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BEYOND THE BASIC FIVE

HOW MANY SENSES ARE THERE?

PROPRIOCEPTION: YOUR PLACE IN SPACE

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The time is now for the scientific community to come together to respond to the challenges of reproducibility and to be part of the solution.

It takes the ingenuity and hard work of the entire research community to move science forward. We build on the ideas and results of our peers to discover new avenues of investigation that in turn inspire ideas for others to advance.

In this way, every new publication acts as a stepping stone, paving the way to a clear and reliable path towards solving a particular scientific problem. Countless diseases have yielded to this approach over the past 100 years, which is a testament both to the expertise and resourcefulness of the community and the strength of its methodology.

Recently, however, there have been reports in journals such as *Nature* and *Science*, stating that the published literature is becoming less reproducible. These reports suggest that the methods that have served us well in the past may now be failing to faithfully guide us.

As a company rooted in science, we are troubled by these reports. Our mission has always been to produce and rigorously validate our products in-house, so they will work dependably in your experiments and be useful to the important experiments they support. Our approach to product development was affirmed by our peers who ranked us as the number one company for reproducibility, sensitivity, specificity and technical support in 2015*. We were honored to be so recognized, and we feel strongly that this recognition comes with a responsibility to act as a leader in addressing the growing reproducibility crisis.

To this end, Cell Signaling Technology is partnering with the Global Biological Standards Institute (GBSI) as well as representatives from industry and the manufacturing, publishing and academic fields. This group is hosting an online discussion with the community, this summer, to generate as many opinions and ideas from the community as it can. The group will then convene this September to review the fruits of the online discussion, draft consensus definitions of reproducibility and its underlying causes, and offer technological and process-oriented solutions.

The reproducibility crisis is undoubtedly a complicated problem, but the challenge is not insurmountable. This is especially true if we address the problem the way we would any other: head-on and as a community.

Together, we can create a means to do better; we can strengthen our faith in the published literature and be confident in our ability to advance biomedical science.

We invite you to join the discussion
www.cellsignal.com/GBSI

Sincerely,

Roberto Polakiewicz

Roberto Polakiewicz, Ph.D.
Chief Scientific Officer
Cell Signaling Technology

**2015 Biocompare Antibody Market Report*

16PADSCORP0091ENG
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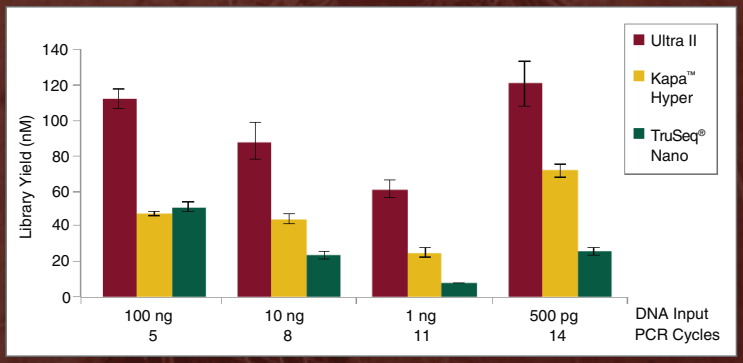

Libraries were prepared from Human NA19240 genomic DNA using the input amounts and numbers of PCR cycles shown. Manufacturers' recommended protocols were followed, with the exception that size selection was omitted.

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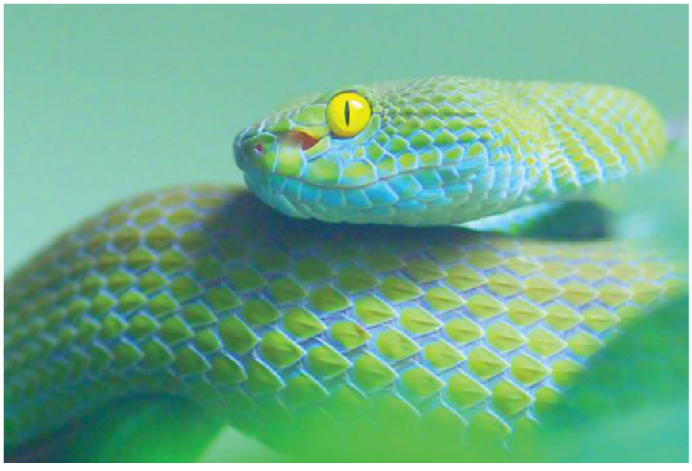

