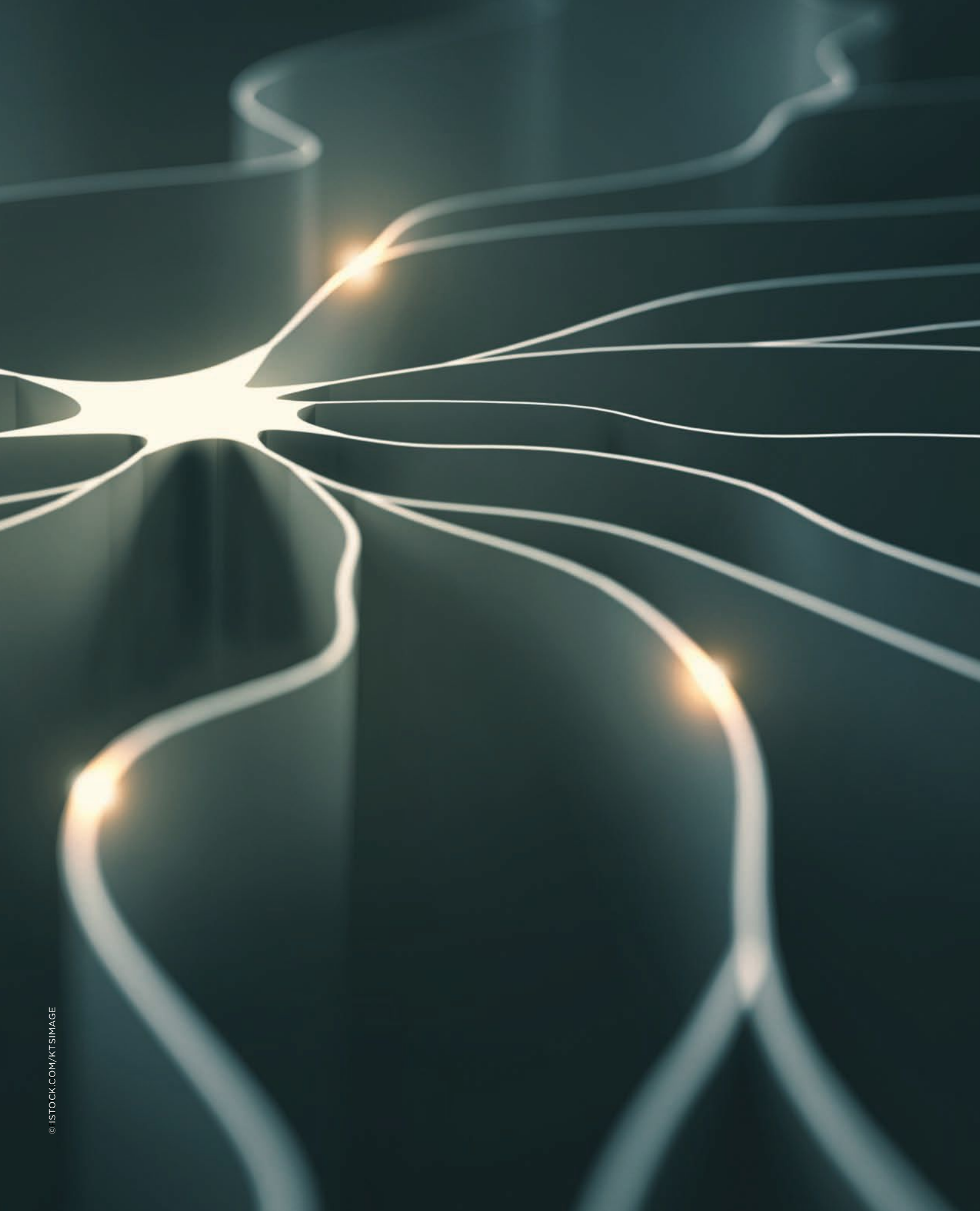
The race to develop analgesic drugs that inhibit sodium channel $Na_V1.7$ is revealing a complex sensory role for the protein.

BY CATHERINE OFFORD

Neurobiologist John Wood has long been interested in how animals feel pain. His research at University College London (UCL) typically involved knocking out various ion channels important in sensory neuronal function from mouse models and observing the effects. But in the mid-2000s, a peculiar story about a boy in Pakistan opened up a new, and particularly human-centric, research path.

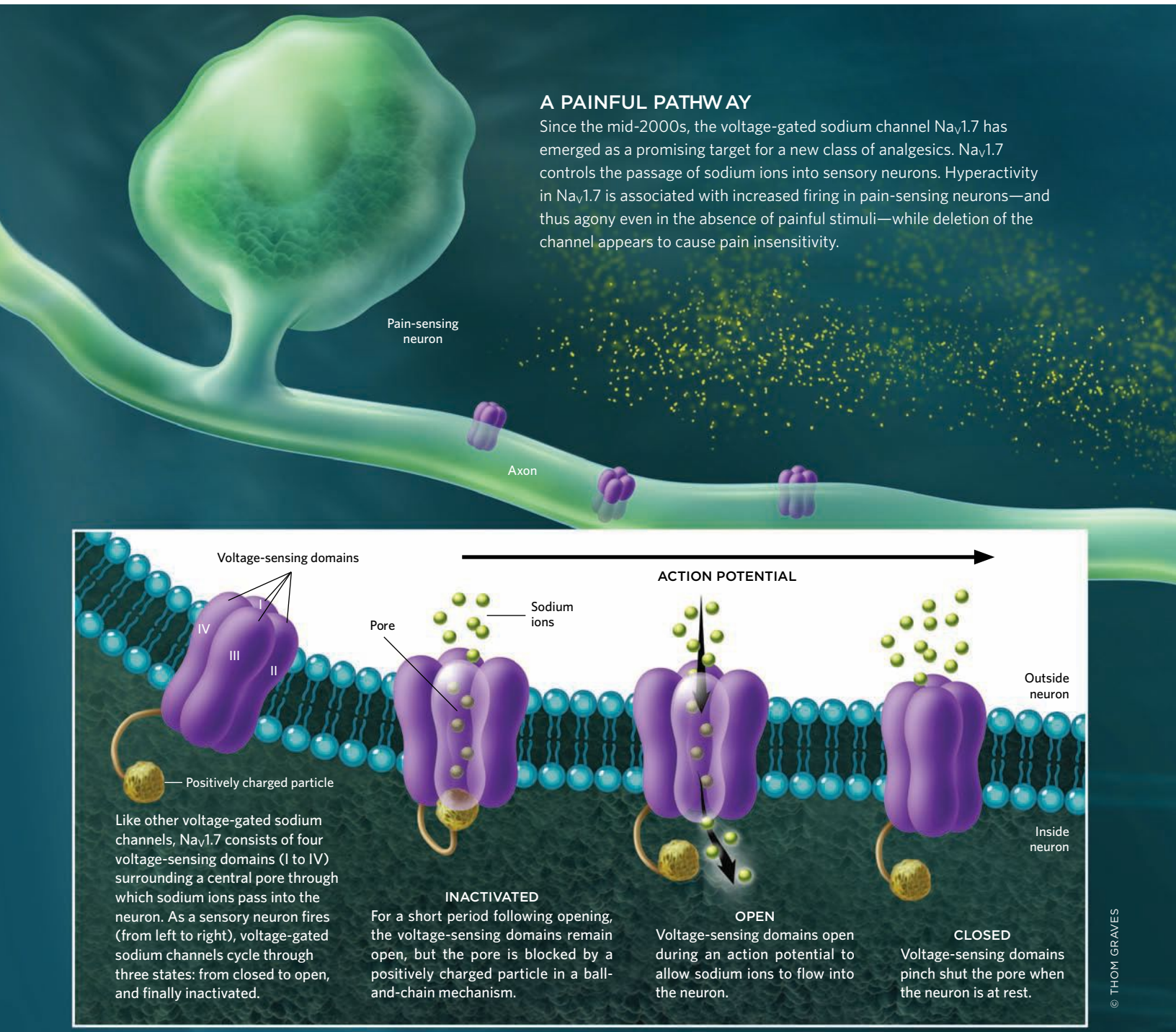
The story was relayed by Geoff Woods, a University of Cambridge geneticist. "Geoff had been wandering round Pakistan looking for consanguineous families that had genes contributing to microcephaly," Wood recalls. During his time there, "somebody came to see him and said that there was a child in the marketplace who was damaging himself for the tourists—and was apparently pain-free." The boy would regularly stick knives through his arms and walk across burning coals, the stories went.

Wood's group at UCL had just published a paper describing a similarly pain-insensitive phenotype in mice genetically engineered to lack the voltage-gated sodium channel $Na_V1.7$ in pain-sensing neurons, or nociceptors. $Na_V1.7$ controls the passage of sodium ions into the cell—a key step in membrane depolarization and, therefore, a neuron's capacity to propa-



gate an action potential. Wood's postdoc, Mohammed Nassar, had shown that mice lacking functional $Na_V1.7$ in their nociceptors exhibited higher-than-normal pain thresholds; they were slower to withdraw a paw from painful stimuli and spent less time licking or biting it after being hurt.[1] Having read the study, Cambridge's Woods reached out to the group in London to discuss whether this same channel could help explain the bizarre behavior of the boy he'd heard about in Pakistan.

The two labs decided to collaborate to learn more about the human phenotype, now known as congenital insensitivity to pain (CIP). Although the boy from the marketplace had died before researchers could study him—he'd sustained fatal head injuries jumping down from the roof of a building on his 14th birthday—Woods located three other Pakistani families with members who displayed the pain-free phenotype. Using a genome-

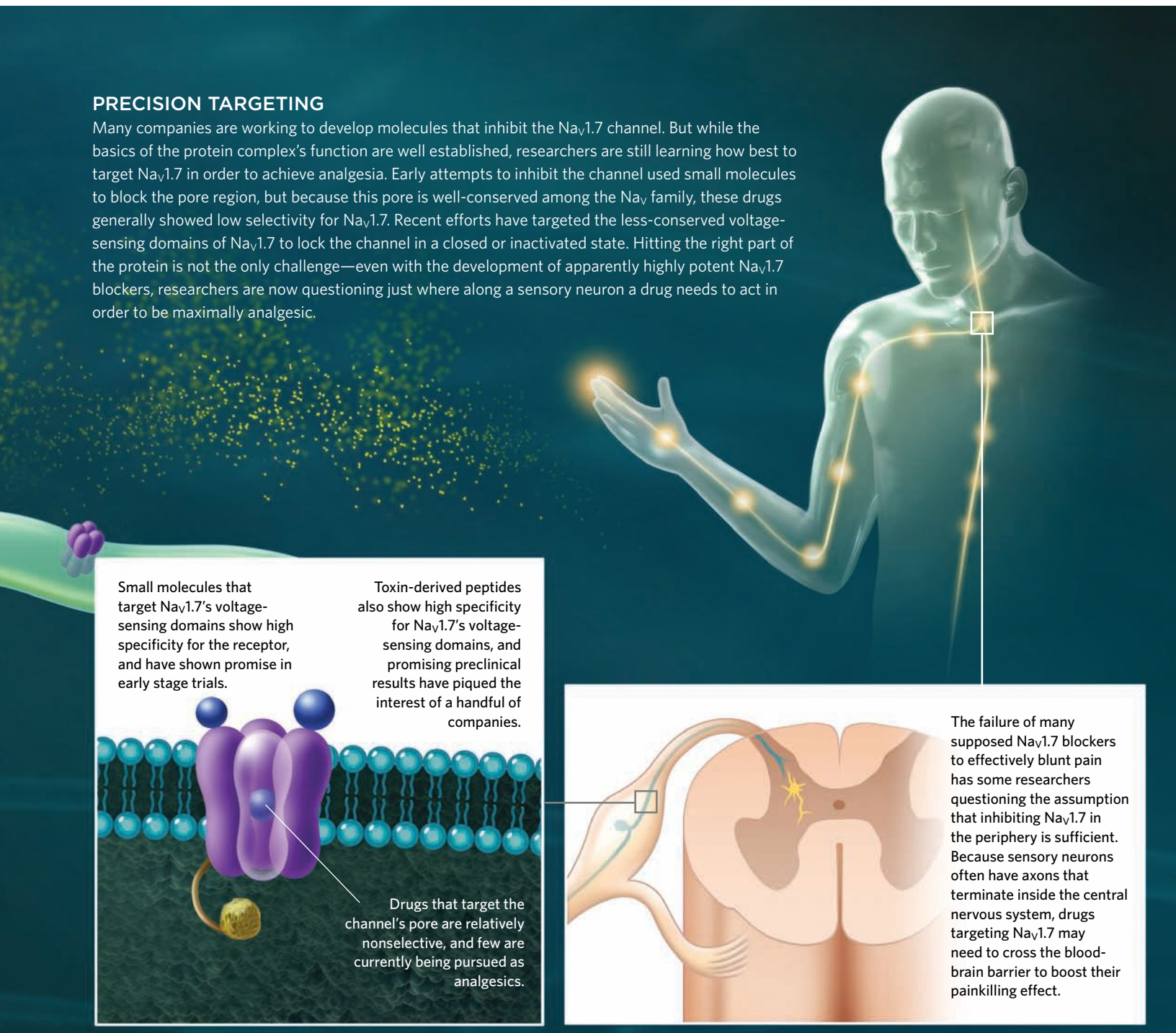


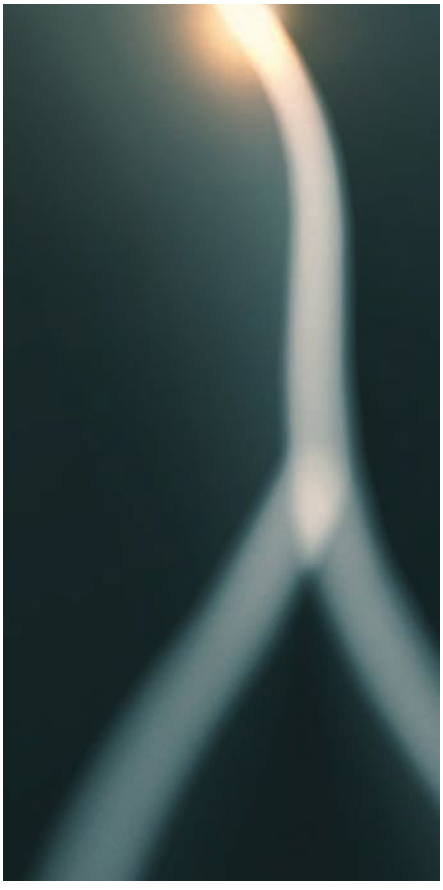
wide scan, a team led by Cambridge postdoc James Cox identified mutations in all three families within a region of *SCN9A*, the gene that codes for $Na_V1.7$. The findings, published in 2006,[2] suggested that "$Na_V1.7$ is absolutely required for humans to feel most sorts of pain," Wood says. "That was a bit of a breakthrough."

The gene itself was not unfamiliar to pain researchers, however; previously, it had been implicated in a different human pain syndrome. In 2004, Chinese researchers linked specific gain-of-function mutations in *SCN9A* to inherited erythromelalgia (IEM)—a condition with symptoms at the opposite end of the spectrum from those of CIP.[3] Patients with IEM, also known as "man on fire" syndrome, "feel searing, excruciating, scalding pain in response to mild warmth," says Stephen Waxman, a neurologist at Yale School of Medicine and the Veterans Affairs Hospital in Connecticut. Triggers include "putting

PRECISION TARGETING

Many companies are working to develop molecules that inhibit the $Na_V1.7$ channel. But while the basics of the protein complex's function are well established, researchers are still learning how best to target $Na_V1.7$ in order to achieve analgesia. Early attempts to inhibit the channel used small molecules to block the pore region, but because this pore is well-conserved among the Na_V family, these drugs generally showed low selectivity for $Na_V1.7$. Recent efforts have targeted the less-conserved voltage-sensing domains of $Na_V1.7$ to lock the channel in a closed or inactivated state. Hitting the right part of the protein is not the only challenge—even with the development of apparently highly potent $Na_V1.7$ blockers, researchers are now questioning just where along a sensory neuron a drug needs to act in order to be maximally analgesic.





It's more complex than the human genetic studies would have suggested.

—Glenn King, University of Queensland

on a sweater, wearing shoes, going into a room at 68 degrees Fahrenheit." Later that year, Waxman's group showed why: $Na_V1.7$ in people with IEM is unusually active, and makes pain-signaling neurons respond to even mild stimuli.[4] "Initially, we found the gain-of-function mutations, which cause excruciating pain, and two years later, the loss-of-function mutations were found" by the UK team, says Waxman. "It's unusual to be dealt a hand that complete."

In addition to providing explanations for two specific pain syndromes in humans, the results attracted the attention of researchers working on treatments for a much broader range of conditions associated with neuropathic pain. A chronic pain state associated with nerve fibers rendered dysfunctional by injury or disease, neuropathic pain is virtually untreatable—even powerful analgesics such as opioids have mixed success in pain management, not to mention a tendency to induce dependence. "The existing drugs either don't work, work only partially, or have unacceptable side effects," says Waxman. "There's a desperate need for better medications."

With the work on CIP and IEM providing a clearer picture of the sodium channel's function, researchers hoped to create improved pain medications by designing $Na_V1.7$ blockers to produce complete analgesia in patients. Scientists also figured that $Na_V1.7$'s almost exclusive presence in peripheral neurons—a property shared by only two other voltage-gated sodium channels in humans, $Na_V1.8$ and $Na_V1.9$—would allow compounds targeting the protein to steer clear of the central nervous system, and thus avoid dependence and other side effects common to opioids. (See "A Safer Poppy" on page 61.)

But the last 10 years have not been smooth sailing for $Na_V1.7$ drug development. A wave of early attempts from the pharmaceutical industry to inhibit the channel were unsuccessful, in part because it has been difficult to design molecules that can block just $Na_V1.7$ and not closely related ion channels that play critical roles outside pain sensing. Moreover, there's a growing appreciation that there's more to the protein than meets the eye. "In principle, it may be a good target," says geneticist Ingo Kurth, who directs RWTH Aachen University's Institute for Human Genetics in Germany. "However, from what we have seen in recent years, [exploiting] it seems to be really complex and difficult."

Closing the gate

Like the other eight proteins in the voltage-gated sodium channel family, $Na_V1.7$ is made up of four voltage-sensing transmembrane domains surrounding a central pore through which sodium ions pass into the neuron. Blocking that pore with a small-molecule drug has been a reliable route to analgesia for well over a century. "We've had sodium channel blockers for donkey's years," says Irina Vetter, deputy director of the Centre for Pain Research at the University of Queensland in Australia. The "prototypical sodium channel blocker," she adds, is cocaine, isolated in 1855 from the leaves of the coca plant—for centuries chewed for their stimulant properties by native South Americans. The compound is still used as a local anesthetic for purposes such as orofacial surgery.