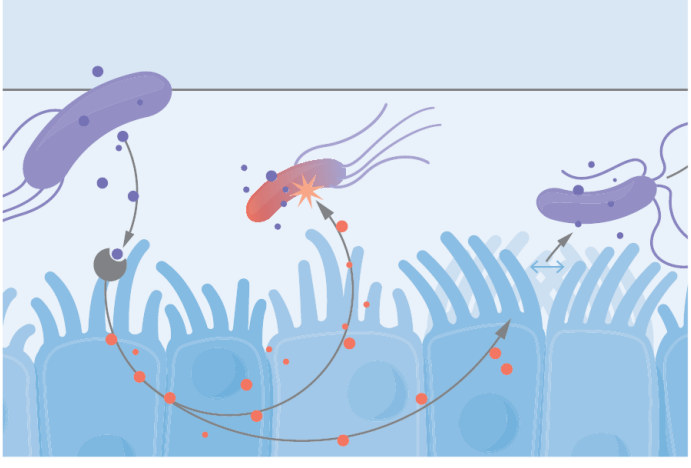

Features

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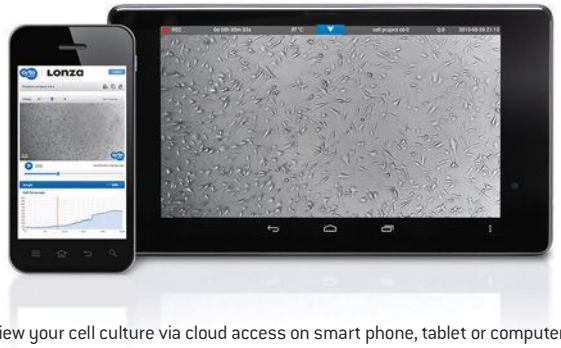

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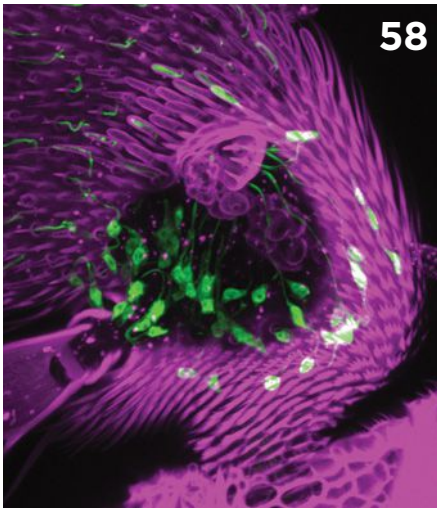

LUND UNIVERSITY, ANDERS ENJIN; COURTESY OF DEAN BUONOMANO; © BRYAN SATALINO

CORRECTIONS:

In "Pinpointing the Cause" (*The Scientist*, July 2016), the micrograph on page 34 does not depict a white fat cell. A reader has identified the cell as a mast cell.

In the last sentence of "Catching a Lift" (*The Scientist*, August 2016, page 47), "by outcrossing species" should read "in outcrossing species."

The Scientist regrets the errors.

Online Contents

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Seeing Heat
Learn how some snakes sense infrared radiation using specialized sense organs in their faces.

SLIDE SHOW
Friends of Hornbills
Meet members of the native tribe in northeastern India who protect the birds they once hunted.

VIDEO
Mag-Neato!
Scientists are unraveling how animals use Earth's magnetic field to navigate.

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

Coming in October

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

- As *The Scientist* turns 30, we look at sequencing, gene editing, neuroscience, microscopy, and stem cells then and now
- Horizontal gene transfer in multicellular hosts
- Imaging single RNA molecules during translation
- Profile: George Church
- Pet medicines

AND MUCH MORE

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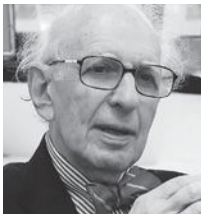
Contributors

Uwe Proske moved from Germany to Australia when he was 10. "At the time, to us, Australia seemed like the Wild West," he recalls. He earned his PhD in 1968 in the lab of A.K. McIntyre at Monash University, where he returned for the bulk of his career, starting as a researcher in 1971 and working his way up to full professor (now emeritus). His research has focused on diverse animals' sensory abilities, from snakes' detection of infrared radiation to the platypus's detection of electric fields. In the latter part of his career, he focused on the mechanism of proprioception in reptiles and mammals, including people. "It is a topic that I have carried into my retirement and for which I continue to have plenty of enthusiasm," he says.