But there's a problem with this sort of drug when it comes to broader applications. The ion-conducting pore targeted by many sodium channel blockers, including several currently in clinical studies, "is extremely well-conserved" across the Na_V protein family, Vetter explains. "That particular part of the channel is almost identical between all the different subtypes, so it's very difficult to find drugs that selectively block one or the other."

This lack of specificity is an obstacle for researchers trying to design therapeutics to systemically treat neuropathic pain, because other sodium channel family members are important for diverse physiological functions. For example, "if you inhibit $Na_V1.5$ in cardiac tissue, you'll end up with a sort of arrhythmia, or worse," says Les Miranda, executive director of research in therapeutic discovery for

Amgen. "If you inhibit [$Na_V1.4$] in muscle tissue, you'll end up with partial paralysis. So clearly, if you're interested in 1.7, you've got to make sure you've got a molecule that does not touch 1.5 or 1.4 or some of the other '1.X' family members."

With this selectivity requirement in mind, many groups have started investigating molecules that target not the channel's pore, but the outer, voltage-sensing domains, which tend to be less conserved between Na_V subtypes. Some small molecules such as aryl sulfonamides, for example, inhibit the domain IV voltage sensor on $Na_V1.7$, and thus prevent the channel from opening in response to changes in voltage. Researchers from Xenon Pharmaceuticals and Genentech recently showed that some members of this class of compounds had good specificity for $Na_V1.7$ over cardiac $Na_V1.5$ and produced analgesia in mouse models of acute and inflammatory pain—although they show poorer specificity for their target over two channels present predominantly in the brain, $Na_V1.2$ and $Na_V1.6$.[5]

Waxman's group, in collaboration with Pfizer, showed in 2016 that a synthetic aryl sulfonamide dubbed PF-05089771 could reduce neuronal hyperactivity in a "pain-in-a-dish" model—sensory neurons grown from induced pluripotent stem cells derived from patients with IEM mutations. The drug was also well-tolerated as a single oral dose in a randomized, double-blind trial of five IEM patients, and temporarily reduced the magnitude and duration of pain attacks in most participants—although the authors noted that there was a high degree of variability in responses among patients.[6]

There's also growing interest in non-small molecules as potential $Na_V1.7$ blockers. In 2014, a group at Duke University Medical Center published a claim that monoclonal antibodies could be designed to selectively target $Na_V1.7$, and provide analgesia in mice.[7] However, the results have not yet been replicated, and for the most part, the approach has not bred much success, notes Miranda. "We have struggled to find antibodies that bind, let alone inhibit, ion channels," he says.

More-promising results have come from experiments with peptides—in particular, ion-channel modulators identified by screening toxins from venomous arthropods. (See "Animal Analgesics" on page 42.) As relatively large molecules, many toxin peptides naturally target "not the pore of the channel, to block ion flow, but the mechanism by which the channel is actually activated," explains University of Queensland biochemist Glenn King, who has worked on venom-derived ion-channel blockers with Vetter, Waxman, and Wood. Like aryl sulfonamides, certain tarantula toxins selectively bind to one of $Na_V1.7$'s four voltage-sensing domains, and can lock the channel in a closed or inactivated state by making it voltage-insensitive.

Several industry groups, including Miranda's team at Amgen, are developing engineered versions of peptides that can capture the selectivity of these toxins and produce pain insensitivity in animal models. One of Janssen Pharmaceuticals's latest drug candidates—a tarantula-inspired synthetic peptide—binds to and inhibits voltage-sensing regions of $Na_V1.7$, keeping the channel from opening regardless of changes in voltage. A study published last year demonstrated that the peptide almost completely suppressed pain behaviors in rats.[8] Microproteins designed by Pfizer and based on another tarantula peptide, meanwhile, show an 80-fold and 20-fold selectivity for $Na_V1.7$ over $Na_V1.2$ and $Na_V1.6$, respectively.[9]

As a result of these efforts, the problem of drug specificity is well on the way

to being solved, says William Catterall, a pharmacologist at the University of Washington in Seattle. "A number of companies have succeeded in making compounds that are surprisingly specific for $Na_V1.7$," he says, noting that such molecules are also invaluable tools in the study of sodium channel function itself. "It's a very difficult task, and it's a great credit to the pharma companies to be able to do that."

Back to basics

Given the broad array of molecules in development and the trend toward ever more-selective $Na_V1.7$ inhibitors, the lack of clinical success from the field over the past decade has surprised and dismayed many researchers. "People have been working on this since 2006," says King. "We still don't have anything in the clinic, and we still don't have clear answers as to why these molecules sometimes work and sometimes don't work. . . . It's proven to be a tremendously more difficult task than everyone appreciated."

Certainly, some of the delay comes from the sheer awkwardness of working with large, membrane-bound proteins such as $Na_V1.7$. The first high-resolution description of a voltage-gated sodium channel's structure was a breakthrough made only in 2011 by Catterall and his colleagues—and that was in a bacterium.[10] "Just getting your hands on the protein is challenging," says Amgen's Miranda. "It's very difficult for us to isolate; it's difficult for us to get cells to express it."

And while Waxman's group has made headway using human cells for drug screening, most groups rely heavily on mouse and rat models, which often pose problems at the drug validation stage due to behavioral and physiological differences in pain sensing between rodents and people. From a structural perspective, human $Na_V1.7$ may be more different from rodent $Na_V1.7$ than it is from other human sodium channels, Catterall says. As a result, "when you home in very specifically on differences between sodium channels [in one animal], you end up with molecules that are so specific they're not very good at inhibiting the channel [in a different animal]."

But there's more at play than just the issue of translating animal research into humans: even molecules considered to be highly selective for human $Na_V1.7$ have produced effects that don't come close to the phenotype of the boy at the Pakistani marketplace. "The human genetic data says that if you inhibit that channel, you should be able to block all types of pain," says King. "But we now have very good inhibitors of the channel, and that's certainly not true. It's more complex than the human genetic studies would have suggested."

Research in the last few years has revealed glimpses of that complexity. For starters, there's a growing appreciation that the channel may play a role in sensory pathways that ostensibly have nothing to do with pain. An early mystery in Wood's lab at UCL, for example, was that mice lacking $Na_V1.7$ from all the cells in their bodies—not just the pain-sensing neurons—died shortly after birth. $Na_V1.7$ knockout humans, by contrast, have no obvious phenotypic abnormalities except, of course, CIP. It wasn't until a few years ago that researchers discovered that $Na_V1.7$ is also present in olfactory neurons, and its knockout causes anosmia—a mild defect in humans but a life-threatening one for lab mice, which rely on smell to find food and potential mates.[1]

ALTERNATIVE TARGETS?

$Na_V1.7$ isn't the only voltage-gated sodium channel being investigated for novel pain treatments. Channels $Na_V1.8$ and $Na_V1.9$ are also predominantly expressed in the peripheral nervous system and have been associated with pain syndromes of their own.

In 2012, an international team of researchers identified two gain-of-function mutations in *SCN10A*—the gene coding for $Na_V1.8$—that altered the channels' activity in a way that rendered sensory neurons hyperexcitable, leading to painful neuropathy (*PNAS*, 109:19444-49). And a couple of years ago, researchers at Pfizer described a $Na_V1.8$-blocking compound that reduced neuronal excitability in human neurons in vitro and apparently produced analgesia in rodent models of inflammatory and neuropathic pain (*Br J Pharmacol*, 172:2654-70, 2015). Scientists have not yet found any loss-of-function mutations leading to a phenotype analogous to pain insensitivity in people with certain mutations in *SCN9A*, the gene coding for $Na_V1.7$.

$Na_V1.9$, by contrast, has been associated with both hypersensitivity and insensitivity to painful stimuli. Like its relatives, $Na_V1.9$ can be rendered hyperactive by gain-of-function mutations in its gene, *SCN11A*. A few years ago, Ingo Kurth at RWTH Aachen University in Germany described a novel mutation in this gene that causes pain insensitivity. Counterintuitively, this particular point mutation causes hyperactivity in $Na_V1.9$ channels; instead of leading to increased pain signaling, the aberrant channel activity means neuronal membranes are consistently depolarized. As a result, cells are unable to generate normal action potentials or communicate properly with other neurons (*Nat Genet*, 45:1399-404, 2013).

Unfortunately, this state of pain-suppressing hyperactivity is likely to be even harder to recreate than $Na_V1.7$-linked insensitivity to pain. For $Na_V1.9$, "the [molecular] mechanisms are very similar between the pain insensitivity phenotype and [the phenotype associated with] more pain," Kurth explains. "It'd be quite difficult to find a drug and concentration to produce a phenotype for pain loss." For now, $Na_V1.7$ remains the leading target among voltage-gated sodium channels for the development of novel analgesics.

In principle, it may be a good target. However, from what we have seen in recent years, exploiting it seems to be really complex and difficult.

—Ingo Kurth, RWTH Aachen University's Institute for Human Genetics

The distribution of the protein throughout the body is also a subject of uncertainty. Although $Na_V1.7$'s predominant presence in peripheral neurons was initially highlighted as a therapeutic advantage, "if you look at the sensory neurons that convey information about tissue damage, their terminals are actually inside the blood-brain barrier, in the central nervous system," says Wood. "We think there's quite a lot of action of $Na_V1.7$ at these central terminals, and it may be that drugs have to get there to be useful." Companies such as Amgen are trying to figure out what effect central nervous system delivery might have on $Na_V1.7$ blockers' analgesic potential, Miranda notes. (See "Getting Drugs Past the Blood-Brain Barrier," *The Scientist*, November 2017.)

More complexity comes from the recent suggestion that $Na_V1.7$'s effects on sensory neurons may go far beyond controlling the passage of sodium ions. While working with knockout mice a few years ago, Wood says, his group made a surprising discovery that implies a role for the channel in regulating transcription. "We found that in the $Na_V1.7$ knockout, opioid peptides—the enkephalins—are upregulated," he says. The group hypothesized that the CIP phenotype in patients lacking functional $Na_V1.7$ from birth might therefore come not only from the lack of sodium channel activity, but also from a boost in endogenous opioid signaling—something that analgesic drugs would have to reproduce to be successful.[12]

To test this theory, Wood and his colleagues administered an opioid blocker to a woman with CIP. "We treated her with naloxone, and she could begin to detect unpleasant stimuli" for the first time, says Wood. "She was thrilled."

More recently, in collaboration with Vetter, King, and Waxman, Wood found that administering a highly selective $Na_V1.7$-blocking spider toxin called Pn3a—which is not by itself analgesic—alongside subtherapeutic doses of opioids produced profound analgesia in mouse models of inflammatory pain, suggesting that a combinatorial drug approach might finally recapture the pain insensitivity researchers are pursuing.[13] "We're very, very keen to carry out proof-of-concept studies in healthy humans," Wood says.

The opioid signaling hypothesis is far from being widely accepted in the field at this point, although several researchers who spoke to *The Scientist* suggested it may well turn out to be correct. Nevertheless, the concept reflects a growing appreciation of the nuance in the $Na_V1.7$ story. Even Cambridge's Geoff Woods, who identified those early extreme phenotypes in $Na_V1.7$-null humans, recently published a case report describing a woman born with CIP who suddenly began reporting pain symptoms after giving birth to her child. "Her case strongly suggests that at least some of the symptoms of neuropathic pain can persist despite the absence of the $Na_V1.7$ channel," the authors write.[14]

King and other researchers, meanwhile, are investigating the possibility that blocking $Na_V1.7$ in combination with at least one other voltage-gated sodium channel is a more effective route to analgesia than targeting $Na_V1.7$ alone. "I think until we really fully understand what's going on, it's going to be really hard to develop molecules that work as well as we might have hoped," King says.

Still, with the attention that the last decade of research has brought to pain-linked sodium channels such as $Na_V1.7$, many researchers are cautiously optimistic that understanding the protein's biology and developing effective molecules against it are achievable goals. "That conviction really drives innovation in this area," Miranda says. "I think given our learning curve around the engineering of molecules, and what we've learned about how to handle and characterize $Na_V1.7$, we are going to be making—collectively, across the industry—progress against $Na_V1.7$ in the near future." ■

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IT TAKES A VILLAGE: Glia (cyan) in the central nervous system are normally considered support for neurons, but research is revealing how these cells can contribute to the aberrant firing of pain pathways. (Rat hippocampus shown here. Neurofilaments in green; DNA in yellow.)

TOM DEERINCK, NATIONAL CENTER FOR MICROSCOPY AND IMAGING RESEARCH

GLIA AND PAIN

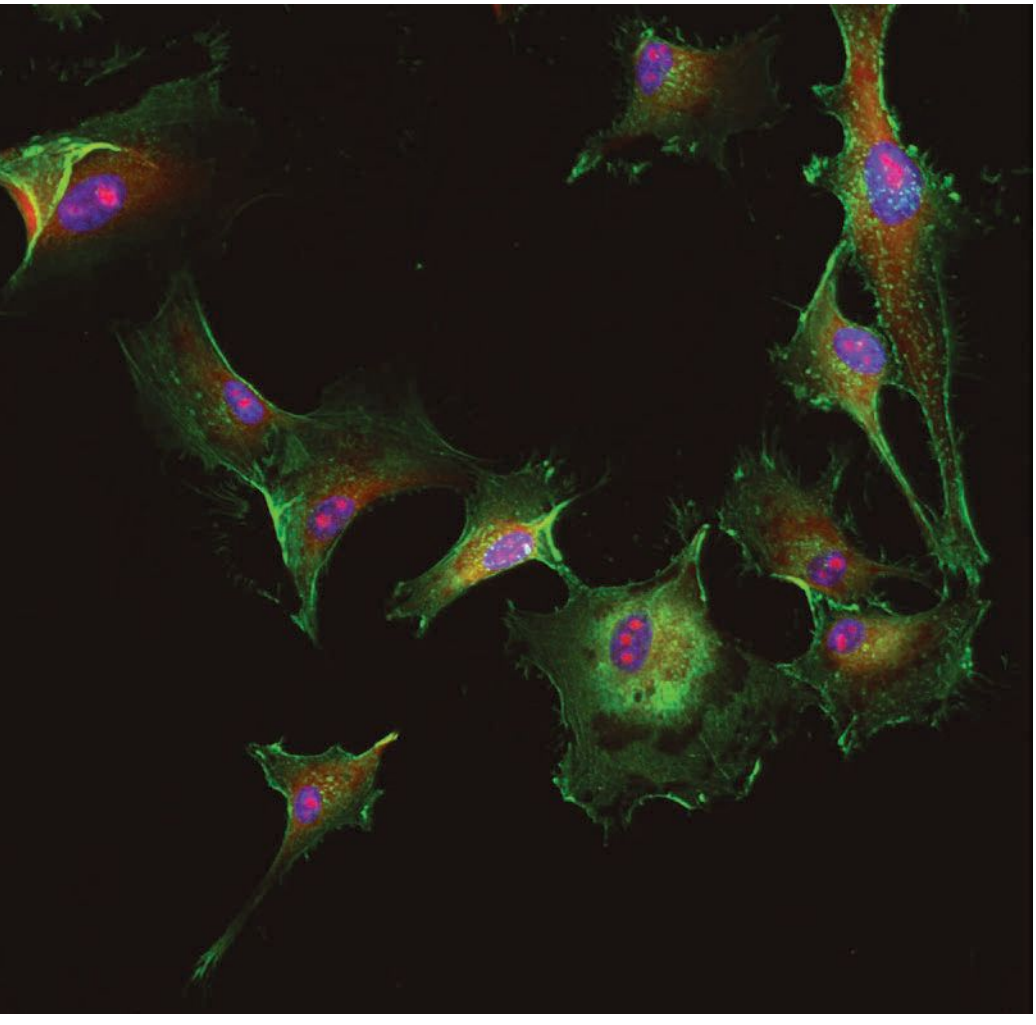
Immune-like cells in the central nervous system are now recognized as key participants in the creation and maintenance of persistent pain.

BY MARK R. HUTCHINSON

When someone is asked to think about pain, he or she will typically envision a graphic wound or a limb bent at an unnatural angle. However, chronic pain, more technically known as persistent pain, is a different beast altogether. In fact, some would say that the only thing that acute and persistent pain have in common is the word "pain." The biological mechanisms that create and sustain the two conditions are very different.

Pain is typically thought of as the behavioral and emotional results of the transmission of a neuronal signal, and indeed, acute pain, or nociception, results from the activation of peripheral neurons and the transmission of this signal along a connected series of so-called somatosensory neurons up the spinal cord and into the brain. But persistent pain, which is characterized by the overactivation of such pain pathways to cause chronic burning, deep aching, and skin-crawling and electric shock–like sensations, commonly involves another cell type altogether: glia.[1]

Long considered to be little more than cellular glue holding the brain together, glia, which outnumber neurons 10 to 1, are now appreciated as critical contributors to the health of the central nervous system, with recognized roles in the formation of synapses, neuronal plasticity, and protection against neurodegeneration. And over the past 15 to 20 years, pain researchers have also begun to appreciate the importance of these cells. Research has demonstrated that glia seem to respond and adapt to the cumulative danger signals that can result from disparate kinds



COURTESY OF CNBP RESEARCH FELLOW LINDSAY PARKER, MACQUARIE UNIVERSITY

COSTARS: Astrocytes, such as these human cells growing in culture, are but one of an array of glial cells, which greatly outnumber neurons.

of injury and illness, and that they appear to prime neural pathways for the overactivation that causes persistent pain. In fact, glial biology may hold important clues to some of the mysteries that have perplexed the pain research field, such as why the prevalence of persistent pain differs between the sexes and why some analgesic medications fail to work.

Importantly, these insights are not just going from the bench to the bookshelf. Rather, large pharmaceutical companies have taken an interest in translating new glia-targeting therapies to the clinic to treat persistent pain, a malady that costs society more than cancer, heart disease, and diabetes combined. A wealth of preclinical evidence supports this translational potential. Every relevant animal and cell model of persistent pain tested to date shows histological and molecular signs of changed glial activity or pharmacological sensitivity to drugs that target these cells. With several new chemical entities and drugs being repurposed to target glia for pain, and with continued laboratory work interrogating the mechanisms underlying glia's involvement in persistent pain processing, the field is poised to make strides in both understanding and treating this enigmatic ailment.

The neuroimmune brain

Until very recently, the brain and spinal cord were thought to be shielded from the body's immune system. But evidence to the contrary has been accumulating for years. And recently, researchers discovered the central nervous system's lymphatic system, which traffics thousands of peripheral immune cells into and out of the healthy brain.[2] (See "Immune System Maintains Brain Health," *The Scientist,* November 2016.) This recognition of the immunocompetence of the central nervous system validates a long-appreciated idea—that in some persistent pain states, peripheral immune cells contribute to the function of somatosensory synapses in the brain and spinal cord. In addition, although they are not themselves considered classical immune cells, glia—which comprise a range of phenotypically different cell types, including astrocytes, microglia, and oligodendrocytes—perform a role similar to that of the peripheral immune system, and can also contribute to exaggerated pain responses.