Simon Gandevia grew up in Melbourne, Australia, with his two physician parents. "From a young age they instilled in me a deep interest in all things biological and in different ways of looking at them," he recalls. He began studying medicine at the University of New South Wales, but his interest in the human body led him to pursue a degree in physiology instead. He later returned to finish his medical degree, training with Ian McCloskey at New South Wales and David Burke at the Prince Henry Hospital for a PhD and an MD, respectively, focusing on proprioception and human movement control. In 1992, Gandevia and his colleagues established the Prince of Wales Medical Research Institute, now called Neuroscience Research Australia (NeuRA), where he now serves as deputy director and works with patients suffering from various motor impairments. "All forms of sensory physiology are fascinating: how can an element of the external world become a subjective experience? This is so much more than just the often extraordinary translation of physical stimuli into nerve signals, especially for proprioception."

Their feature "Proprioception: The Sense Within" appears on page 36.



Columbia University neuroscientist **Eric Kandel** was born in the ashes of the Austro-Hungarian Empire. But even in 1929, just 11 years after the Allied victory in World War I, his hometown of Vienna still celebrated its historically vibrant cultural scene, with authors, painters, composers, and thinkers mingling in the city's cafes and boulevards. Steeped in this rich milieu, the young Kandel developed a profound appreciation for art, culture, and intellectualism. But by 1939, as the Nazi regime rose to power in the region, the Kandel family (and countless other Viennese Jews) were forced to relocate.

"My early experiences in Vienna almost certainly contributed to my curiosity about the contradictions and complexities of human behavior," Kandel wrote in his Nobel Prize autobiography. "How could a highly educated and cultured society, a society that at one historical moment nourished the music of Haydn, Mozart, and Beethoven, in the next historical moment sink into barbarism?" After doing an undergraduate dissertation at Harvard University on the contribution of European intellectuals to the rise of the Nazis and then studying psychiatry at New York University, Kandel switched to neurobiology. His work on the basic neurological components of memory netted him a Nobel Prize in 2000.

He and his wife are avid collectors of art and antiques. In "Tapping Into the Artful Brain" (page 74), Kandel muses on the reductive similarities between mid-century abstract art and modern neuroscience, the subject of his latest book, *Reductionism in Art and Brain Science: Bridging the Two Cultures.*

Born and raised in East Los Angeles, editorial intern **Alison F. Takemura** opted for an undergraduate experience different from many of her UCLA– and UC, Berkeley–bound classmates: Rice University in Houston. The small school appealed to her, especially its ability to give students hands-on research exposure from the get-go. She first worked on making nanoparticles out of gold, then engineering *E. coli* to better break down plant material. For graduate school, Takemura chose to study microbiology at MIT.

Under the leadership of Martin Polz, Takemura investigated the metabolic requirements underlying *Vibrio* bacteria's ability to be either vegetarians or carnivores. During her PhD, Takemura earned a fellowship in the Biological Engineering Communication Lab, where she mentored fellow students in conveying their science, whether in a manuscript, poster, or presentation. Upon completing her doctorate, she headed back to California for the science-writing program at the University of California, Santa Cruz, and graduated in June.

In reporting on hawkmoths' sense of smell (page 23), Takemura became enamored of the massive insects. And after learning that they live near her in the Boston area, "it's my dream to find one," she says. Fortunately, Takemura will be sticking around—later this month she returns to MIT to be a Communication Lab manager.

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Sensory Overload

There's far more to the world than what humans perceive.

BY MARY BETH ABERLIN

Having devoted one issue per year to each of the five basic senses—taste, touch, smell, sight, and hearing—herein you have the something "extra" I promised in my September 2015 editorial: an issue that examines senses beyond those basic five. But how many "extra" senses are there, really?

Tongues, fingers, noses, eyes, and ears are obvious gatherers of sensory information. But other sensory systems are more subtle. Our first feature (page 36) describes the current state of research on a sense that offers few external clues about how it functions. Proprioception, often referred to as the sixth sense, allows us to process where our limbs are in space and in relation to each other so we can coordinate their movements. "While we have learned a lot in recent years about the peripheral signals responsible for the senses of limb position and movement, the picture continues to evolve. . . . Yet we still know relatively little about the central processing of the incoming information," write researchers Uwe Proske and Simon Gandevia. They explain their long-term fascination thus: "Our very sense of self is believed to be generated in association with the central processing of proprioceptive information."

The human body also contains sensory receptors where you would never expect them. It seems fantastical to say that skin, kidneys, muscles, and even sperm can smell; that light-sensitive pigments in blood vessels can make those vessels relax; that taste receptors in our guts can go "yuck" to pathogens and bacteria. But there's no nonsense involved in freelancer Sandeep Ravindran's feature article about seemingly out-of-place sensory receptors (page 51), and there may be potential therapeutic benefit from researching such unexpected physiological phenomena. One researcher Ravindran interviewed touted an advantage of activating taste receptors to stimulate a person's immune cells: "you wouldn't expect to get drug-resistant bacteria."