While synapses were once thought to involve just two participants—the pre- and postsynaptic neuronal terminals—researchers now recognize that upward of 90 percent of neural connections include

one, two, and sometimes even three additional types of cellular players.

In persistent pain, if glial function is modified in and around synapses, the transmission of nociceptive signals can be augmented in a way that will result in exaggerated pain responses. For example, projections from astrocytes known as endfeet closely monitor synaptic activity for changes in neuronal firing. When the glial cells detect an increase in the extracellular concentrations of neurotransmitters, they begin to take up greater amounts of the molecules in an attempt to bring the hyperactive synapses under control. Under states of persistent pain, however, there is a significant downregulation of the molecular transporters on astrocytes that are responsible for maintaining excitatory neurotransmitter homeostasis, resulting in less removal of excess excitatory neurotransmitters.

Microglia, meanwhile, survey the synaptic space for local and distant paracrine signals such as cytokines, chemokines, and trophic factors that drive neuronal adaptations at the level of the synapse to continue to refine their likelihood of firing.[3] Some glial cells also release their own proinflammatory cytokines and other mediators, such as reactive oxygen and nitrogen species. Along with additional proinflammatory factors from peripheral immune cells, these compounds can prime the synapse for heightened neuronal firing by increasing the release of excitatory neurotransmitters from neurons.

In addition, glial cytokines and chemokines are known to drive increased production of neuronal receptors that the neurotransmitters bind to on the postsynaptic terminal, as well as the modification of receptor subunits, to promote a state of enhanced neuroexcitability and, therefore, pain sensitivity. And if all that weren't bad enough, these glial interactions also cause a loss of inhibitory control measures in somatosensory neuronal networks, further heightening and spreading nociceptive signal transmission.

It is abundantly clear that glia can enhance the firing of neurons in pain-sensing pathways to promote exaggerated responses. But how important to persistent pain are misbehaving glia? In 2016, Linda Watkins and Peter Grace of the University of Colorado Boulder and their colleagues answered this question, using a new technology known as designer receptors exclusively activated by designer drugs (DREADDs). Watkins, Grace, and their colleagues constructed an exclusively microglia-targeting viral vector that would introduce into rats an engineered mutant form of a G protein–coupled receptor that can only be activated by the DREADD-selective ligand clozapine-N-oxide (CNO). Injecting CNO, the researchers observed the activation of microglial proinflammatory responses and surmised that this response was sufficient to elicit heightened pain in the animals, even in the absence of neuronal injury.[4]

Glial biology may hold important clues to some of the mysteries that have perplexed the pain research field.

Hence, glia are critical to the exaggeration of pain signals that results from aberrant neuronal firing—but these immune-like cells appear capable of triggering persistent pain symptoms on their own, at least in animal models. And recent neuroimaging studies by Harvard University's Marco Loggia and colleagues provide the first evidence that the extent of glial reactivity may be related to the severity of persistent pain in humans. The researchers employed integrated positron emission tomography–magnetic resonance imaging and a recently developed radioligand that binds to the glial translocator protein (TSPO), an anti-inflammatory molecule whose upregulation is thought to be triggered by periods of heightened glial activity to control local inflammation and reduce pain. Indeed, the team found in patients with chronic lower back pain that increased TSPO levels in the thalamus, a key higher brain region in the somatosensory pathway, negatively correlated with clinical pain scores as well as with circulating levels of the proinflammatory cytokine interleukin-6.[5] These data indirectly support the role of glia signaling in persistent pain, not just in animal models but in humans as well, and provide hope that if we can find a way to regain control over glial hyper-responsiveness, we may be able to develop an effective treatment.

Glial mediators

Now that we know glia can modulate aberrant pain responses caused by somatosensory dysfunction, and can even misbehave on their own to drive persistent pain, we can ask what signals these cells are responding to. While some such signals are well known, mechanisms governing glial involvement in pain processing remain to be discovered.

In early 2016, Allan Basbaum of the University of California, San Francisco School of Medicine led a team that identified a fascinating mechanism by which sensory neurons communicate danger to the spinal cord. The team discovered that colony-stimulating factor 1 (CSF1) is produced inside mouse primary sensory neurons after injury and that the protein is

Pharmaceutical companies have taken an interest in translating new glia-targeting therapies to the clinic to treat persistent pain.

physically transported to the spinal cord along the neuronal axon. Once at the heart of the spinal somatosensory processes, this neuronal payload is released to selectively target the microglial CSF1 receptor (CSF1R), triggering a cascade of signaling events that drive microglial cell proliferation and enhanced inflammatory cytokine production, and, as a result, an exaggerated pain response in the animals.[6]

Another mediator of glial contributions to persistent pain appears to be the pattern-recognition receptor systems of the innate immune system, which detect conserved features in and on invading pathogens. The Toll-like receptor (TLR) system can trigger an immune response after detecting a wide range of molecular patterns—called pathogen-associated molecular patterns (PAMPs)—on invading viral, fungal, and bacterial species. This same receptor system also enables communication with the microbiome, via detection of so-called microbiome-associated molecular patterns (MAMPs). Under persistent pain conditions, however, this detection system is upregulated on glia in the somatosensory system, and is activated by endogenous signals from stressed or damaged cells termed danger-associated molecular patterns (DAMPs). The consequences of DAMP-induced microglial TLR activation serve as a first-line trigger of microglial inflammatory cytokines, which can initiate a cascade that drives heightened pain responses.

Based on the role that glia play in exaggerated pain states, the last thing that a doctor would want to do to a persistent pain patient is heighten the reactivity of the glial innate immune pattern recognition systems, or increase proinflammatory signals. But evidence has accrued over the last two decades that opioids—the gold standard for the management of acute and cancer pain, and increasingly used for the management of chronic pain—drive precisely this unwanted reactive phenotype in spinal glia and cause increased and protracted pain sensitivity. And recent research points to the innate immune system's involvement in the unwanted side effects of opioid analgesics, with preclinical and clinical studies implicating glial responses in both tolerance and dependence. Additionally, the presentation of opioid-induced hyperalgesia—heightened pain following exposure to opioids—has been linked to glial responses triggered by the activation of pattern recognition receptors by what's termed xenobiotic-associated molecular patterns (XAMPs). These new discoveries will soon force us to change the way we use opioids and to consider new approaches to treating persistent pain.

Glial-targeted therapies

The growing recognition that glia are key players in persistent pain has raised interest in mining this system for novel targets to alleviate the condition. So far, however, glial-targeted treatments have fallen short. In fact, the 2009 failure of a closely watched Phase 2 clinical trial of propentofylline—a glial modulator that had shown efficacy for treating persistent pain in animal models—all but killed off drug development in this space for years.

The lack of a successful glial-targeted therapy thus far can be attributed to a couple of key factors. First is the lack of a translational rodent-to-human experimental model that captures the mechanisms underpinning persistent pain, including both neuronal and glial components. Indeed, ongoing exploration into the 2009 trial, which failed to reduce pain in patients with post-herpetic neuralgia, a complication of shingles, pointed to differences in how human and rodent microglia responded to propentofylline in vitro.[7] Secondly, the field still lacks objective biomarkers of persistent pain that allow for enriched subject recruitment

TWO PAIN PATHS DIVERGE IN THE BODY

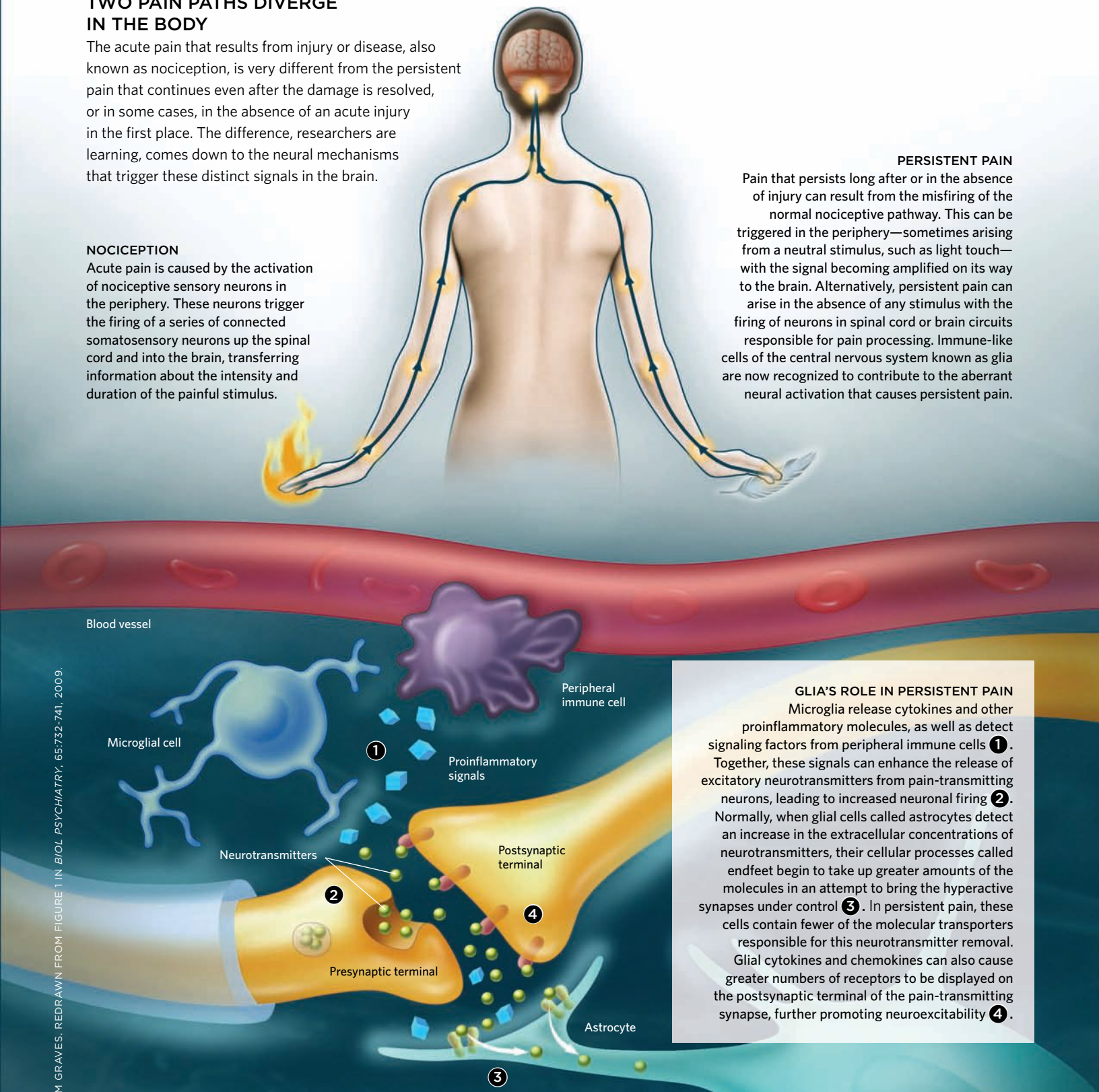
The acute pain that results from injury or disease, also known as nociception, is very different from the persistent pain that continues even after the damage is resolved, or in some cases, in the absence of an acute injury in the first place. The difference, researchers are learning, comes down to the neural mechanisms that trigger these distinct signals in the brain.

NOCICEPTION

Acute pain is caused by the activation of nociceptive sensory neurons in the periphery. These neurons trigger the firing of a series of connected somatosensory neurons up the spinal cord and into the brain, transferring information about the intensity and duration of the painful stimulus.

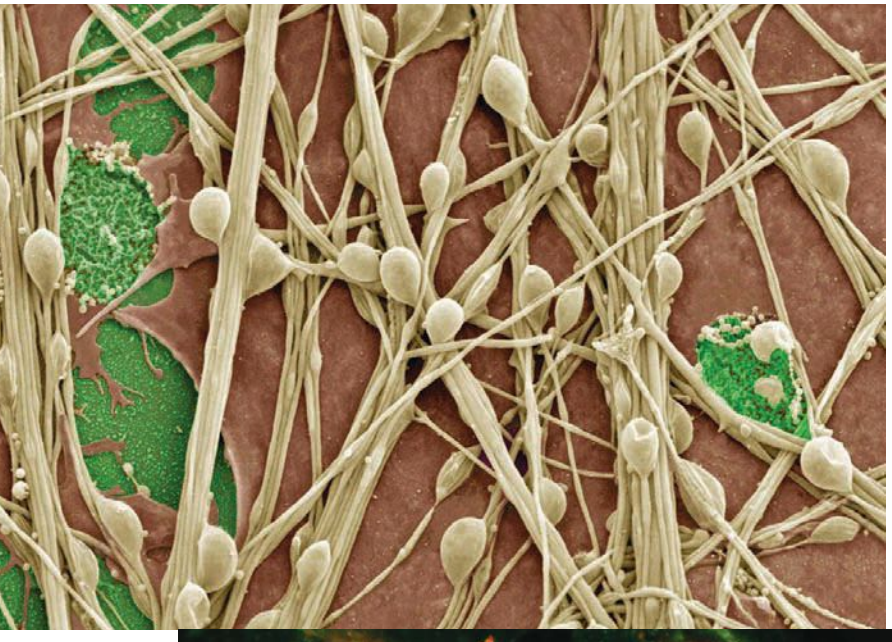
PERSISTENT PAIN

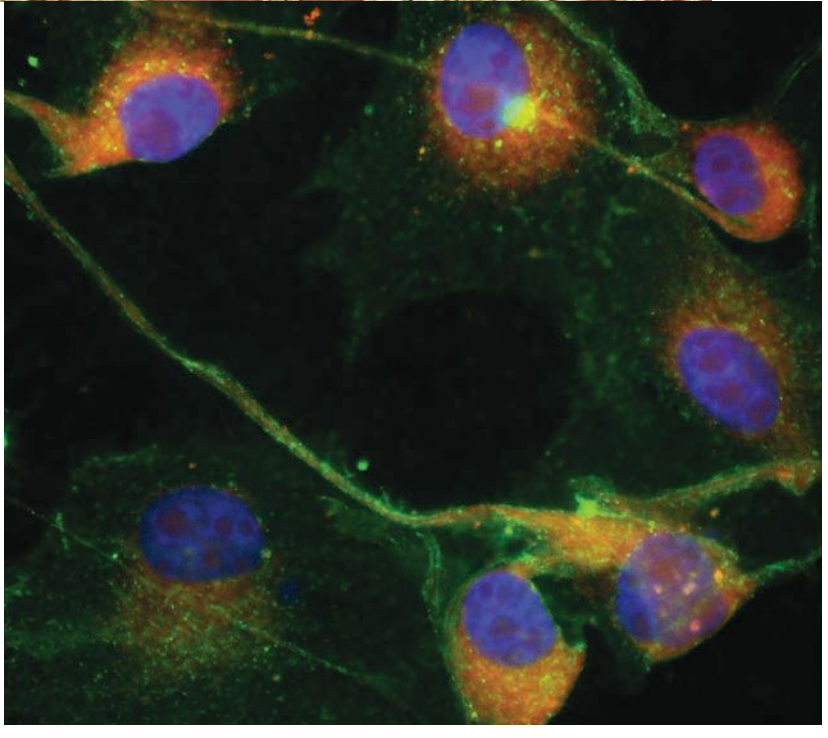
Pain that persists long after or in the absence of injury can result from the misfiring of the normal nociceptive pathway. This can be triggered in the periphery—sometimes arising from a neutral stimulus, such as light touch—with the signal becoming amplified on its way to the brain. Alternatively, persistent pain can arise in the absence of any stimulus with the firing of neurons in spinal cord or brain circuits responsible for pain processing. Immune-like cells of the central nervous system known as glia are now recognized to contribute to the aberrant neural activation that causes persistent pain.

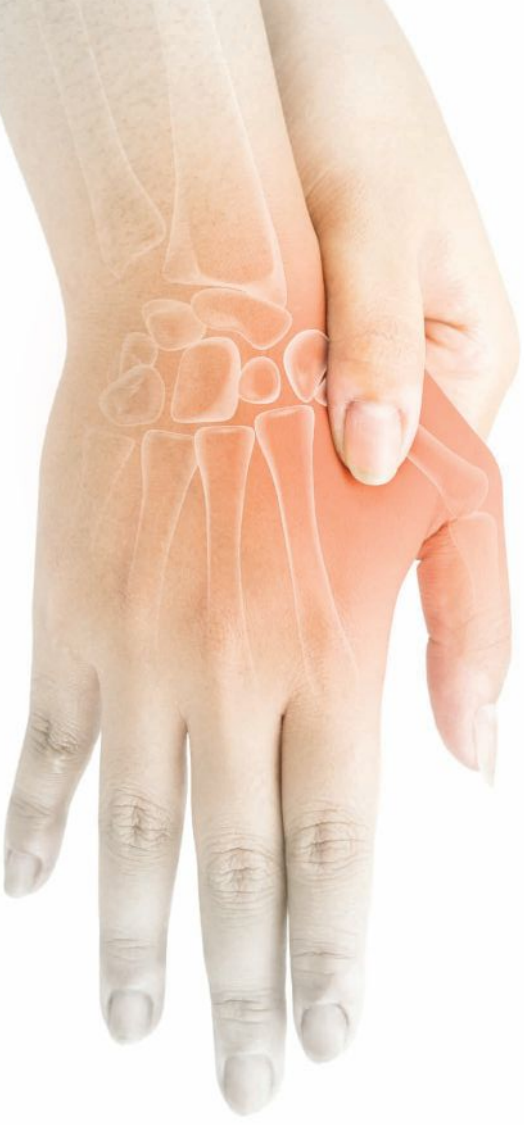


GLIA'S ROLE IN PERSISTENT PAIN

Microglia release cytokines and other proinflammatory molecules, as well as detect signaling factors from peripheral immune cells ❶. Together, these signals can enhance the release of excitatory neurotransmitters from pain-transmitting neurons, leading to increased neuronal firing ❷. Normally, when glial cells called astrocytes detect an increase in the extracellular concentrations of neurotransmitters, their cellular processes called endfeet begin to take up greater amounts of the molecules in an attempt to bring the hyperactive synapses under control ❸. In persistent pain, these cells contain fewer of the molecular transporters responsible for this neurotransmitter removal. Glial cytokines and chemokines can also cause greater numbers of receptors to be displayed on the postsynaptic terminal of the pain-transmitting synapse, further promoting neuroexcitability ❹.







SYNAPTIC MODULATORS: Astrocytes (top image: brown mat below a cultured network of neurons shown in tan; bottom image: alone in culture) can remove neurotransmitters as they cross synapses (round tan balls in top image), affecting the likelihood that postsynaptic neurons will fire. Under states of persistent pain, however, astrocytes are less effective at taking up excess neurotransmitters.

into trials and objective quantification of the pain experience.

Sex differences in pain processing also complicate the search for effective glial-targeted analgesics. While healthy males and females don't have substantial differences in sensitivity to acute pain, women are significantly more susceptible than men to persistent pain. (See "His and Hers Analgesia" on page 17.) The field has yet to reach a consensus on the underlying reason for this observation, but one possible culprit is variation between the sexes in the activity of the immune cell types responsible for the creation and maintenance of persistent pain. Scientists will need to be conscious of this difference and, when it comes to the application of glial-targeted therapies, consider developing sex-specific treatment approaches for persistent pain in men and women.

More broadly, the new glial view of persistent pain is also changing the way

ASTROCYTES: TOP—NATIONAL CENTER FOR MICROSCOPY AND IMAGING RESEARCH; BOTTOM—COURTESY OF CNBP RESEARCH FELLOW PHILLIPP REINECK, RMIT; HAND: © ISTOCK.COM/HORILLAZ

The lack of a definitive clinical test for persistent pain does not mean that the condition has no biological basis.

we think about other prevalent pain conditions. Classically viewed peripheral inflammatory pathologies associated with persistent pain, such as rheumatoid arthritis and osteoarthritis, are now being recognized as having previously unexplored central nervous system glial contributions. In these conditions, disease-modifying antirheumatic drugs are successfully blocking the peripheral manifestation of the debilitating diseases. However, clinical data demonstrate that exaggerated and persistent pain continues in the absence of any ongoing disease progression, and recent preclinical rodent studies have demonstrated that this persistent pain has its origins in the central nervous system and can be controlled by glial-targeted therapies. For example, spinal delivery of glial-targeted drugs diminished pain behaviors in rodent models of rheumatoid arthritis and osteoarthritis.[8]

No one can question that persistent pain is a very complex, multicellular disease state. There is no neuronal loss or profound lesion; the triggering injury or disease has resolved, or never existed peripherally in the first place. As a result, often nothing can be measured histologically or via blood tests, leading some medical professionals to incorrectly tell their patients that "the pain is all in your head." But the lack of a definitive clinical test for persistent pain does not mean that the condition has no biological basis. Indeed, we now know that an alteration in the homeostasis of multiple cellular systems changes the reactivity of somatosensory pain pathways, causing persistent, unremitting agony. Pain physician and pharmacologist Paul Rolan of the University of Adelaide calls this condition a "cancer of the soul."

But there is hope. Despite the clinical failures to date, recent breakthroughs point toward new molecular players in the glial-driven persistence of pain, and researchers continue to pursue drugs that target these mechanisms. Along with continued research into the biological basis of persistent pain, these efforts may one day lead to disease-modifying treatments for a painful and costly medical problem. ■

Mark R. Hutchinson is the director of the Australian Research Council Centre of Excellence for Nanoscale BioPhotonics at the University of Adelaide Medical School.

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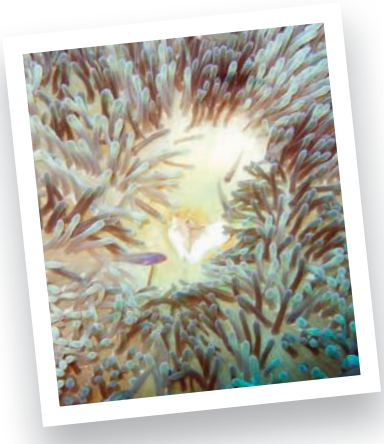

Animal Analgesics

A cornucopia of toxins in the animal kingdom could provide inspiration for novel painkillers, but so far, effective drugs have proven elusive.

BY *THE SCIENTIST* STAFF

Beyond the usual suspects of snakes, spiders, and scorpions, the animal kingdom is filled with noxious critters: snails, frogs, fish, anemones, and more make toxins for defense or predation. The noxious chemicals these animals produce are potent, and they often strike where it hurts: pain pathways. These compounds have long captivated researchers hoping to understand their effects and use that knowledge to develop drugs that suppress pain in a wide variety of ailments affecting humans.

Paradoxically, some of these toxins are themselves analgesic, and researchers have worked to develop synthetic derivatives that can be tested as painkillers. Such is the case for the only toxin-derived analgesic to be approved by the US Food and Drug Administration (FDA): ziconotide (Prialt), a compound 1,000 times more potent than morphine that was inspired by a component of the venom of the cone snail *Conus magus*. Other toxins elicit pain, and researchers have used these compounds to identify inhibitors of ion channels on the pain-sensing neurons they target.

Despite more than half a century of research in this field, however, scientists have had a frustrating time developing effective analgesics. Challenges include ensuring that the drugs are highly specific to their targets—each family of ion channels involved in pain sensing in humans contains several conserved proteins—and getting them to those targets, which often lie beyond the blood-brain barrier in the central nervous system. Nevertheless, several toxin-derived candidates are beginning to prove their worth in preclinical experiments and a handful of clinical trials, and bioprospectors continue to mine the animal kingdom's vast library of venoms and poisons for more leads. The next big thing in painkillers could soon be slithering, creeping, hopping, or swimming into the pipeline.

CENTIPEDE: EIVIND UNDHEIM; SHELL: DENIS FINNIN, AMERICAN MUSEUM OF NATURAL HISTORY; SEA ANEMONE: TAM WARNER MINTON/WIKIMEDIA COMMONS

WHAT'S IN A TOXIN?

Animals produce toxins of two varieties: poisons and venoms. Poisons cause pain or illness when ingested, whereas venoms are injected via a bite or a sting to cause ill effects.

FROGS: © ISTOCK.COM/CYNOCLUB; SCORPION: © FMPORTELLA/SHUTTERSTOCK.COM; SPIDER: BASTIAN RAST

By Cones Alone

IN THE 1970S, UNIVERSITY OF UTAH RESEARCHER Baldomero Olivera heard stories of Filipino fishermen dying after pulling in their nets. Their catches turned out to contain *Conus geographus*, a marine mollusk that produces some of the most potent venom of any cone snail species. The ultimate cause of death, then, seemed clear. But the details of how the cone snail toxin had killed the fishermen were more curious. According to medical reports, the men were not writhing in agony as their lives slipped from their grasp, leading researchers and clinicians to dub the tragic outcome a "painless death."

"They didn't cry out in pain, they weren't doubling over, they weren't getting swollen like you kind of do from a wasp sting or from a snakebite, where you get this massive inflammation," says Mandë Holford, a biochemist at Hunter College and CUNY Graduate Center and the American Museum of Natural History who was a postdoc in Olivera's lab in the early 2000s. "They were just sort of withering away."

These painless deaths led researchers to wonder how cone snail venom was behaving inside the body. "A lot of [insect or snake] venom has acetylcholine in it, which reacts with pain receptors, so you get throbbing pain," Holford says. "That doesn't happen with cone snail venoms. Instead, it sort of restricts the diaphragm and the person sometimes dies from a heart attack because they can't breathe, and that shock of not being able to breathe is what kills them."

In the 1980s, researchers isolated a novel conotoxin peptide from the venom of a species, *Conus magus*, related to the fishermen's killers, and derived a synthetic version of the peptide called ziconotide. Extensive functional studies revealed that ziconotide blocked $Ca_V2.2$, or N-type, voltage-gated calcium channels, and so inhibited the release of pain-transmitting chemical messengers, including glutamate and calcitonin gene–related peptide, in the central nervous system. In 2004, the drug, which is 1,000 times more potent than morphine, was approved for sale in the United States by the FDA for the treatment of intractable pain, especially neuropathic pain and pain in cancer patients, under the trade name Prialt.

> It is a very expensive treatment, and it's only for certain types of patients.
>
> —Frank Mari, National Institute of Standards and Technology

But because ziconotide cannot cross the blood-brain barrier, it must be delivered directly into patients' spinal cords via a surgically implanted pump. "It is a very expensive treatment, and it's only for certain types of patients" for whom opioids and other painkillers have failed, says Frank Mari, a biochemist at the National Institute of Standards and Technology (NIST) who studies cone snail venoms. "It's a boutique drug."

Even though ziconotide didn't turn out to be a blockbuster pain medication, it did shine a

light on a novel pain pathway, says Richard Lewis, a University of Queensland, Australia, molecular pharmacologist and director of the school's Institute for Molecular Bioscience Centre for Pain Research. "[The drug's developers] basically showed that a specific calcium channel was analgesic if you blocked it. That was proof of concept for that target."

Despite years of continued research into conotoxins as potential analgesics and a handful of clinical trials testing promising derivatives, however, no cone snail–inspired drugs other than ziconotide have made it to FDA approval. One reason is safety concerns, as venom peptides are extremely potent; another is the tendency for peptides to degrade quickly in the body. And in at least one case, a mid-course change in targeted indication is to blame, Lewis says.

About a decade ago, Lewis cofounded Xenome, a Brisbane-based biotech, to test a novel conotoxin-derived drug called Xen2174 for the treatment of cancer pain. Xen2174 blocked the reuptake of noradrenalin, a hormone that acts as a neurotransmitter and can be overproduced in some chronic pain cases. But after the FDA decided to reassign the drug to the treatment of post-surgical pain—in part to speed recruitment—Lewis says it failed to produce analgesic effects as promising as those the company had seen in patients with cancer-related pain. "It was disappointing that a promising molecule didn't survive the change in direction at the clinical trial level."

But researchers aren't giving up. Lewis says that cone snails, of which there are believed to be about 750 or 800 species, represent fertile ground for the search for novel analgesics. Each species has its own unique blend of peptides that make up its venom, and he estimates that, so far, "we're at about 1 percent of knowing the major components."

Holford's work, which seeks to get a handle on the chemical diversity of cone snail venom, could inform that search. The research—known as venomics—involves using genomics to assemble phylogenetic trees of known cone snail species. By targeting the venoms of species that are related to cone snails such as *C. magus* that have already yielded promising conotoxins, Holford says she can streamline the search for newer peptides that may be able to cross the blood-brain barrier or target peripheral pain receptors. "I like to call it 'from mollusks to medicine' or 'from beach to bedside,'" Holford says.

Meanwhile, Lewis is also using sequencing to hunt for novel conotoxins that may inspire a new class of pain drugs. "All of the approaches used for the human genome and proteome, we're applying those now to the venom of cone snails and starting to unravel and untangle that story."

—Bob Grant

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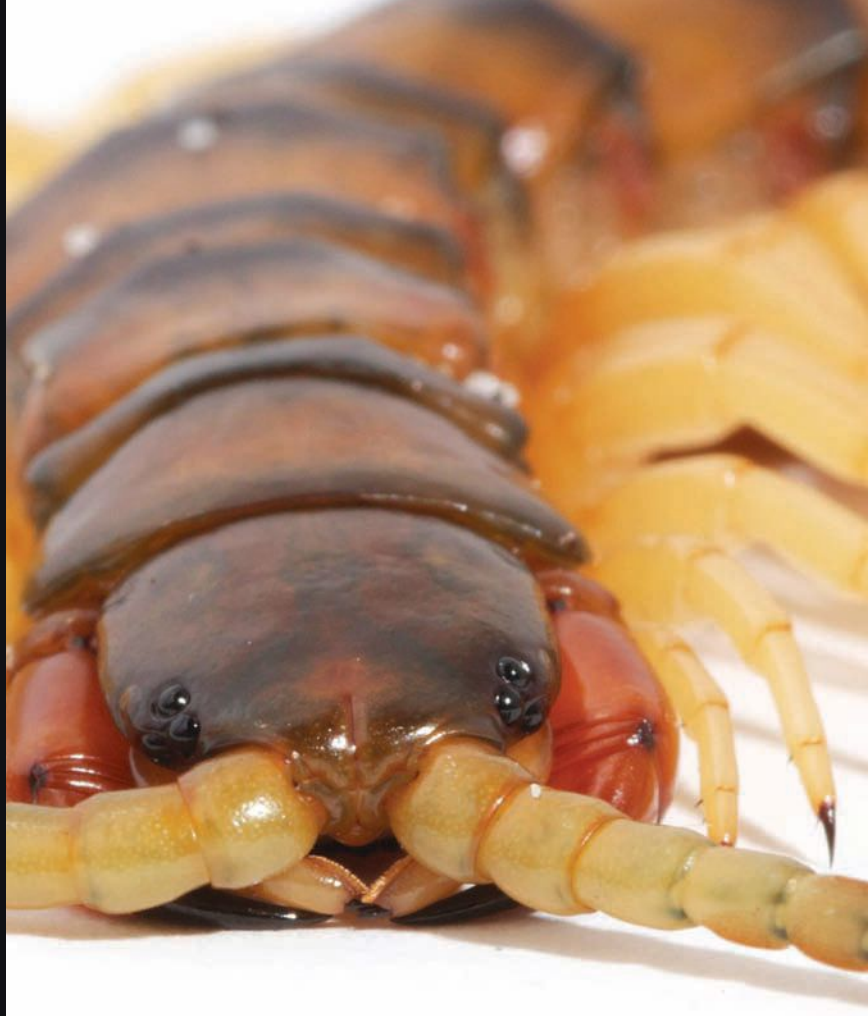

Leggy Laboratories

CENTIPEDE VENOM HAS A LONG HISTORY AS A PAIN-killer—toxins from the Chinese red-headed centipede (*Scolopendra subspinipes mutilans*) have been used in Chinese medicine for hundreds of years. But the leggy creature's arsenal has only recently gained attention as inspiration for modern analgesics. "There's on the order of 3,000 venomous centipedes," says Glenn King, a biochemist at the University of Queensland, Australia. "Yet they're massively underexplored in terms of what's in the venom."

In 2013, King and colleagues reported that one centipede-made peptide, Ssm6a, selectively inhibits $Na_V1.7$—a voltage-gated sodium channel implicated in pain sensing in mammals, including humans (*PNAS*, 110:17534-39). In rodent models, Ssm6a was more potent than morphine, though the finding has yet to be reproduced. Last year, a Chinese team showed that another centipede peptide, potassium channel blocker SsmTX-I, has analgesic properties in mice (*J Ppt Sci*, 23:384-91, 2017).

"These venoms are full of ion-channel modulators," says King. From a pain-killing perspective, "some of those molecules may turn out to be more or less useful therapeutically—we just don't know yet."

—Catherine Offord

The Spider's Bite

COMPRISING MORE THAN 40,000 SPECIES—ALMOST all of which produce venom—spiders are a treasure trove of nerve-attacking molecules. "They're basically little combinatorial peptide libraries walking around on a bunch of legs," says David Julius, a physiologist at the University of California, San Francisco. "They've had millions of years to evolve peptide sequences that interact with the functionally most important parts of protein targets."

Those targets are often ion channels in the membranes of pain-sensing neurons. In a recent screen of multiple venoms, Julius's group identified two toxins from the Togo starburst tarantula (*Heteroscodra maculata*) that selectively activated the mammalian voltage-gated sodium channel $Na_V1.1$, which behavioral experiments with mice revealed plays a previously unknown role in mechanical pain (*Nature*, 534:494-99, 2016). The team also characterized one of the toxin's binding sites, opening a route to developing selective $Na_V1.1$ inhibitors for analgesia. (Read a profile of Julius on page 54.)

Other toxins inhibit, rather than activate, these channels, providing more-direct inspiration for analgesic drug design. In 2017, a group of researchers in Australia reported that Pn3a—a peptide from the giant blue bloom tarantula (*Pamphobeteus nigricolor*)—inhibits the pain-associated sodium channel $Na_V1.7$ by binding to its voltage-sensing domains, and is analgesic in rodents when administered with subtherapeutic levels of opioids (*Sci Rep*, 7:40883). The same group recently identified another $Na_V1.7$ inhibitor, Cd1a, from the African rear-horned baboon spider (*Ceratogyrus darlingi*).

The specificity of such toxins is appealing to industry, notes Lachlan Rash, a pharmacologist at the University of Queensland. Pfizer researchers recently synthesized $Na_V1.7$-targeting "microproteins" inspired by peptides from the straight-horned baboon tarantula (*Ceratogyrus marshalli*). And last year, Janssen scientists engineered a variant of protoxin II, originally isolated from Peruvian green velvet tarantula (*Thrixopelma pruriens*) venom, that made rats insensitive to pain by blocking $Na_V1.7$ activation (*Sci Rep*, 7:39662, 2017).

Spider venoms are "really showing us pathways that we can target," explains Rash. Then, "we can try and mimic them with molecules that might have more-favorable pharmacokinetic properties, or be easier and cheaper to manufacture."

—Catherine Offord

BASTIAN RAST

Eating on the Edge

IT TAKES ONLY A MILLIGRAM OF TETRODOTOXIN (TTX) from improperly prepared fugu—typically made from one of a number of genera of pufferfish in Japanese or Korean cuisine—to kill an unlucky diner. Just 20 minutes after it passes a person's lips, the tongue goes numb. Then, headache, vomiting, paralysis, and difficulty breathing can follow, and victims might die within a few hours. While tragic in the culinary setting, TTX has been a windfall for neuroscience and, if all goes well with ongoing clinical trials, may one day serve as a potent painkiller.

Since the 1960s, TTX has been a favored tool among neurophysiologists to understand cellular structure and function. It blocks sodium channels on the surface of neurons, effectively stopping their electrical transmission. Among these channels is $Na_V1.7$, whose inhibition is known to help stop pain.

Vancouver-based Wex Pharmaceuticals is pursuing advanced clinical trials using TTX, which it harvests from the ovaries of the oblong blowfish (*Takifugu oblongus*), to treat cancer- and chemotherapy-related pain. In a recent Phase 3 trial with 149 participants, those receiving 30 μg of TTX twice a day for four days were more likely to report reduced pain than were those who received a placebo (*Pain Res Manag*, 2017:7212713, 2017).

The study didn't hit all the statistical marks, but Wex's CEO Christopher Gallen says there is enough promise in the results to pursue another Phase 3 study. Most appealing is the apparent lack of serious side effects, something Gallen attributes to "sheer luck": the charge and shape of the molecule keep it from crossing the blood-brain barrier. "The molecule does not look like anything a chemist would want to design," he says. "Because it's restricted to the peripheral compartment, we think that's why it's so safe. . . . It's blocking pain from ever getting to the brain."

—Kerry Grens

Because it's restricted to the peripheral compartment, we think that's why it's so safe. It's blocking pain from ever getting to the brain.

—Christopher Gallen, Wex Pharmaceuticals

Stinging Scorpions

STUDYING SCORPIONS COMES WITH its share of danger, as biologist Bryan Fry of the University of Queensland knows all too well. On a 2009 trip to the Brazilian Amazon, Fry was stung while trying to collect the lethal Brazilian yellow scorpion (*Tityus serrulatus*), and for eight hours he says it felt as though his finger was in a candle flame. Meanwhile, his heart flipped between racing and stopping for up to five seconds at a time. "At least the insane levels of pain helped keep my mind off my failing heart," Fry writes in an email to *The Scientist*.