Because many members of the animal kingdom can perceive sensory signals humans can't (but often wish they could), we also decided to take a look at nonhuman sensory apparatuses, some of which, like the hair cells of the fish lateral line system, are precursors of human cell types. A staff-written feature ("Senses Census," page 43) reports on some of these sensory organs and the responsible anatomy. We offer a whole universe of sensations beyond what humans experience: electro-, magneto-, thermo-, and mechanoreception (via the lateral line), and gravity detection by invertebrates, some of which, like the comb jellies, don't even have a nervous system.

In the future, researchers armed with a deeper understanding of these (to us) exotic animal senses may be able to enhance the human sensory repertory, writes hearing researcher Bernd Fritzsch in a Thought Experiment, "Acquiring Extra Senses" (page 30).

Other articles in this "extra-sensory" issue include two Notebooks: one on a wearable smart skin that integrates touch, temperature, and magnetic-field sensors (page 17), and the other on finding olfactory sensors on a sphinx moth's proboscis, not just in the usual spots on the insect's antennae (page 23). All three articles in The Literature section (page 58–59) report on recent papers involving unusual sensing mechanisms: polarized light perception by orchid bee ocelli (three simple eyes on the top of the head), humidity sensing by insects, and pH sensing in the vertebrate central nervous system. And a profile of UCLA neurobiologist Dean Buonomano recounts his lifelong dedication to figuring out how the brain senses time (page 60). A Foundations article (page 80) delves into early studies (and subsequent debunking) of a different kind of extra sense: ESP.

One day, science may endow us humans with more senses than we currently employ to interface with our world. But for now, sit back and enjoy what your eyes already allow you to do: read this beautiful issue. ■

MBA

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

ANDRZEJ KRAUZE

Speaking of Science

Mantis shrimp, or stomatopods, are well known for aggressive temperaments and complex visual systems, but until now we've known very little about whether and how they use color to communicate with other mantis shrimp. Our experiments demonstrate that they use a complex signaling system that combines the UV reflectance of an important spot of color as well chemical cues to help them judge their opponent's state of aggression, fighting ability, and the presence of a stomatopod in a refuge.

—**Amanda Franklin,** Tufts University grad student, on the *Royal Society Open Science* paper she recently coauthored describing a novel sensory behavior in a marine invertebrate (*TuftsNow*, August 3)

MOOD DETECTOR: A rock mantis shrimp (*Neogonodactylus oerstedii*) may communicate its fitness and aggression to rivals through the use of UV-reflective spots on the sides of its body.

The most amazing thing is that it's not like seeing light. It's almost a feeling, at the threshold of imagination.

—Physicist **Alipasha Vaziri** of Rockefeller University talking about his recently published *Nature Communications* paper showing that human beings could detect flashes of light from a single photon (*Nature*, July 19)

It's part of our evolutionary history. Magnetoreception may be the primal sense.

—California Institute of Technology geophysicist **Joe Kirschvink**, on his quest to show experimentally that human beings posses sensory apparatuses that help them perceive Earth's magnetic field (June 23)

Large, publicly owned publishing companies make huge profits off of scientists by publishing our science and then selling it back to the university libraries at a massive profit (which primarily benefits stockholders). It is not in the best interest of the society, the scientists, the public, or the research.

—University of Cambridge animal behaviorist **Corina Logan**, on the need for open access in science publishing (From "The 7 biggest problems facing science, according to 270 scientists," *Vox*, July 14)