His symptoms were caused by an arsenal of toxins in the animal's sting, which contribute to one of the most painful attacks in the animal kingdom. But at least one mammal—the southern grasshopper mouse (*Onychomys torridus*)—regularly chows down on Arizona bark scorpions (*Centruroides sculpturatus*) and doesn't seem to experience pain, despite receiving plenty of stings. In 2013, Ashlee Rowe, now of Michigan State University, and colleagues showed that bark scorpion venom interacts with the $Na_V1.8$ voltage-gated sodium channel in grasshopper mice, in addition to activating the $Na_V1.7$ channel as it does in other mammals (*Science*, 342:441-46).

During more than 400 million years of evolution, scorpions have developed an efficient venom arsenal, composed of extremely diverse active components.

—Peng Cao
Nanjing University of Chinese Medicine

Rowe's team showed that grasshopper mice have evolved amino acid changes in $Na_V1.8$ that allow it to bind scorpion venom components, and in turn prevent the channel's activation. Because $Na_V1.8$ is responsible for transmitting pain signals to the central nervous system following $Na_V1.7$ binding, blocking its activation prevents the sensation of pain. In other mammals, scorpion venom has no effect on $Na_V1.8$.

Rowe and her collaborators have since isolated several candidate peptides that inhibit the firing of grasshopper mouse $Na_V1.8$ to various degrees. "[Our goal is] to understand the molecular and the biophysical interactions between the channel and those peptides," she says. "We ultimately hope to use them as a framework to design peptides that would do the same thing in a human channel."

On the other side of the world, Peng Cao, a biologist at Nanjing University of Chinese Medicine, is studying the interactions of $Na_V1.8$ with components from the venom of the Chinese scorpion (*Buthus martensii* Karsch), which for thousands of years has been used in traditional Chinese medicine to treat many ailments, including pain. "During more than 400 million years of evolution, scorpions have developed an efficient venom arsenal, composed of extremely diverse active components," writes Cao in an email to *The Scientist*. "In recent years, my team has isolated a range of scorpion analgesic peptides which target voltage-gated sodium channels."

—Abby Olena

MATTHEW P. ROWE

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Healing Anemones

IN THE EARLY DAYS OF SEARCHING FOR INTERESTING compounds in animal venoms, researchers regularly discovered molecules that blocked voltage-gated sodium channels. While these channels play critical roles in pain perception, the protein family that makes up the channels also mediates other physiological functions, so the challenge became to block channels only on cells involved in pain. "It's all about selectivity," says Christine Beeton, an immunologist at Baylor College of Medicine.

In the mid-1990s, researchers discovered an alternative—stichodactyla toxin (ShK) from a Caribbean sea anemone (*Stichodactyla helianthus*), one of the first peptide toxins identified that blocks a voltage-gated potassium channel. And not just any potassium channel; ShK targets $K_v1.3$, which is upregulated on autoreactive T lymphocytes—cells that drive numerous autoimmune diseases, Beeton explains. "That makes this channel a very, very good target, because now you can target autoreactive T lymphocytes without targeting all the T lymphocytes in the body, so you're not completely immunocompromising the patients."

In 2001, Beeton started working on ShK as a postdoc at the University of California, Irvine. There she helped develop the analog ShK-186—so called because it was the 186th compound tested—which was highly selective for the $K_v1.3$ channel and bound to it with a high affinity. Now known as dalazatide, the drug is being developed by Seattle-based Kineta for the treatment of multiple autoimmune disorders, including psoriasis (the only indication for which dalazatide has progressed to early-stage human trials), inclusion-body myositis, and lupus. While dalazatide is not an analgesic per se, "each time you have an inflammatory disease, if you manage to reduce this inflammation, as an effect you're going to reduce pain also," Beeton says.

—Jef Akst

Leapfrogging Pain

WHEN THE LATE ORGANIC CHEMIST John Daly was on the hunt for poisonous frogs, he employed an unadvisable method: "It involved touching the frog, then sampling it on the tongue. If you got a burning sensation, then you knew this was a frog you ought to collect," he once told a National Institutes of Health (NIH) newsletter writer. Daly survived to gather frogs from South America, Madagascar, Australia, and Thailand, and he extracted more than 500 compounds from their skin (many of which the frogs in turn had harvested from their insect diets). One of these compounds, the toxin epibatidine, turned out to have an analgesic effect 200 times more potent than morphine in rodents, Daly and his colleagues reported in 1992 (*J Am Chem Soc,* 114:3475-78, 1992); and rather than working through opioid receptors, epibatidine bound to nicotinic receptors.

"To have a drug that works as well [as opioids] but is actually targeting a completely independent receptor system is really one of those holy grails of the drug industry," says Daniel McGehee, who studies nicotinic receptors at the University of Chicago. But an epibatidine-related compound tested by Abbott Labs as an analgesic in the late 2000s caused uncontrollable vomiting, McGehee says. Although research on nicotinic receptors continues, he's not aware of any epibatidine analogs currently in the drug development pipeline.

But frogs may yet hold clues to killing pain. At least one frog does deploy an opioid: the waxy monkey tree frog (*Phyllomedusa sauvagii*), whose skin is laced with the peptide dermorphin. Although the compound does not appear to be a toxin that wards off predators, dermorphin has about 40 times the potency of morphine in a guinea-pig ileum assay, but it doesn't effectively cross the blood-brain barrier, says pharmacologist Tony Yaksh of the University of California, San Diego. Dermorphin also boasts an unusual chemical property: the inclusion of a D-amino acid in its sequence. Almost all amino acids found in natural compounds are L-isomers, and dermorphin's stereochemistry makes it resistant to metabolism and "certainly renders it more potent," Yaksh writes in an email to *The Scientist*.

In the mid-1980s, Yaksh and Craig Stevens, now of Oklahoma State University, experimented with delivering dermorphin to rodents via the spinal cord; the researchers reported it was hundreds of times more potent than morphine delivered the same way. The drug was even delivered through the spine for some patients during post-surgical recovery in one study, but at the time, "it was hard to get people to think seriously about developing spinal drugs," Yaksh says.

But interest in spinally delivered drugs has picked up in the past decade, he notes. Dosing opiates through an implanted spinal catheter would avoid many of the side effects associated with systemic use, he says, but repeatedly delivering morphine in this way has caused another complication: development of a mass called a granuloma at the injection site. Because it is more potent and requires less volume, dermorphin or an analog of the compound, such as [Dmt[1]]DALDA, may turn out to be better suited for this use, says Yaksh. He and a collaborator recently got a grant to further explore this possibility. Other researchers are now conducting animal studies for various kinds of pain with DALDA.

—Shawna Williams

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Taking a Bite Out of Pain

SNAKES REPRESENT SOME OF THE deadliest venomous animals in the world, killing between 81,000 and 138,000 people each year, according to the World Health Organization. But in low doses, some of their toxins can produce analgesia.

For instance, a toxin from the black mamba (*Dendroaspis polylepis*) inhibits pain by blocking acid-sensing ion channels on the surface of pain-transmitting neurons in mice (*Nature*, 490:552-55, 2012). Another compound, taken from the venom of the king cobra (*Ophiophagus hannah*), likewise diminishes pain but acts in an unusual way. R. Manjunatha Kini of the National University of Singapore has found that so-called hannalgesin likely disrupts nitric oxide synthase in neurons, thereby reducing nitric oxide production, which is involved in pain. "There are many ways of skinning a cat, and there could be many ways to block pain," says Kini.

Although both toxins helped launch companies looking to develop analgesics in recent years, neither progressed, principally because of economic and other circumstances beyond the science, Kini says.

The University of Queensland's Bryan Fry says snake venoms could contribute to pain therapeutics in ways other than by tapping into their analgesic components: by zeroing in on venom compounds that *cause* pain. In 2016, Fry's group described the mechanism by which the toxin of the long-glanded blue coral snake (*Calliophis bivirgatus*) acts upon the $Na_V1.4$ voltage-gated sodium channel (*Toxins*, 8:303, 2017), "therefore revealing a new target for drug design," he says in an email to *The Scientist*. "Toxins that cause pain can be just as useful as those which block pain, because they can teach us more about how pain works."

Toxins that cause pain can be just as useful as those which block pain because they can teach us more about how pain works.

—Bryan Fry, University of Queensland

—Kerry Grens

The Literature

NEUROSCIENCE

Phases of Fear

THE PAPER

F. Klumpers et al., "How human amygdala and bed nucleus of the stria terminalis may drive distinct defensive responses," *J Neurosci*, 37:9645-56, 2017.

When Floris Klumpers zapped people with electricity while working toward his PhD in the late 2000s, he expected his volunteers' amygdalae—key emotion centers in the brain—to light up in anticipation of a shock. "There was this idea that the amygdala is the most important structure in emotion processing—especially in fear processing," says Klumpers, then at Utrecht University in the Netherlands. "We were quite surprised, using fMRI studies, to *not* find amygdala activity when people were anticipating an adverse event."

Klumpers assumed he'd made a mistake, but after replicating the finding in further experimental work, he began thinking about the different stages of animals' fear responses. First, there's anticipation, during which an individual becomes alert and plans reactions to possible danger. Then there's confrontation, when it has to act to avoid imminent danger. Perhaps, Klumpers reasoned, the brain's fear-processing regions treat these two phases differently.

To investigate, Klumpers, now a neuroscientist at Radboud University Medical Center, and colleagues recently collected data from more than 150 volunteers, who received mild electrical shocks to their fingers as they viewed a computer. "We have a simple cue on the screen that can predict the occurrence of an electrical stimulation," Klumpers says. In one set of experiments, for example, a yellow square meant a shock was likely, while a blue square signaled no shock for the time being. Meanwhile, the researchers monitored participants' heart rates and imaged their brains using fMRI.

Most people's heart rates dropped on seeing the shock-predicting cue—a response "linked in previous studies to an enhanced state of alertness" and reaction planning, says Klumpers. Plus, neural activity increased in the bed nucleus of the stria terminalis, a tiny brain region associated with anxiety in rodents. Upon being shocked, though, heart rate accelerated—a response that "helps you mobilize resources" to react, Klumpers says—and neural activity shifted to regions associated with fear, including the amygdala.

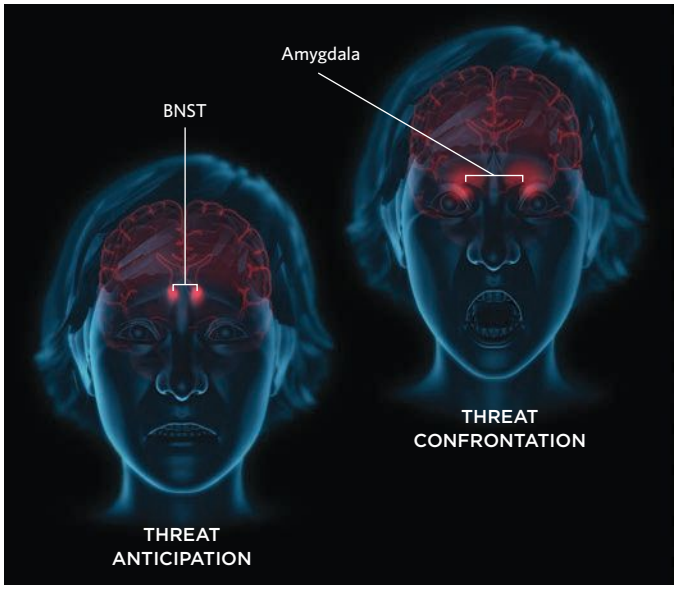
The results indicate that threat anticipation and confrontation phases are separated in the brain, Klumpers notes, adding there's substantial individual variation. For example, in people who reported childhood maltreatment on a questionnaire administered after the study, "we found that the amygdala was more active during threat anticipation," he says. "The alarm system goes off a little bit earlier."

Caltech neuroscientist Dean Mobbs says that while the group's main findings are plausible, he suspects a higher-resolution study would reveal finer-grained variation in activity within the regions studied. "I think it's correct, but I don't think it's the full picture," he says. He'd also prefer a more ecologically relevant stimulus than electricity, he adds; his group monitors people's responses to a live tarantula.

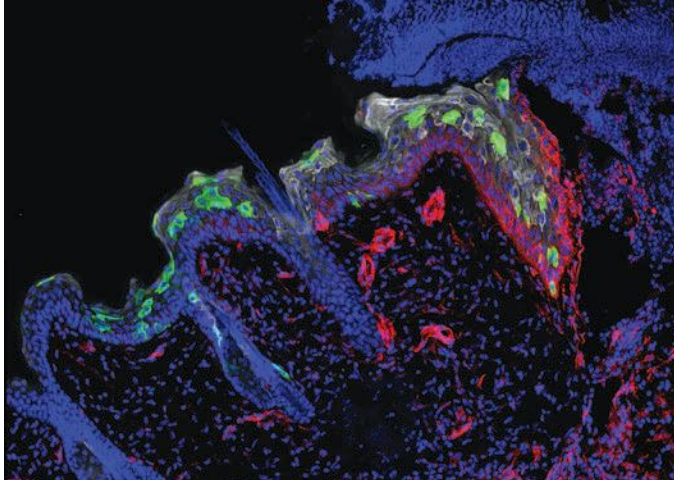
Klumpers says he now wants to study people's brains when they are planning how to avoid a threat. "In the real world, when we're anticipating something we still have a window of opportunity to deal with it."

—Catherine Offord

ON EDGE: Researchers trained participants in two studies to associate visual cues with a mild electric shock to the finger. Following a visual cue suggesting a shock might be imminent—i.e., during threat anticipation—the volunteers' brains showed higher activity in the bed nucleus of the stria terminalis (BNST), a region of the brain associated with defensive responses in uncertain situations. When participants were shocked—i.e., during threat confrontation—they showed higher activity in their amygdalae, two almond-shaped clusters of nuclei associated with fear and emotional stimulation.



OMEGA-6 FIELDS: Linoleic acid is plentiful in soy and other common components of modern Western diets.



FIX-IT CREW: Epithelial stem cells (green) migrate into a wound in mouse skin to repair the damaged tissue barrier.

CELL & MOLECULAR BIOLOGY

Overly Sensitive

THE PAPER

C.E. Ramsden et al., "A systems approach for discovering linoleic acid derivatives that potentially mediate pain and itch," *Sci Signal*, doi:10.1126/scisignal.aal5241, 2017.

PLUSES AND MINUSES

Linoleic acid, also known as omega-6 fatty acid—abundant in many vegetable and seed oils—is essential for forming our skin's waxy, waterproof barrier, and has become an increasingly common component of modern Western diets. Research has linked it to pain and chronic headaches, and Christopher Ramsden, a nutrition researcher at the National Institute on Aging, wanted to know why.

SCRATCHING THE ITCH

Ramsden and colleagues explored eight linoleic acid derivatives in human and rat skin. They prospected for the compounds in inflamed psoriatic skin lesions in humans, and injected them into mice to see if they would induce itching.

FEELING PAIN

One of the derivatives was significantly more concentrated in psoriatic skin than in healthy skin, and induced scratching in mice. Two of the other compounds sensitized rat sensory neurons in vitro, supporting the hypothesis that such molecules could spark pain sensations. "That's the first step in feeling itch or pain," explains Ramsden. In line with this, when the researchers analyzed data from a clinical trial that had tested strategies to manage chronic headache, they found that patients reported fewer painful episodes when they cut back their intake of linoleic acid.

MOLECULAR MYSTERY

The receptors that bind these derivatives, once identified, might provide novel pharmacological targets for treating pain. Tamara Rosenbaum, who studies pain at the National Autonomous University of Mexico and who was not involved in the study, tells *The Scientist* in an email that a lot of work is still needed to understand the molecular mechanisms at play, but that the study "provides us with important pieces to a puzzle."

—Katarina Zimmer

CELL BIOLOGY

Forget Me Not

THE PAPER

S. Naik et al., "Inflammatory memory sensitizes skin epithelial stem cells to tissue damage," *Nature*, 550:475–80, 2017.

INFLAMMATORY MEMORY

Our body is routinely assaulted by ultraviolet radiation, irritants, and pathogens. Shruti Naik, an immunologist at Rockefeller University, wondered: "Do these stressors have any kind of lasting impact on cells?" Immune cells are known to "remember" infections and inflammatory events so that they can respond faster to future insults, but what about the epithelial stem cells that maintain the skin and promote wound healing?

MULTIPLE ASSAULTS

Naik and her colleagues induced inflammation in mice by exposing the animals' skin to chemicals, fungal infection, or mechanical wounding. Then they measured the time it took for the skin to heal after injuring it in the same place a second time. On average, regardless of the type of injury, skin that had been previously inflamed healed about 2.5 times faster than the skin of mice that were wounded for the first time.

SWIFT REPAIR

To uncover the genetic basis for an "inflammatory memory," the researchers searched for genetic loci in the epithelial stem cells that were maintained in a chromosomally accessible state after the first injury. Multiple regions of chromatin were left "open" for up to 180 days after an assault, allowing rapid transcription of key stress response genes following a second injury.

BENEATH THE SKIN

Epithelial stem cells are the first nonimmune cells found to have a memory, and the findings point to "a primitive basic response to jazz up the cells quickly and make them heal the wound," says George Cotsarelis, a dermatologist at the University of Pennsylvania's Perelman School of Medicine who was not involved in the study. "It changes the way people think about the skin now."

—Katarina Zimmer

PROFILE

Painstakingly Perceptive

David Julius's fascination with natural products guided him to important insights into the molecular basis of pain and related sensory processes.

BY ANNA AZVOLINSKY

David Julius entered the biochemistry graduate program at the University of California, Berkeley, in 1977. "It was all one foot in front of the other. I wasn't trying to figure out what I would be doing in 10 years," says the University of California, San Francisco (UCSF) professor of physiology. "When I arrived, I thought, 'Classes are pretty much over. This is like a real job, and I can just go in the lab and do my thing.'"

Julius joined the UC Berkeley lab of Jeremy Thorner, who was studying hormonal signaling and trying to understand how budding yeast cells switch mating type. Randy Schekman, a Berkeley researcher who worked on protein secretion and vesicular transport, served as Julius's coadvisor. "What was great about Jeremy and Randy was that they were both trained as biochemists and then had decided to take advantage of the yeast genetic system to understand the biochemistry of cellular signaling."

No one in my family had gone to a private college, so I assumed I would go to SUNY Stony Brook.

Haploid yeast cells can be either "type a" or "type α," and mate with cells of the opposite type. Julius worked on the synthesis of alpha factor, one of two mating hormones produced and secreted by yeast. His graduate studies produced three *Cell* papers. The first, published in 1983, reported that a class of enzymes, the dipeptidyl aminopeptidases, is necessary to cleave a longer precursor of alpha factor into the final 13-amino-acid peptide. To identify the specific dipeptidyl aminopeptidase and elucidate its role, Julius took advantage of yeast mutants, including one called *ste13* (for sterile 13), which cannot produce normal alpha factor. It was the first time anyone had characterized the biochemical functioning of one of the yeast sterile mutants.