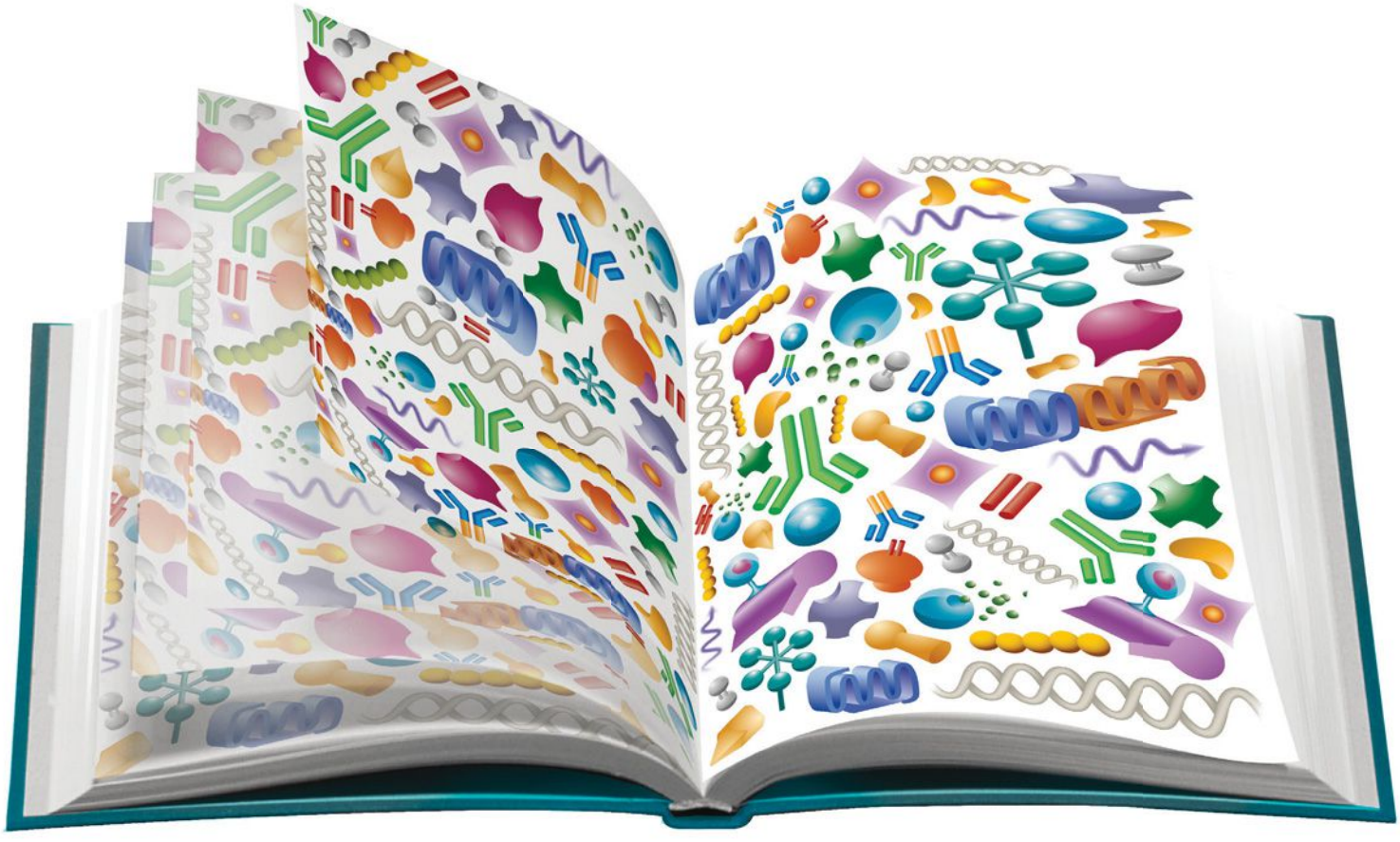
I believe in science. I believe climate change is real and that we can save our planet while creating millions of good-paying clean-energy jobs.

—Democratic presidential candidate **Hillary Clinton**, accepting her party's nomination during the Democratic National Convention (July 28)

The concept of global warming was created by and for the Chinese in order to make U.S. manufacturing noncompetitive.

—Republican presidential candidate **Donald Trump**, from a 2012 tweet

A. M. FRANKLIN, ET AL., *ROY SOC OPEN SCI*, DOI:10.1098/RSOS.160329, 2016.



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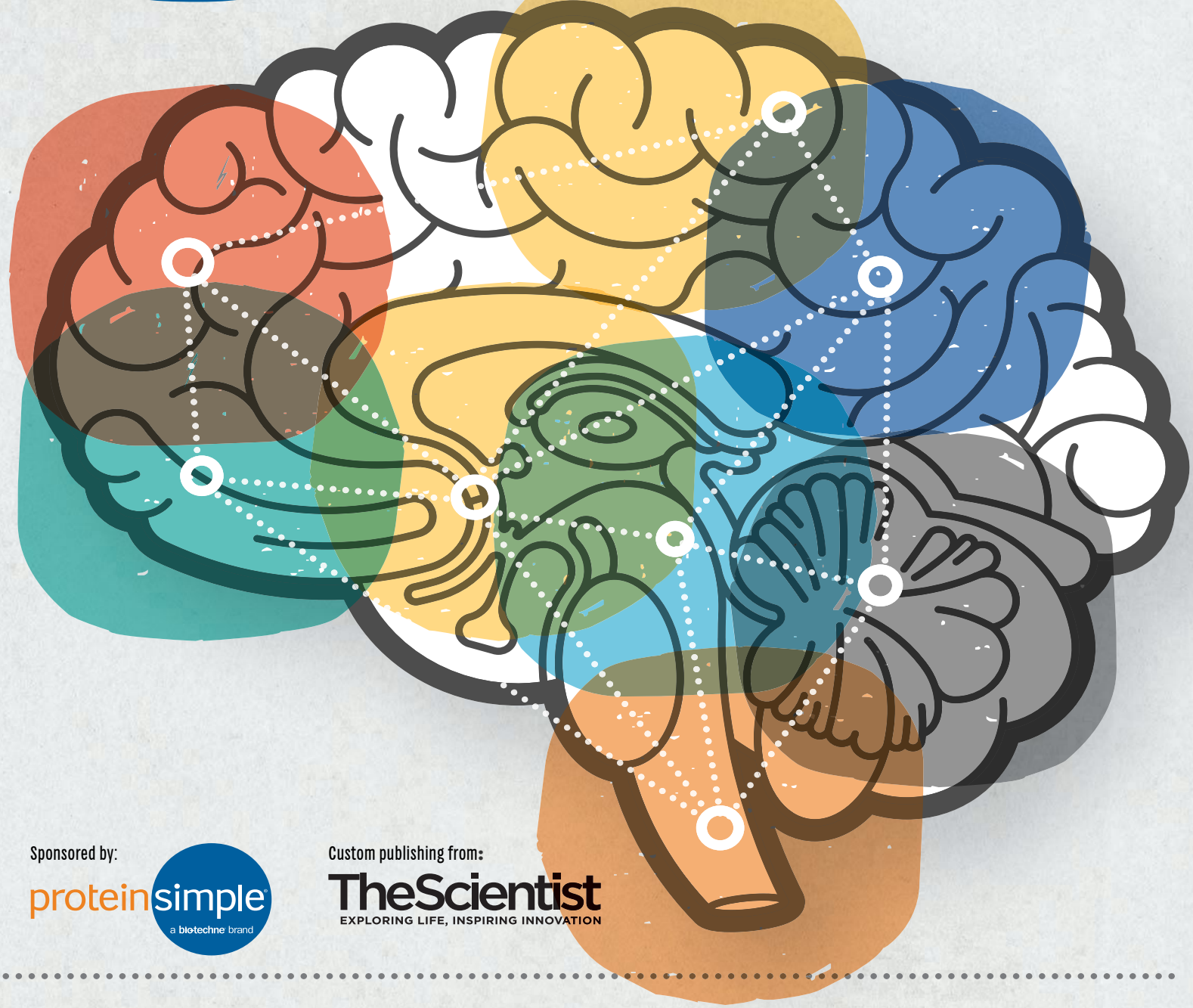

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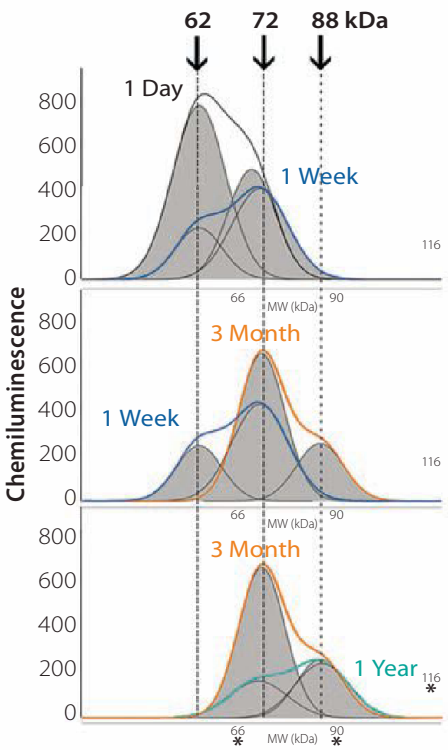
Wes sets a new pace for Alzheimer's research

At the Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center in San Antonio, researchers study the basic biology of aging. One project in particular focuses on how age-associated changes in normal physiology alter the expression and function of tau, a biomarker for many neurodegenerative disorders, including Alzheimer's disease. Using traditional Western blotting to study the correlation between tau's expression and aging proved to be challenging, particularly with small sample size collected from brain sub-regions.