In another of the *Cell* papers, Julius and his coauthors reported the discovery of a pro-protein convertase called KEX2 that cleaves polypeptide precursors between certain pairs of basic amino acids, resulting in an active hormone. Again, Julius worked with yeast strains that had mutations, this time in the gene *kex2*, using a bevy of peptide substrates to assay for dibasic residue cleavage. "I was able to get linear activity using this assay in wild-type yeast and didn't see any activity in my mutants," Julius says. "I thought that was super exciting, so I dove right in, working like a maniac for three months."

While the findings Julius generated as a PhD student aided science's understanding of yeast mating type systems, more broadly they shed light on how peptide hormones, such as insulin and endorphins, are synthesized. "Peptide hormones are generally made from large precursors, and one [major goal] was to identify the enzymes that cleave between basic residues, such as an arginine and lysine," he says—as KEX2 does.

"I remember Randy had been on sabbatical when I was doing the work," Julius says. "When he came back he said to Jeremy, 'When did he get all of this done?'" The research set the stage for subsequent identification by others of mammalian pro-hormone convertases, something Julius didn't expect to accomplish as a grad student.

As a postdoc and in his own lab at UCSF, Julius went on to study the biochemical and neurological processes of the mammalian nervous system. Here, Julius talks about his choice to attend his neighborhood New York City high school over a prestigious, selective one; why he was drawn to studying sensory systems; and how science has always fostered an international community.

JULIUS JUMPS

Back to the neighborhood. "Brighton Beach was a good place to grow up," says Julius of the Coney Island neighborhood where he spent his boyhood. "It was New York City, so we had access to everything, but also felt out of the city because there was plenty of green." As a teenager, Julius decided to try testing into the STEM-focused Stuyvesant High School and was accepted. But it was a long haul from Brighton Beach. The commute "was a grind, but what was even worse was the constant testing. I noticed that many of the kids were burning out before college. I remember going in my first day of sophomore year and just looking around and deciding, 'I can't do this anymore.'" So Julius—much to his father's disappointment—switched to Brighton Beach's Abraham Lincoln High School, which boasts notable alumni such as Arthur Miller (one of Julius's favorite writers), Neil Diamond, and Nobel laureates Paul Berg and Arthur Kornberg. "The move was transformative for me. I met interesting people, and we got out of school early, so would take the train into the city and actually enjoyed museums, shows, and music."

Julius also recalls an agent of scientific inspiration from his high school days—his physics teacher, Herb Isaacson. "He made me think that science might be something I wanted to do. I wrote about him for an autobiography tied to an award I received. Afterwards, I received letters from others who attended Lincoln and had similar fantastic experiences with this one teacher who encouraged them to go into medicine or science."

SUSAN MERRILL, UCSF

DAVID JULIUS

Professor and Chair, Department of Physiology, University of California, San Francisco, School of Medicine
2010 Shaw Prize in Life Sciences and Medicine
2013 Paul Janssen Prize for Biomedical Research
2017 Canada Gairdner International Award

Greatest Hits

- In budding yeast, first identified a gene for a peptidase that cleaves peptides between two basic amino acids and is required to synthesize the yeast peptide hormone alpha factor
- Cloned the mammalian serotonin 1c receptor using a function-based screen
- With Michael Caterina, cloned the receptor that is found in pain-processing neurons and binds to capsaicin, the molecule that makes chili peppers taste spicy
- Along with collaborators, identified the first cold-sensing receptor, TRP melastatin 8 (TRPM8), which is activated by menthol and other chemical cooling agents as well as cold temperatures
- With Yifan Cheng's lab at UCSF, resolved the structure of the TRPV1 ion channel with electron cryomicroscopy

Fitting in. "No one in my family had gone to a private college, so I assumed I would go to SUNY [State University of New York] Stony Brook," says Julius. But on a whim, he applied to MIT, was accepted, and started as an undergrad there in 1973. "It was disorienting at first. There were a of lot smart people, which was intimidating." But Julius says that as he settled into the university, he realized that he wasn't too different from other students. "I remember a chemistry class where I couldn't understand one of the professors and started to panic because I saw lots of people just sitting in the lecture reading the newspaper. I thought, 'Wow, I guess they know all of this stuff.' So I asked one of them, and he said, 'Nah, I can't understand a thing, so I gave up 20 minutes ago.' I realized that everyone was in the same boat except for the few geniuses among us, and I began to relax more." Julius decided to focus on biology, enrolling in MIT's Undergraduate Research Opportunities Program. "This was the late '70s, and not many colleges had opportunities for undergraduate students to engage in research. At MIT, through this program, they even placed people over the summer at companies. So MIT undergrads, instead of just saying, 'One day I will be an engineer or a scientist,' actually started living that life right away."

Escape from class. In 1974, Julius began research in Joel Huberman's DNA replication lab with then–grad student Janis Fraser. Fraser asked him to help with a pulse-chase experiment—radiolabeling DNA and putting it through a sucrose gradient and then chasing it to see when the Okazaki fragments from replication got incorporated into the longer, newly replicated DNA strand. "I set up a device to hold the pipette really still so that I could gently layer the sample on top of the gradient and spin it." The result was beautiful, according to Julius, and got him excited about hands-on lab work. "I thought, 'Wow, I can figure out how to do this.'"

While he was making strides in the lab, Julius admits that classes were not his forte. "Laboratory research is what got me through college. I liked that combination of hands-on experiments and abstract problem solving to figure out what your data mean." In his junior year, he switched to Alexander Rich's biophysics lab, where he became interested in the mechanics of protein synthesis and worked with Fraser's husband Tom, a chemical biologist who synthesized transfer RNA (tRNA) analogs. Using the analogs, Julius and Tom Fraser worked on understanding the specificity of protein-synthesis enzymes for particular tRNAs and amino acids.

JULIUS JOURNEYS

Head trip. One night, after Julius had left Boston for the Bay Area, he was lying on a bench outside the lab at Berkeley at 11 o'clock, waiting for his yeast cultures to grow, when he was approached by "two typical Berkeley denizens" who claimed that a scientist had made and sold them LSD a few years back. That these guys were still recalling an LSD experience they had years ago got Julius thinking about what actually happens when the brain is exposed to hallucinogenic drugs. "So I started reading about this topic, and that sparked the beginning of my interest in understanding how chemicals and natural products interact with the nervous system."

In 1984, Julius joined Richard Axel's lab at Columbia University as a postdoc to attempt to clone a serotonin receptor. "This was believed to be the target for many hallucinogens," Julius says. "No one had yet identified genes for receptors in the brain. I was in Richard's lab for four years before there was any glimmer that something was going to work." But gene expression technology caught up, and in 1988, Julius used a functional *Xenopus* oocyte screen to clone the serotonin 1c (5-HT1c) receptor from rat brain tissue.

Hot, hot, hot. Julius joined the faculty of UCSF in 1989 and continued his study of neurotransmitter receptors. In 1990, his team cloned another member of the serotonin receptor family, the 5-HT3 receptor, and they published their findings in 1991. Julius and his collaborators also developed their own knockout mouse models to study the functions of these and other receptors. Because many of these receptors were expressed in somatosensory neurons, Julius became interested in understanding mechanisms underlying somatosensation and pain. "A big question in the somatosensory field was: Can one find functional markers for somatosensory neurons that are involved in pain sensation? And the Holy Grail in this area was the mythical capsaicin receptor," says Julius. Capsaicin is the chemical that gives chili peppers their kick. "The somatosensory system was less well understood compared to other sensory systems, and there were comparatively fewer biochemical or genetic leads to go after." Julius chose a pharmacological approach, which also satisfied his proclivity for natural products.

While the project initially seemed like a blind alley, Julius's postdoc Michael Caterina cloned the receptor in 1997. Called vanilloid receptor type 1, it was among the first identified pain-specific ion channels, but it also demonstrated an unexpected functionality. "We started throwing nonchemical stimuli at the receptor and found, to our surprise, that heat could activate the channel," opening the way to understanding the molecular biology of somatosensation.

Sticking to pain. That surprising discovery got Julius interested in thermosensation. In 2007, his lab used the menthol molecule, which is perceived as a cool sensation, to identify the TRP (transient receptor potential) melastatin 8 (TRPM8) ion channel, activated by menthol but also by cold temperatures. Along the way, Julius's lab searched for toxins from spiders and snakes that activate various receptors on pain-sensing neurons, and that have become valuable tools for studying these receptors and the pathways they activate. "There are lots of toxins we know now from plants and animals that zero in on TRP channels to generate pain," says Julius.

I realized that sensory systems are beautiful things to work on because it is basically how we view the world. The colors we can see and the things we can smell are just a product of the molecular detectors that we have.

More recently, Julius's lab has completed the atomic structure of the TRPV1 ion channel as well as of the TRPA1 ion channel, which is activated by wasabi as well as other forms of horseradish, mustard, and other pungent phytochemicals. "It's been thrilling to obtain the three-dimensional atomic structures of TRP channels," says Julius. "These have stood as among the last mountains to climb because there was no structural information about these channels. For many TRP channels, we still don't understand what activates them, so spices have given us a big pharmacological advantage for mechanistic and structural studies of TRPV1 and other receptors."

JULIUS JAMS

NYC childhood. Julius credits a childhood spent marinating in the cultural milieu of 1960s and early 1970s New York City with his lifelong love of film, art, and music. "I loved being in New York in high school, getting to see shows on Broadway or at Lincoln Center for $5. I remember seeing *Death of a Salesman* with Lee J. Cobb, and *A Streetcar Named Desire* with James Farentino. My brothers and I would go down to the Village and sneak into music shows and just take in the scene. That was fantastic."

Tuning his senses. Julius found that tackling his preferred neuroscience topic of sensory biology has been psychologically satisfying. "I realized that sensory systems are beautiful things to work on because it is basically how we view the world. The colors we can see and the things we can smell are just a product of the molecular detectors that we have. Every animal sees and perceives the world in a different way based on its biophysical detectors. The systems are nice because you can really understand mechanisms of signal detection and how circuitry begets specificity."

Open horizons. Julius doesn't shy away from sharing his scientific opinions, or his political ones. "I gave a talk at a neuroscience meeting at the time of the result of the [2016] presidential election and was compelled to say that the rise of nationalism in politics is so antithetical to the life of a scientist. We as scientists live in this fantastic international community, and we have to protect that. We have scientists come to the U.S. to train, and we go to other places to train and be hosted. This is the lifeblood of scientists, and we need to resist closed-border policies." ■

Annina Schmid: Pain Pursuer

Associate professor, Nuffield Department of Clinical Neurosciences, University of Oxford. Age: 40

BY CATHERINE OFFORD

As a physiotherapist at University Hospital Zurich in the mid-2000s, Annina Schmid often encountered people with chronic pain. "My interest in research got sparked while I was seeing my patients," she says. "It was very difficult to treat them, or to understand why pain persists in some people, while it doesn't even occur in others."

Schmid, who grew up in Switzerland, had earned her master's degree in clinical physiotherapy in 2005 at Curtin University in Perth, Australia, and she was keen to return down under. In 2008, she secured an Endeavour Europe Scholarship from the Australian Government and moved to the University of Queensland in Brisbane for a PhD in neuroscience.

"She's very motivated," says Schmid's colleague and collaborator Brigitte Tampin, a musculoskeletal physiotherapist at Curtin University and at Osnabrück University of Applied Sciences in Germany. Tampin adds that Schmid's physiotherapy background was an asset for her PhD work and beyond. "She can think as a clinician and as a researcher."

For her PhD, Schmid focused on animal models of mild nerve compression, also called entrapment neuropathy, in which pressure on nerve fibers—from bone, for example—can cause pain and loss of motor function. Using a tube to compress the sciatic nerves of rats, Schmid was able to replicate not only local symptoms seen in humans, but also inflammation at distant sites, a possible explanation for why patients often report pain in other parts of the body.[1]

By the time she earned her doctorate in 2011, Schmid says she was "hooked" on research. During a subsequent internship with Claudia Sommer at the University of Würzburg, Germany, Schmid processed skin biopsies from patients with the pain syndrome fibromyalgia, and found degeneration in small nerve fibers—a finding she says helped shape her future focus.[2]

Shortly afterward, Schmid began a postdoc with neurobiologist David Bennett at the University of Oxford's Nuffield Department of Clinical Neurosciences (NDCN). "She's always been a joy to work with," says Bennett. "She's got a nice way of thinking about interesting and important questions in neuropathic pain."

Working on patients with carpal tunnel syndrome—a common entrapment neuropathy affecting the wrist—Schmid described reductions in small fiber density and showed that changes in nerve structure occurred even far away from the site of compression.[3]

In 2015, Schmid won an early career research grant from the International Association for the Study of Pain. Now an associate professor at NDCN, she continues to collaborate with Bennett to study nerve regeneration following compression. She also practices physiotherapy part-time and shares her expertise with students. "Her teaching is always very well received," says Bennett. "She's a very good communicator."

Beyond the University of Oxford, Schmid teaches at Oxford Brookes University and leads international courses for physiotherapists with Tampin. "I'm quite passionate about trying to translate findings from research to clinicians," Schmid says. "Physiotherapy is a profession that's still very close to my heart." ■

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Syncing Signals

High-throughput technologies enable scientists to link metabolites to epigenetic effects.

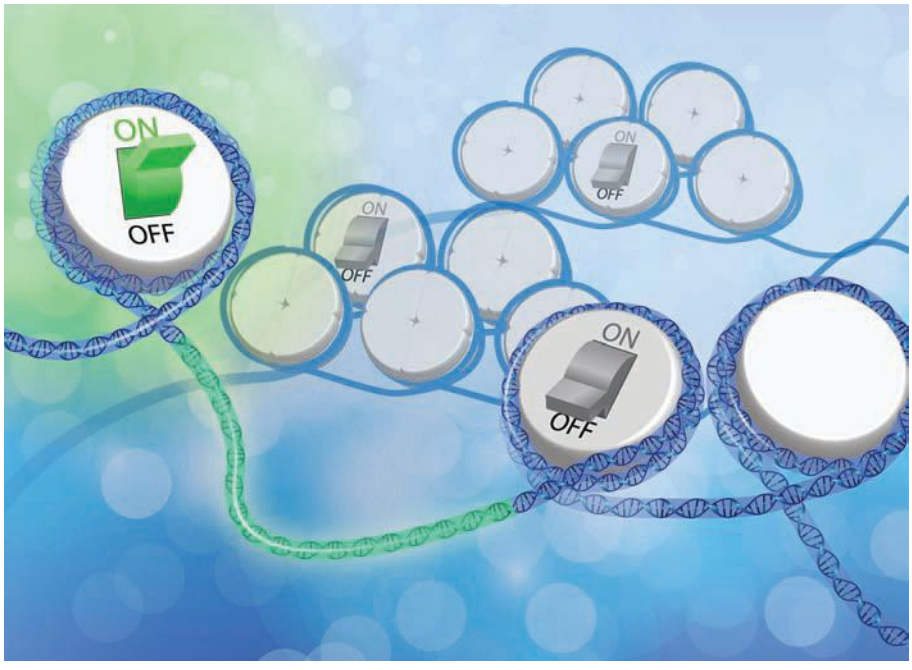
BY JYOTI MADHUSOODANAN

Chemical groups tacked onto DNA or histone proteins regulate how and when genes are expressed. Environmental signals can change the placement of these epigenetic tags, but researchers have had trouble pinning down how phenomena such as diet, inflammation, or social stress are converted into instructions that tweak gene functions.

Researchers have known for decades how some aspects of metabolism can wield epigenetic effects: breakdown products formed during sugar or protein digestion, for example, can be converted into chemical tags that epigenetically modify DNA or histones. But even a process as fundamental as turning glucose into cellular fuel can occur via distinct pathways that dynamically change based on a cell's immediate environment and state. So a cancer cell and a healthy one might digest sugars in distinct ways—and thus have different metabolites available for making epigenetic marks.

Knowing which metabolites can regulate gene function—and whether they do so by epigenetic means, binding to transcription factors, or other routes—is crucial to developing better drugs. Fortunately, studies demonstrating precisely how metabolism alters epigenetics have recently emerged, triggered in part by better technologies. Whole genome sequencing methods now enable researchers to map epigenetic changes across the entire genome, and high-throughput approaches such as liquid chromatography and mass spectrometry can track changes in hundreds of metabolites at once.

Also, recent research has revealed connections between aberrant metabolism and epigenetic changes in cancer cells, leading scientists to examine whether metabolic changes may be driving epigenetic ones. "These are two fields that operated independently for years," says Jason Locasale, a cancer researcher at Duke University. "In the last 10 years, a confluence of different observations of altered metabolism in cancer cells and developmental biology has led to many of these new studies."



DARRYL LEJA/NHGRI/SCIENCE SOURCE

Here, *The Scientist* surveys the landscape of research aimed at understanding how metabolites drive epigenetic changes.

T-CELL TOGGLE

INVESTIGATOR: Ming Li, immunologist, Memorial Sloan Kettering Cancer Center

PROJECT: How sugars control T-cell activation

PROBLEM: Cells typically digest sugars by breaking them down into carbon dioxide and water in the presence of oxygen. But cancer cells and activated T cells funnel glucose down different pathways. One of these is aerobic glycolysis, in which glucose gets converted to the 3-carbon lactate molecule that's typically produced during anaerobic processes, even in the presence of oxygen. Although this digestion is a hallmark of activated T cells and is known to influence the expression of pro-inflammatory genes, precisely why those cells employ this metabolic route—and how it controls gene expression—isn't well understood.

STUDY: Li and his colleagues generated mouse lines with T cells deficient in lactate dehydrogenase A (LDHA), an enzyme that catalyzes one of the key

steps in aerobic glycolysis. Mutant helper T cells consumed approximately 70 percent less glucose than wildtype cells did and expressed lower levels of interferon gamma (IFNγ), a cytokine crucial to helper T-cell function. In a previous study, researchers reported that blocking aerobic glycolysis increased binding of another enzyme to IFNγ mRNA transcripts at the 3' untranslated region (UTR), thus decreasing translation of the gene. But when Li's team deleted the 3' UTR, inhibiting aerobic glycolysis still reduced expression of IFNγ. LDHA did not exert its effects at the translational stage, but rather facilitated the gene's transcription.

Seeking an alternate link between the gene's expression and sugar metabolism, the team turned to chromatin immunoprecipitation (ChIP-Seq) and found that wildtype and LDHA knockout cells had different histone acetylation and gene expression patterns. (Histone acetylation furthers gene expression by rendering DNA more accessible to transcription factors.) In the knockouts, 86 percent of downregulated genes, including *IFN*γ, showed reduced histone acetylation. When LDHA drives the energy-producing process of aerobic glycolysis, mitochondria—which would normally fuel a cell's activities—can dial down respiration and instead export citrate to produce acetyl-CoA, a cofactor that's needed to acetylate histone proteins. Supplementing cells with acetate restored histone acetylation and *IFN*γ expression, suggesting that aerobic glycolysis controls interferon expression via an epigenetic mechanism (*Science,* 354:481-84, 2016). Now, the team is looking for other inflammatory cytokines that might be similarly regulated. "IFNγ was a clean model to use to differentiate epigenetic gene regulation from translational mechanisms," Li says.

APPROACH: When trying to elucidate a metabolite's role in an epigenetic effect, look for specific changes such as DNA methylation or histone acetylation, then search for metabolic pathways that might produce the ingredients needed to make these marks, Li suggests.

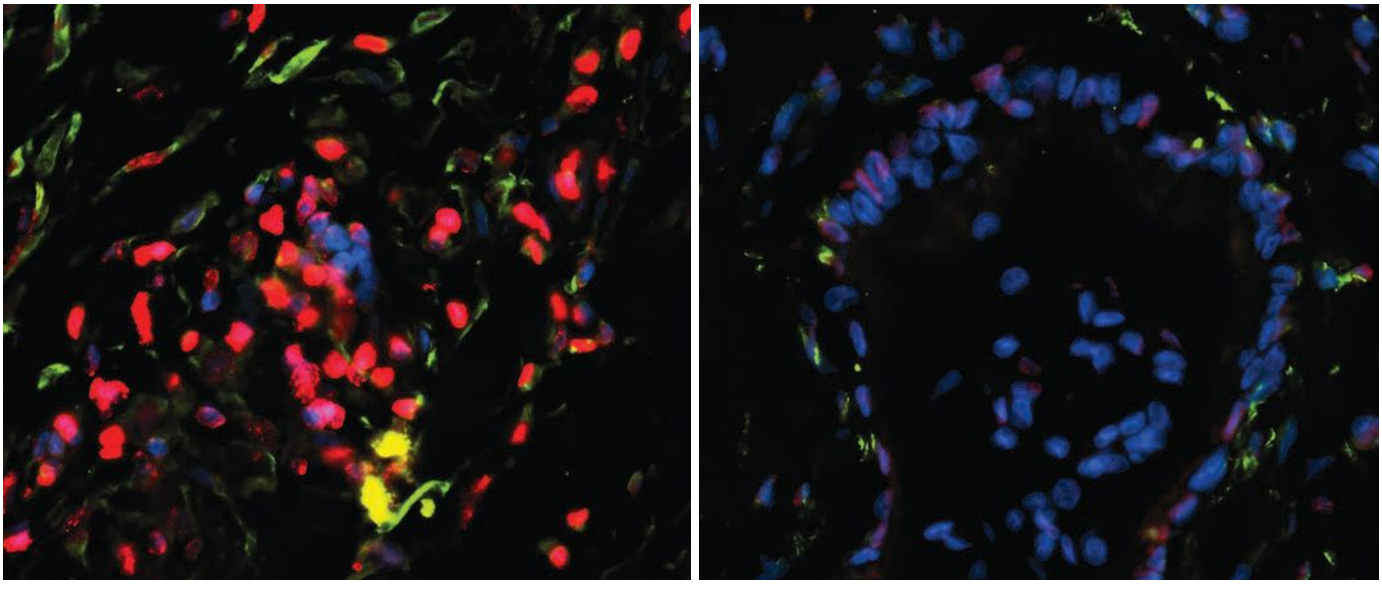
CANCER CHEMISTRY

INVESTIGATOR: Oliver McDonald, pathologist, Vanderbilt University

PROJECT: How glucose metabolites control genes involved in metastasis

PROBLEM: Primary tumors and metastatic cells in pancreatic ductal adenocarcinoma—the most common form of pancreatic cancer—are genetically similar to each other. But metastatic cells lodge in environments that are richer in nutrients than the primary tumor's niche, and grow in distinctly different ways. The genetic controls that enable the spread and growth of metastatic cells are unclear. But these cells show signs of demethylation across large swaths of their genome, suggesting an epigenetic mechanism at work. After spending a year knocking down assorted chromatin-modifying machinery in mouse tumors with no results, McDonald's team devised an alternate hypothesis: that such large epigenetic changes were regulated by equally large changes in metabolism.

STUDY: When the researchers compared paired samples from primary and metastatic human tumors for methylated histone proteins, they found that metastatic cells from distant sites such as liver and lung carried distinct methylation and acetylation marks on their histones. Unlike primary tumors,



SUGAR RUSH: Cells that first form a pancreatic tumor (left panel) show high levels of histone methylation (red). Methylation is lost in cancer cells that metastasize to distant locations such as the liver (right panel).

CHRISTINE IACOBUZIO-DONAHUE

which can supplement or replace glucose with various "alternative fuels," metastatic cells rely heavily on glucose consumption to form tumors. So the researchers used liquid chromatography and high-resolution mass spectrometry to track which metabolic pathways the sugars followed in metastases. Then they looked for steps in those pathways on which the cancer cells might be most dependent. Surveying metabolite profiles across tumors, the team found that distant metastatic cells had strikingly low levels of one chemical, known as 6-phosphogluconic acid (6PG), made during glucose digestion. 6PG is broken down further by an enzyme known as 6-phosphogluconate dehydrogenase (PGD), suggesting that the enzyme was overactive and had consumed all its substrate (*Nat Genet,* 49:367–76, 2017).

Knocking out the PGD gene did not change the expression of other enzymes in the same pathway, but it reversed changes to chromatin methylation and acetylation in distant metastatic cells. Inhibiting this enzyme blocked tumor formation in 3-D cultures. So PGD might act as a nutrient-sensing mechanism: by constantly chowing down on 6PG, it forces cells to consume more glucose. Then, cells can deploy the excess metabolites produced by digesting glucose to acetylate or demethylate histones in chromatin regions needed by malignant cells to form tumors, causing the large-scale epigenetic changes seen in metastatic growths. "We don't know how [the PGD signal is] targeted to these sites yet," McDonald says. "It could be through transcription factors that are pre-bound to these regions." When the researchers knocked down PGD in tumor-forming cells in experimental assays, primary tumor cells still grew, but metastatic cell growth stalled.

APPROACH: McDonald relies on manually sifting through data to find links between metabolite levels and chromatin changes. Large chromatin domains such as those altered in metastatic pancreatic cancer can appear as hundreds of different signals, and metabolite levels can change because of many different cellular pathways. Glucose, for example, could be digested by any one of several routes, and each would produce different intermediates. "So bioinformatics programs might not detect all interesting changes," McDonald says. "Hand-graphing metabolite data into spreadsheets is what led us to PGD, because it was wiped out across several different samples."

In the last 10 years, a confluence of different observations of altered metabolism in cancer cells and developmental biology has led to many of these new studies.

—Jason Locasale, Duke University

For those without the means to manually analyze such large datasets, McDonald suggests finding metabolite patterns that are likely to be relevant to the disease being studied, then working with a bioinformatician to tailor algorithms so they reflect the biology at hand.

STEM CELL STABILITY

INVESTIGATOR: Jason Locasale, pharmacologist and cancer biologist, Duke University

PROJECT: Understanding how the amino acid methionine affects histone methylation in embryonic stem cells

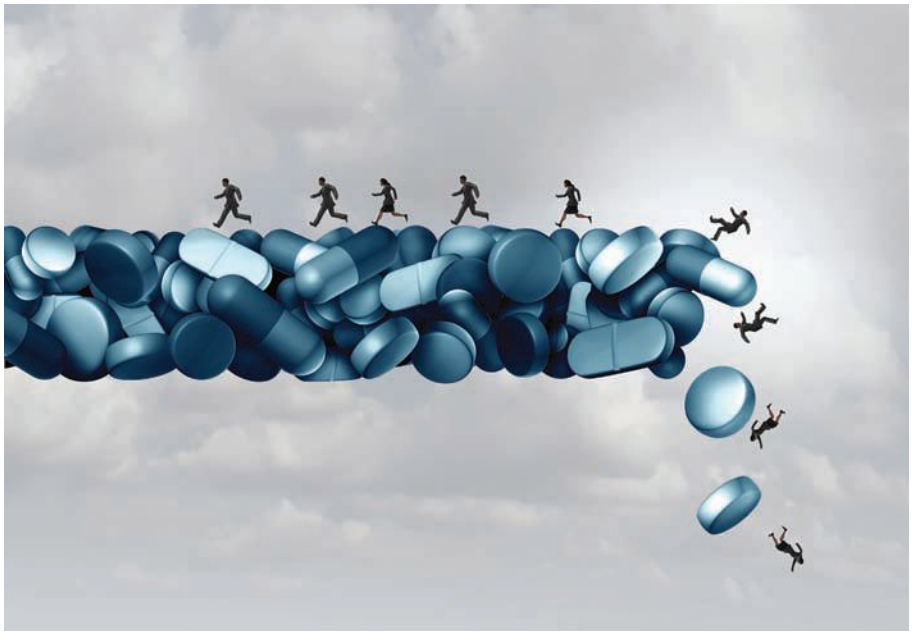
STUDY: Methionine is an essential amino acid, meaning that it must be supplied by dietary proteins. Among other functions, it's required to make S-adenosylmethionine (SAM), a molecule that helps to methylate DNA and histones. Locasale began studying methionine metabolism as a way to understand how cells sensed metabolic changes and whether they could communicate that information to chromatin. In a recent study, his team reported that SIRT1, a highly conserved mammalian enzyme that regulates methionine metabolism in mouse embryonic stem cells, could also tweak epigenetic markers that control differentiation. When the researchers deleted or knocked out SIRT1 in mouse embryonic stem cells, mutant cells had high levels of methionine but low levels of SAM—and consequently, many histone methylation marks were lost, leading to a loss of pluripotency. But other SIRT1 activities were unaffected. The team suggested that stem cells lost pluripotency because the enzyme that converts methionine to SAM is controlled by SIRT1 (*EMBO J,* 36:3175–93, 2017). "We knew that SAM and acetyl-CoA interact with chromatin via enzymes to establish these epigenetic modifications, but the extent to which it happened and how wasn't resolved," Locasale says.

APPROACH: To study which amino acid metabolic pathways were affected by knocking SIRT1 out, Locasale's team turned to high-resolution mass spectroscopy, which allowed the researchers to measure the concentrations of hundreds of metabolites in less than an hour. Then, they applied an algorithm to the data that compared metabolites from the experiment to a standardized library of metabolites to infer how nutrients flowed from one metabolic pathway to another. They put the data from a mass spectroscopy run to work in many ways: to measure all the metabolites that reacted with chromatin at a given time point, to identify all the metabolites that were responsive to changes in methionine levels, and to measure how much variation in metabolism contributed to chromatin changes. "When we have this level of detail [on metabolite fluxes], we can quickly test hypotheses and generate alternate possibilities as needed," Locasale says. "Ten years ago, this sort of work would have taken a lot more time and effort." ■

A Safer Poppy

From basic research in academic labs to Phase 3 clinical trials, myriad efforts are underway to divorce opioids' pain-relieving potential from their undesirable downsides.

BY JENNY ROOD



Opioid drugs are well-established double-edged swords. Extremely effective at analgesia, they cause an array of harmful side effects throughout the body, including itching, constipation, and respiratory depression—the slowed breathing that ultimately causes death in overdose cases. What's more, the body's interaction with opioids is dynamic: our receptors for these compounds become desensitized to the drugs' activity over time, requiring ever larger doses to suppress pain and eventually provoking severe dependence and protracted withdrawal.

In the past few years, these side effects have plagued growing numbers of US citizens, plunging the country into the throes of a devastating opioid crisis in which nearly 100 people die from overdoses every day. Even so, opioids are still among the most effective pain-relief options available. "Over hundreds of years, [opioid receptors] have remained a target," says Laura Bohn, a biochemist at the Scripps Research Institute in Jupiter, Florida. "Therapeutically, it works."

Since the early 2000s, intriguing evidence has emerged suggesting that opioids' useful properties could be separated from their harmful attributes. (See "Pain and Progress," *The Scientist*, February 2014.) In 2005, Bohn, then at the Ohio State University College of Medicine, and colleagues showed that shutting down one of the signaling pathways downstream of the opioid receptor targeted by morphine not only amped up the drug's painkilling effects in mice, but also reduced constipation and respiratory depression (*J Pharmacol Exp Ther*, 314:1195-201).

That research opened the door to developing a new type of opioid: a "biased agonist" that could trigger analgesia without tripping the switches on other pathways that cause side effects. Now, more than a decade later, Trevena Inc.'s Olinvo (oliceridine)—a drug based on this principle and designated by the US Food and Drug Administration (FDA) as a breakthrough therapy—has completed Phase 3 clinical trials.

Olinvo is just one of many such drugs under development. From compounds that act only in specific regions of the body to those that engage multiple receptor types, researchers and pharmaceutical companies are trying many different tactics to produce less-dangerous opioids.

Hitting where it hurts

Although the main types of opioid receptors—mu, delta, and kappa—and the nociceptin receptor (NOP), previously called the opioid receptor–like receptor, are located on multiple cell types throughout the body, the ones in the central nervous system (CNS) are the most critical for managing body-wide pain. Hitting CNS receptors, however, can also light up reward centers in the brain, causing an immediate high, drug-seeking behavior, and eventual dependence if use is continued.

Some types of pain are localized, though. For example, when immune response–mediated inflammation around damaged tissue triggers pain-sensing neurons, pain may be felt only at the site of the inflammation. Because inflamed tissues are more acidic than their surroundings, a group led by anesthesiologist Christoph Stein of the Charité university hospital in Berlin, Germany, wondered if it would be possible to exploit pH to restrict an opioid's activity to the site of pain, thus avoiding the CNS altogether. Using computer simulations of interactions between the mu opioid receptor and the drug fentanyl, the researchers designed molecules that were active only under acidic conditions, including a new opioid receptor agonist

called NFEPP, a modified version of fentanyl. Tested on rats with foot injuries, NFEPP treated pain without addicting the rodents to the drug or slowing their breathing (*Science*, 355:966-69, 2017). Although the road to the clinic is long, Stein notes that the compound could be useful in both surgical settings and as a replacement for anti-inflammatory drugs (NSAIDs) such as ibuprofen, which carry their own toxicities.

Cara Therapeutics's compound CR845, a kappa agonist, is also designed to target pain at the source—although rather than being specifically targeted to peripheral nerves, it avoids the CNS thanks to a peptide structure that reduces the compound's ability to cross the blood-brain barrier, according to the company. Intravenously administered CR845, currently in Phase 3 trials, has been tested for both chronic and acute pain, as well as itching, and shows relatively minimal opioid side effects, the company reports; the oral formulation, meanwhile, is in Phase 2 trials. Bob Twillman, executive director of the Academy of Integrative Pain Management (AIPM) tells *The Scientist* that such a drug could be particularly valuable for pain caused by inflammation, such as in arthritis, or in a post-operative setting.

Meanwhile, rather than blocking entry into the CNS altogether, Nektar Therapeutics is trying to slow it down. The company strategy aligns with research from Nora Volkow of the National Institute on Drug Abuse (NIDA). Her work has demonstrated that cocaine and other abused drugs cause rapid dopamine spikes in the brain's reward center, the nucleus accumbens.

Nektar's drug NKTR-181 is active throughout the brain, but consists of a morphine scaffold with short polyethylene glycol chains that slow the compound's crossing of the blood-brain barrier to avoid inciting wild surges in dopamine. This slowed entry makes the drug a poor choice for acute pain, says Nektar's chief scientific officer Steve Doberstein, but an excellent one for chronic pain.

The company hopes that NKTR-181's trial results so far—demonstrating efficacy in treating chronic back pain, with minimal side effects, no withdrawal symptoms, and low abuse potential even at triple the maximum drug dose—will convince the FDA that the compound is ready for a New Drug Application in early 2018. If Nektar successfully clears that regulatory hurdle, the company hopes to launch the drug by early 2019, Doberstein says.

> There's truly a complexity here that hasn't been scratched.
>
> —Gavril Pasternak
> Memorial Sloan Kettering Cancer Center

A one-two punch

Another approach to managing side effects is to tweak opioid biology by engaging multiple receptors simultaneously. For example, "there's a whole range of other receptors that modulate mu dependence," says pharmacologist Andrew Coop of the University of Maryland. Combining a mu opioid agonist with a compound targeting NOP, delta, or kappa receptors can increase analgesia and decrease side effects such as tolerance and dependence—the downregulation and desensitization of receptors so that the drug fails to produce a "high" even at increasing levels and withdrawal causes acute psychological and physiological distress. As a result, several teams are now developing compounds that can hit two receptors at once.

One such molecule is BU08028—an altered version of the existing drug buprenorphine—which serves as a partial agonist of both the mu (buprenorphine's original target) and NOP receptors. Developed by medicinal chemist Stephen Husbands of the University of Bath in the U.K., BU08028 became the first opioid to successfully treat acute pain in macaques without triggering the side effects of drug-seeking behavior, respiratory depression, or itching (*PNAS*, E5511-E5518, 2016). As primates are a better model than rodents for human biology when it comes to opioid side effects, the findings suggest that this or a similar drug may stand a good chance of success in people, says study coauthor Mei-Chuan Ko, a pharmacologist at Wake Forest University. Husbands plans to follow up on a similar compound that differs enough from buprenorphine to be patentable.

Meanwhile, Coop is pursuing UMB425, a mu agonist/delta antagonist combo that prevents these receptors from interacting. The compound doesn't cause dependence, but does lead to drug-seeking behavior in animals (as measured by self-administration of the drug), a side effect Coop is currently working to eliminate. Other efforts underway include Jane Aldrich's studies at the University of Florida of a cyclic four-amino-acid peptide called CJ-15,208 that hits both kappa and mu receptors, reducing the activation of the reward system.

Yet other multitarget drugs may help identify new target receptor pairings that trigger different downstream pathways to increase pain relief and reduce side effects. For example, Philip Portoghese at the University of Minnesota is studying compounds that bring together targeting of receptors on neurons and on glia—the brain's immune cells—which become activated during inflammation and sensitize pain neurons. To make analgesia more effective, "we not only have to target and activate the opioid receptors in neurons so that they will inhibit pain, but we have to block glia," he says. (See "Glia and Pain" on page 34.)

MMG22, a compound designed and generated by Portoghese's University of Minnesota collaborator Eyup Akgün, does exactly that. It contains an opioid agonist tethered to an antagonist for the metabotropic glutamate receptor 5 (mGLuR5), which in turn targets receptors present in both neurons and glial cells called astrocytes, Portoghese says. The compound has been shown to be effective for both neuropathic and inflammatory pain in mice (*Pain*, 158:2431-41, 2017). In addition, the drug doesn't appear to produce tolerance or respiratory depression in rodent models, Portoghese says.

One of Portoghese's former students, Ajay Yekkirala, is pursuing a different early-stage receptor pair–targeting drug, BLUE-181, at the Massachusetts-

based startup Blue Therapeutics. The compound appears to be substantially more potent than morphine, but doesn't seem to trigger drug-seeking, withdrawal symptoms, tolerance, or respiratory depression, Yekkirala says; it's currently undergoing preclinical safety and toxicity studies.