With Wes, they run 24 independent samples and get fully analyzed data in about 3 hours. All that with 95% less tissue and antibody. Data was reproducible and reliable. Furthermore, they discovered a novel high molecular weight isoform of tau protein that is expressed in the brains of the naked mole-rat (NMR). The results showed that tau undergoes a progressive shift in molecular weight during the first year of NMR brain development (M.E. Orr et al., Neurobiology of Aging, 36, 2015).

Don't let old technologies slow down your research. Win the race to discovery with Wes.



Detection of tau in nakedmole rats (NMR) in different stages of life development using Wes. A progressive molecular weight shift in NMR tau is observed during development. (HT7 antibody recognizes tau at an epitope corresponding to human tau 159-163).

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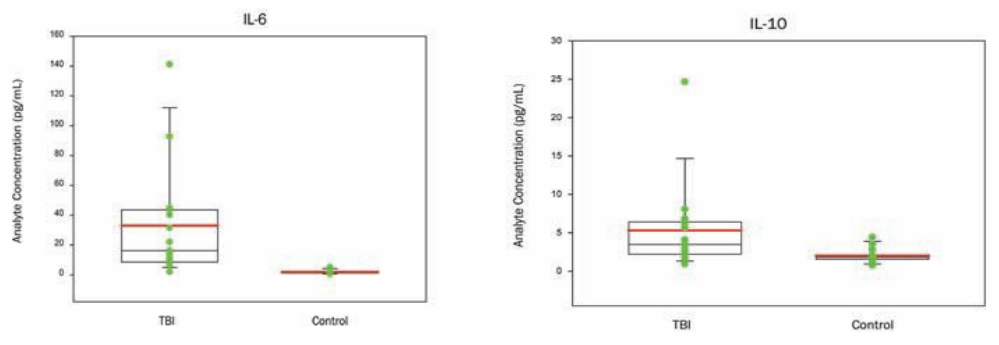

Proteins that could act as markers for Traumatic Brain Injury. IL-6 and 10 are just two of many analytes with increased concentrations in TBI samples compared to control samples.

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Diagnosing Disease

EXPLOITING BIOMARKERS TO IDENTIFY & MONITOR BRAIN DYSFUNCTION

The central nervous system (CNS) is exquisitely tuned for performing its many core functions, but disease and dysfunction can impede its work. Disease biomarkers are chemical signatures of pathologic processes, detection of which enables diagnosis and progression analysis from blood, cerebrospinal fluid, or tissue biopsy. Biomarkers of CNS disorders have been successfully exploited for their diagnostic and prognostic value, becoming ever more valuable in the fight against insidious diseases that invade and damage our most essential organ system.

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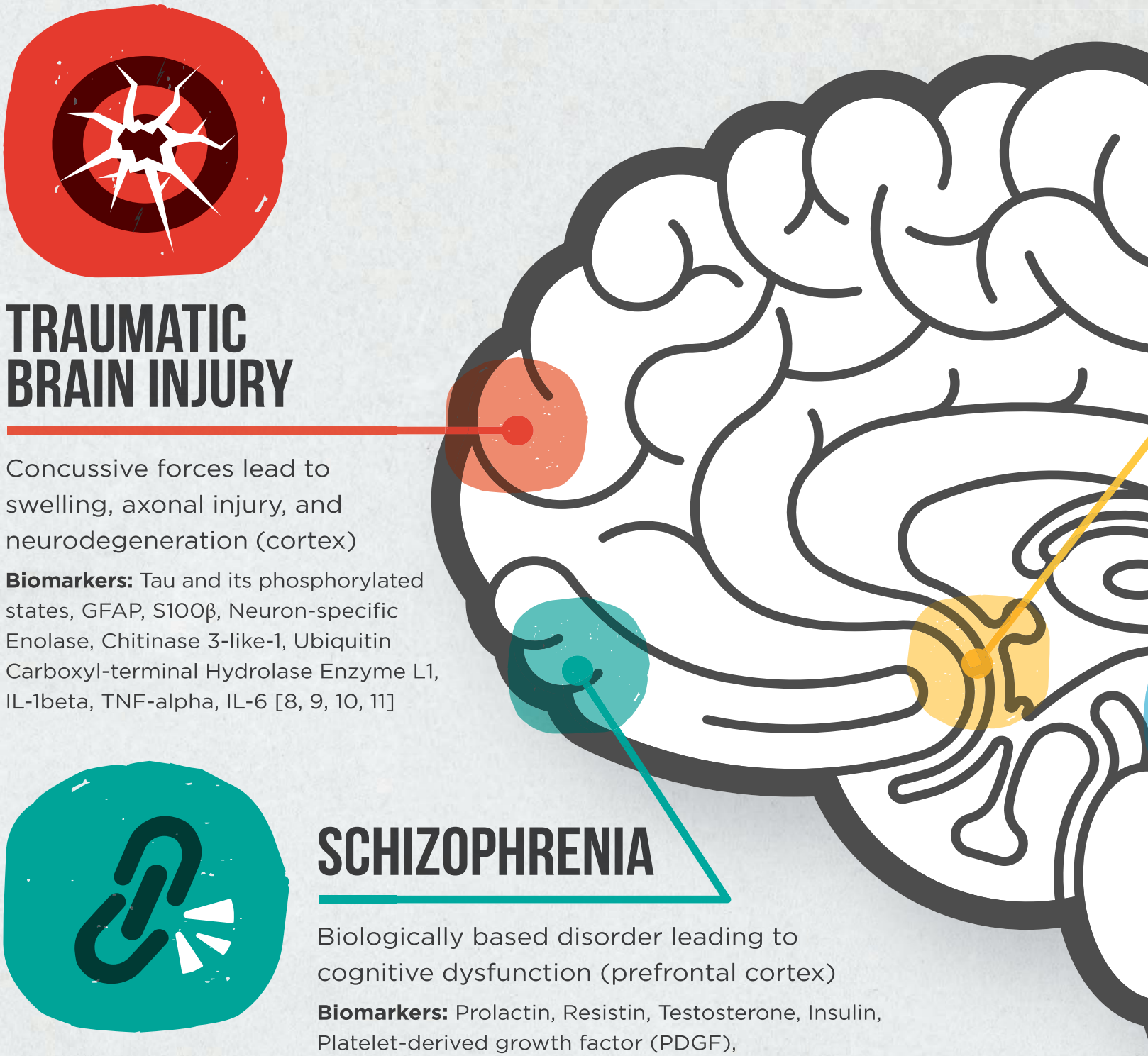
A physical, psychological, and behavioral need for an exogenous chemical (global)