Picking a pathway

The biased agonism approach suggested by Bohn's early-2000s mouse studies is also a hot area of research. Bohn herself is investigating a biased agonist targeting the kappa opioid receptor that prevented pain and itch in rodents without causing sedation, increased dopamine levels, or another side effect associated with kappa agonists, dysphoria—the opposite of a high (*Sci Signal*, 9:ra117, 2016). Such compounds are useful both as potential drugs and as tools to help further unravel the complex biology of opioid receptors, says opioid researcher Gavril Pasternak of Memorial Sloan Kettering Cancer Center. Plus, combining biased agonists with new receptor classes could open the door to new drugs, he adds. "There's truly a complexity here that hasn't been scratched."

To explore that complexity, some researchers are using bioinformatics to find new biased agonists. The University of California, San Francisco team of Brian Shoichet and Aashish Manglik recently unveiled PZM21, a compound effective

A SELECTION OF POTENTIALLY SAFER ALTERNATIVES TO CONVENTIONAL OPIOIDS

Drug developers are investigating three main approaches to creating safer opioid drugs. Biased agonists (found in dark tan boxes) are designed to activate only the pathways that cause analgesia, and not those leading to harmful side effects. Drugs employing specific targeting mechanisms (light tan) aim to localize opioid activity to the site of pain and avoid activation of the central nervous system pathways that lead to dependence. Combinatorial compounds (white) target multiple receptors at once to modify receptor activity and reduce off-target effects.

Drug	Lead scientist or company	Target	Reported mechanism	Stage of development
Olinvo (oliceridine, TRV130)	Trevena Inc.	Mu opioid receptor	Selectively activates the G protein pathway, as opposed to all downstream signaling pathways	Completed Phase 3
NKTR-181	Nektar Therapeutics	Mu opioid receptor	Enters brain more slowly than typical opioids	Phase 3
CR845	Cara Therapeutics	Kappa opioid receptor	Designed to act only in peripheral nervous system, supposedly does not cross blood-brain barrier	Phase 3
BLUE-181	Blue Therapeutics	Unspecified heteromer	Targets not specified	Preclinical
PZM21	Brian Shoichet and Aashish Manglik, University of California, San Francisco	Mu opioid receptor	Selectively activates the G protein pathway	Basic research (to be further developed by Epiodyne)
BU08028	Stephen Husbands, University of Bath	Mu opioid receptor and NOP receptor	Serves as partial mu and NOP receptor agonist	Basic research
UMB425	Andrew Coop, University of Maryland	Mu and delta opioid receptors	Serves as a mu opioid receptor agonist and a delta opioid receptor antagonist	Basic research
Triazole 1.1	Laura Bohn, Scripps Florida	Kappa opioid receptor	Selectively activates the G protein pathway	Basic research
MMG22	Philip Portoghese, University of Minnesota	Heteromer of mu opioid receptor and mGluR5 (on glia cells in the brain)	Engages both neuronal opioid receptors and receptors on glia cells	Basic research
CJ-15,208	Jane Aldrich, University of Florida	Mu and kappa opioid receptors	Balances activation of mu and kappa receptors	Basic research
NFEPP	Christoph Stein, Charité Hospital, Berlin	Mu opioid receptor	Activated only in acidic tissues, thus targeting inflammation sites	Basic research

for pain relief in mice that they identified through a computational screen of three million drugs that bind to opioid receptors' crystal structures (*Nature*, 537:185-90, 2016). Their startup, Epiodyne, will seek to commercialize the compound. Pasternak calls the work "an extraordinary step forward," and notes that the approach allows researchers to identify new compounds that might look nothing like existing opioids—although he cautions that crystal structures might not always reflect a protein's native conformation.

Further along still is Trevena's injectable Olinvo, which targets acute postsurgical pain, and could reduce the opioid risk profile by widening the "therapeutic window"—the range between doses that relieve pain and cause side effects, explains Trevena cofounder Jon Violin. So far, five studies—spanning Phase 1, 2, and 3 clinical trials—suggest that Olinvo triggers fewer instances of respiratory depression and constipation than morphine while still providing potent analgesia (*Pain*, 157:264-72, 2016; *J Pain Research*, 10:2413-24, 2017).

Nevertheless, Charité's Stein remains unconvinced that it is possible to separate side effects from analgesia by activating different pathways. Beyond animal studies, "there's practically no evidence that this hypothesis is true," he says. Dave Thomas, a program official at NIDA and a member of the National Institutes of Health Pain Consortium, agrees there's work to be done. "We're hopeful, but I think we're still a long way away from a magic bullet," he says. "We haven't been able to separate the good and bad of opioids, and it's not for lack of trying."

A broad approach

If one or more of these drugs does break through—and a few, including Nektar's and Trevena's compounds, appear poised to do so—how might they change pain management and drug abuse? "If we can stop the abuse that leads to addiction, we can cut that cycle off," says Nektar's Doberstein. However, he adds that even if NKTR-181 is successful, there will have to be alternatives on the market—50 million people in the U.S. experience chronic pain severe enough to need opioid therapy, and there is unlikely to be a one-size-fits-all solution to their needs.

New opioid medications that reduce harmful side effects would certainly be a useful addition to the toolbox, says AIPM's Twillman, though he is skeptical that a truly non-addictive opioid will ever exist. Instead, he and NIDA's Thomas advocate for a multipronged approach to treating pain that also incorporates non-opioid management strategies. "How we got into [the opioid] crisis," Thomas says, "is by putting all our eggs in the opioid basket." ■

F*ck, That Hurts

Why pain and swearing are a match made in neurological heaven

BY EMMA BYRNE

It was pain that got me hooked on swearing.

I was working as a computational neuroscientist, based in London's Science Museum, and I was looking for interesting experiments to demonstrate to visitors. I read about a study that needed no more than a stopwatch, a bowl of ice water, and volunteers who were willing to keep their hands submerged as long as possible in the freezing water—once while saying a neutral word, and once while swearing.

My version of the study was due to be run at a late-night event that included access to a bar, so I already knew that our results would be a curiosity at best. But in the original experiment, carried out under more-controlled (and less alcohol-soaked) conditions by Richard Stephens at Keele University in 2009, the results were nevertheless striking and similar to my own. Using a swearword rather than a neutral word had two significant effects: it allowed the volunteers to keep their hands in ice water for about half again as long, and swearing subjects reported that the water actually felt less painful.

At that point, it was a toss-up whether I'd end up writing a book on the science of swearing or one on pain, because something about that experiment really intrigued me, and still does. As my pile of research findings on strong language steadily grew higher, I decided *Swearing Is Good for You* was the book I wanted to write.

While writing the chapter on pain and swearing, I realized that pain is not a purely neurological phenomenon. Sure, peripheral sensory neurons give you information about a stimulus, but the way you process that pain is as much psychologically constructed as it is neurologically formed. Our anticipation of pain, our gender roles and social expectations, even whether we're feeling lonely or sad, all change the way we feel pain. Swearing is just one of those factors. So how does it work?

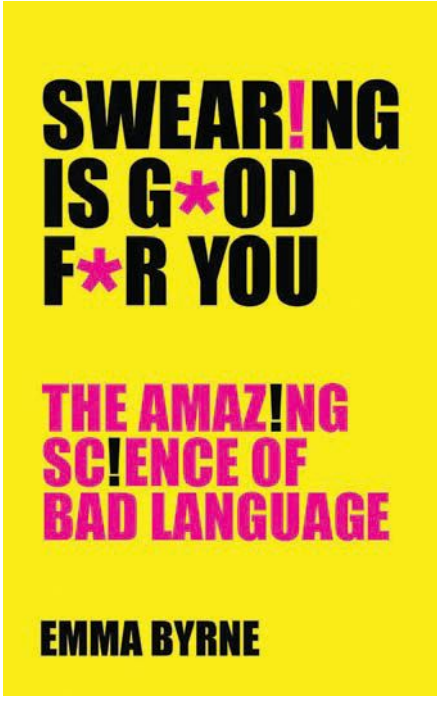
Swearing is a unique part of our language, bound up with our emotions, our communication, our sensory experiences, and our societies.

In Stephens's experiment, he took care to rule out some purely cognitive effects. He wanted to be sure that the volunteers weren't distracting themselves with more creativity or varied language in one trial versus the other, so he allowed them only one word on the swearing trial (such as "shit") and one word on the neutral trial (such as "wooden").

To try to minimize the effect of one word being more difficult to recall than the other, Stephens asked each volunteer for five words they would use if they dropped a hammer on their thumbs, and five words to describe a table. Then he took the first word on each list.

The study clearly showed that swearing affected the volunteers' perception of pain, reducing its intensity. Stephens's lab is now using video games, measures of people's background levels of aggression, and different types of swearing to try to uncover why swearing is such a powerful analgesic.

Follow-up experiments suggest that "minced oaths"—those socially palatable curses we trot out when we think we might be overheard—just don't work as well as the real thing. Intriguingly, the same is true in patients with Tourette syndrome. Using a softer form of swearing gives them much less relief from the urge to tic, like rubbing an itch instead of scratching it.



Profile Books, November 2017

We're still not entirely certain what it is that makes swearing an effective painkiller, but in the research for *Swearing is Good for You*, I discovered that it is a unique part of our language, bound up with our emotions, our communication, our sensory experiences, and our societies. ■

Emma Byrne researches artificial intelligence for 10x Future Technologies. Her research has been published in Science *and* The BMJ*, among other publications. Read an excerpt of* Swearing Is Good for You: The Amazing Science of Bad Language *at the-scientist.com.*

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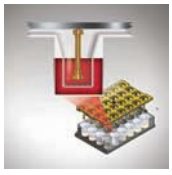

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photo: course picnic at CSHL beach

Meetings

Systems Biology: Global Regulation of Gene Expression
March 20 - 23

The PARP Family & ADP-ribosylation
April 3 - 6

Neuronal Circuits
April 11 - 14

Protein Homeostasis in Health & Disease
April 17 - 21

Gene Expression and Signaling in the Immune System
April 24 - 28

Nuclear Organization & Function
May 1 - 5

Biology & Genomics of Social Insects
May 5 - 8

The Biology of Genomes
May 8 - 12

Regulatory & Non-Coding RNAs
May 15 - 19

Retroviruses
May 21 - 26

Brains & Behavior: Order & Disorder in the Nervous System
May 30 - June 4

Glia in Health & Disease
July 19 - 23

Mechanisms & Models of Cancer
August 14 - 18

Genome Engineering: The CRISPR/Cas Revolution
August 22 - 25

Single Biomolecules
August 28 - September 1

Translational Control
September 4 - 8

Epigenetics & Chromatin
September 11 - 15

Molecular Mechanisms of Neuronal Connectivity
September 25 - 29

Mechanisms of Aging
October 1 - 5

Germ Cells
October 9 - 13

Nutrient Signaling
October 25 - 28

Transposable Elements
November 1 - 4

Probabilistic Modeling in Genomics
November 4 - 7

Biological Data Science
November 7 - 10

Neurodegenerative Diseases: Biology & Therapeutics
November 28 - December 1

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Courses

Cryoelectron Microscopy
March 1 - 14

Cell & Developmental Biology of Xenopus
April 4 - 17

Expression, Purification & Analysis of Proteins & Protein Complexes
April 4 - 17

Quantitative Imaging: From Acquisition to Analysis
April 4 - 17

Advanced Bacterial Genetics
June 5 - 25

Ion Channels in Synaptic and Neural Circuit Physiology
June 5 - 25

Workshop on Schizophrenia & Related Disorders
June 6 - 13

Mouse Development, Stem Cells & Cancer
June 6 - 25

Metabolomics
June 9 - 25

Statistical Methods for Functional Genomics
June 29 - July 12

Advanced Techniques in Molecular Neuroscience
June 29 - July 14

Single Cell Analysis
June 29 - July 14

Drosophila Neurobiology: Genes, Circuits & Behavior
June 29 - July 19

Frontiers & Techniques in Plant Science
June 29 - July 19

Computational Neuroscience: Vision
July 9 - 22

Synthetic Biology
July 24 - August 6

Chromatin, Epigenetics and Gene Expression
July 24 - August 12

Imaging Structure & Function in the Nervous System
July 24 - August 13

Yeast Genetics & Genomics
July 24 - August 13

Cellular Biology of Addiction (in UK)
July 29 - August 5

Genetics & Neurobiology of Language
July 30 - August 5

Brain Tumors
August 7 - 13

Proteomics
August 7 - 21

Programming for Biology
October 15 - 30

X-Ray Methods in Structural Biology
October 15 - 30

Advanced Sequencing Technologies & Applications
November 6 - 18

Computational Genomics
November 28 - December 5

The Genome Access Course
March 26 – 28 & September 23 - 25

Professional Development: Workshop on Leadership in Bioscience
March 23 - 26

Scientific Writing Retreat November 14 - 18

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Fake News: Mars Edition, circa 1877

BY DIANA KWON

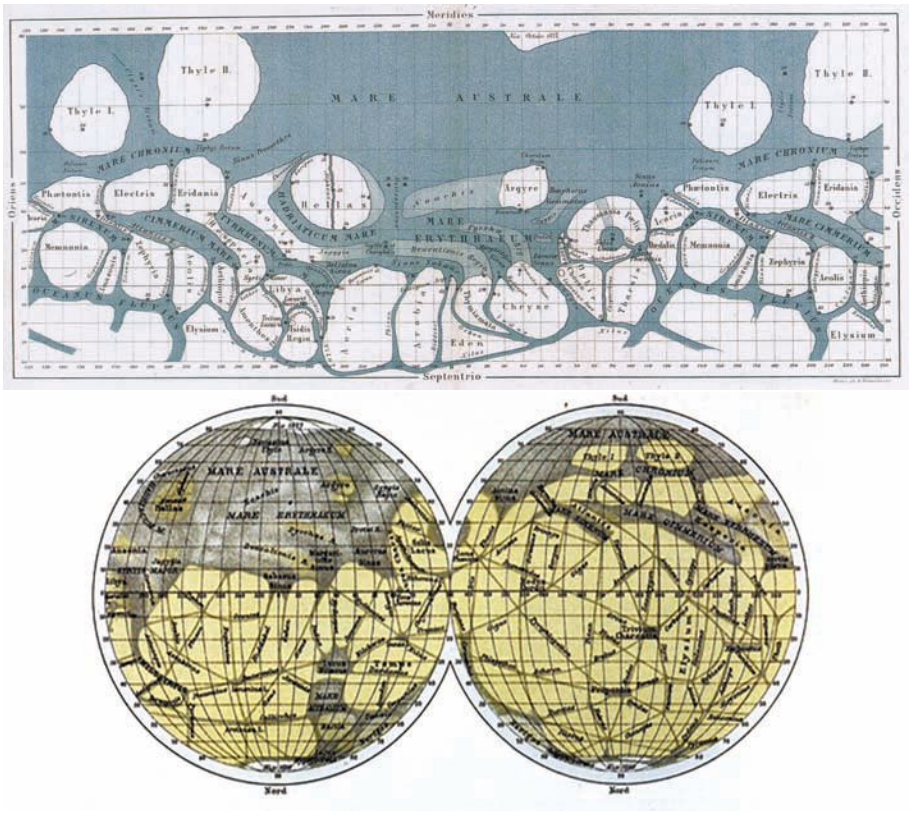
When Italian astronomer Giovanni Schiaparelli peered at Mars through a powerful telescope in the late 1800s, he observed dark channels raked across its surface. These features became the key characteristics in his detailed maps of the planet, which fueled more than a decade of wild speculation regarding alien-built canals.

Schiaparelli's drawings were provocative. His first detailed map of Mars, published in 1877, laid out an intricate network of channels (or *canali* in Italian) that were colored in blue, in sharp contrast to other representations at the time that marked the Martian surface in the reddish-orange shades that more closely approximate the planet's actual hue. His later diagrams became more abstract—winding waterways became straight, dark lines—partly in response to criticism from other astronomers, says Maria Lane, a historical geographer at the University of New Mexico who wrote *Geographies of Mars*, a book about Schiaparelli's and others' maps.

These 19th-century diagrams set the stage for the work of Percival Lowell, a wealthy American businessman and amateur astronomer. Lowell built his own private observatory, one of the best for viewing Mars at the time, and drew his own detailed maps of the planet.

Lowell believed the canals Schiaparelli observed were made by intelligent beings, and proposed that Mars was covered in an intricate irrigation system that brought water from the poles to grow vegetation on the rest of the planet. Using his own observations as evidence, Lowell championed this hypothesis in lectures, books, and stories in the popular press. His extraordinary claim made headlines in both North America and Europe. *The New York Times*, for example, published the 1911 headline, "Martians build two immense canals in two years."

These concepts also influenced popular culture: from H.G. Wells's *War of the Worlds* in 1898 to Ray Bradbury's *Martian Chronicles* in 1950, the notion of an intelligent Martian race persisted for decades in science fiction.



OTHERWORLDLY CARTOGRAPHY: In Schiaparelli's first detailed map of Mars, finished in 1878 (top), he colored channels blue and their surroundings white. His later schematics (bottom, completed in 1888) became more abstract, with straight lines and dustier shades. Despite the detail in these drawings, it was incredibly hard to see the features of Mars through telescopes of the time. Astronomers created intricate diagrams like these by compiling small sketches from multiple nights of observation.

Many scientists at the time were extremely skeptical of Lowell's claims. The belief in Martian engineering received a huge blow in 1909, when the French astronomer Eugène Antoniadi, who was observing Mars through one of the most powerful telescopes of the time, published a detailed map showing that up close, the canals were actually smaller, irregular features more indicative of natural origins.

Some historians attribute the belief that the Red Planet was inhabited by intelligent beings to mistranslation of the Italian word *canali* to "canals" in English—the latter conveying artificiality. But Lane maintains the notion sprang mainly from Schiaparelli's maps themselves. "It's a cartographic process that I think is really influential," she says. "Once you saw these linear features—that's what convinced people that they were probably canals."

Even today, it's still unclear what, exactly, Schiaparelli was seeing. Telescopes at the time were much weaker than today's. "The big limitation was that Schiaparelli was working visually," says Steven Dutch, a professor emeritus of geology at the University of Wisconsin–Green Bay. "Even through a very large telescope, Mars looked about the size of the full moon to the unaided eye." ■

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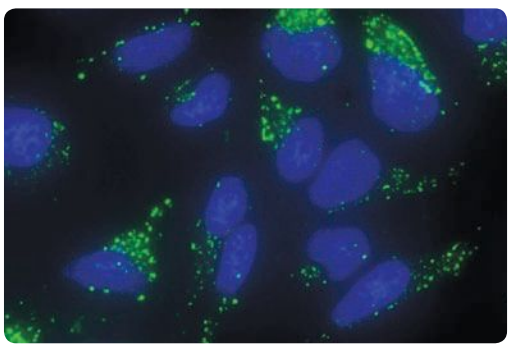

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