Biomarkers: Heat-shock protein 70, Peroxiredoxin-6, n-Methylserotonin [22]

1. K. Henriksen et al., "The future of blood-based biomarkers for *Alzheimer's disease,*" Alzheimer's & Dementia, doi:10.1016/j.jalz.2013.01.013, 2014. 2. A. Hartz et al., "Aβ40 reduces P-glycoprotein at the blood-brain barrier through the ubiquitin-proteasome pathway," *J Neurosci*, doi:10.1523/JNEUROSCI.0350-15.2016, 2016. 3. M.I. Kester et al., "Cerebrospinal fluid VILIP-1 and YKL-40, candidate biomarkers to diagnose, predict and monitor Alzheimer's disease in a memory clinic cohort," *Alzheimer's Research & Therapy,* doi:10.1186/s13195-015-0142-1, 2015. 4. V.V. Giau et al., "Emergence of exosomal miRNAs as a diagnostic biomarker for Alzheimer's disease," *J Neurol Sci,* doi:10.1016/j.jns.2015.12.005, 2016. 5. C-H. Lin et al., "Biomarkers of cognitive decline in Parkinson's disease," *Parkinsonism Relat Disord,* doi:10.1016/j.parkreldis.2015.02.010, 2015. 6. L.V. Kalia et al., "Parkinson's disease," *Lancet,* doi:10.1016/S0140-6736(14)61393-3, 2015. 7. G. Esposito et al., "Synaptic vesicle trafficking and Parkinson's Disease," *Developmental Neurobiology,* doi:10.1002/dneu.20916, 2012. 8. K. Kawata et al., "Blood biomarkers for brain injury: What are we measuring?," *Neurosci Biobehav Rev,* doi:10.1016/j.neubiorev.2016.05.009, 2016. 9. C.A. Wiley et al., "Role for mammalian chitinase 3-like protein 1 in traumatic brain injury," *Neuropathology,* doi:10.1111/neup.12158, 2015. 10. J. Li et al., "Serum ubiquitin C-terminal hydrolase L1 as a biomarker for traumatic brain injury: A systematic review and meta-analysis," *Am J Emerg Med,* doi:10.1016/j.ajem.2015.05.023, 2015. 11. J. Zhang et al., "Biomarkers of Traumatic Brain Injury and Relationship to Pathology," *Translational Research in Traumatic Brain Injury,* D. Laskowitz, G. Grant, eds., Boca Raton, Florida: CRC Press/Taylor and Francis Group, 2016, Chapter 12, 2016. 12. R.



TRAUMATIC BRAIN INJURY

Concussive forces lead to swelling, axonal injury, and neurodegeneration (cortex)

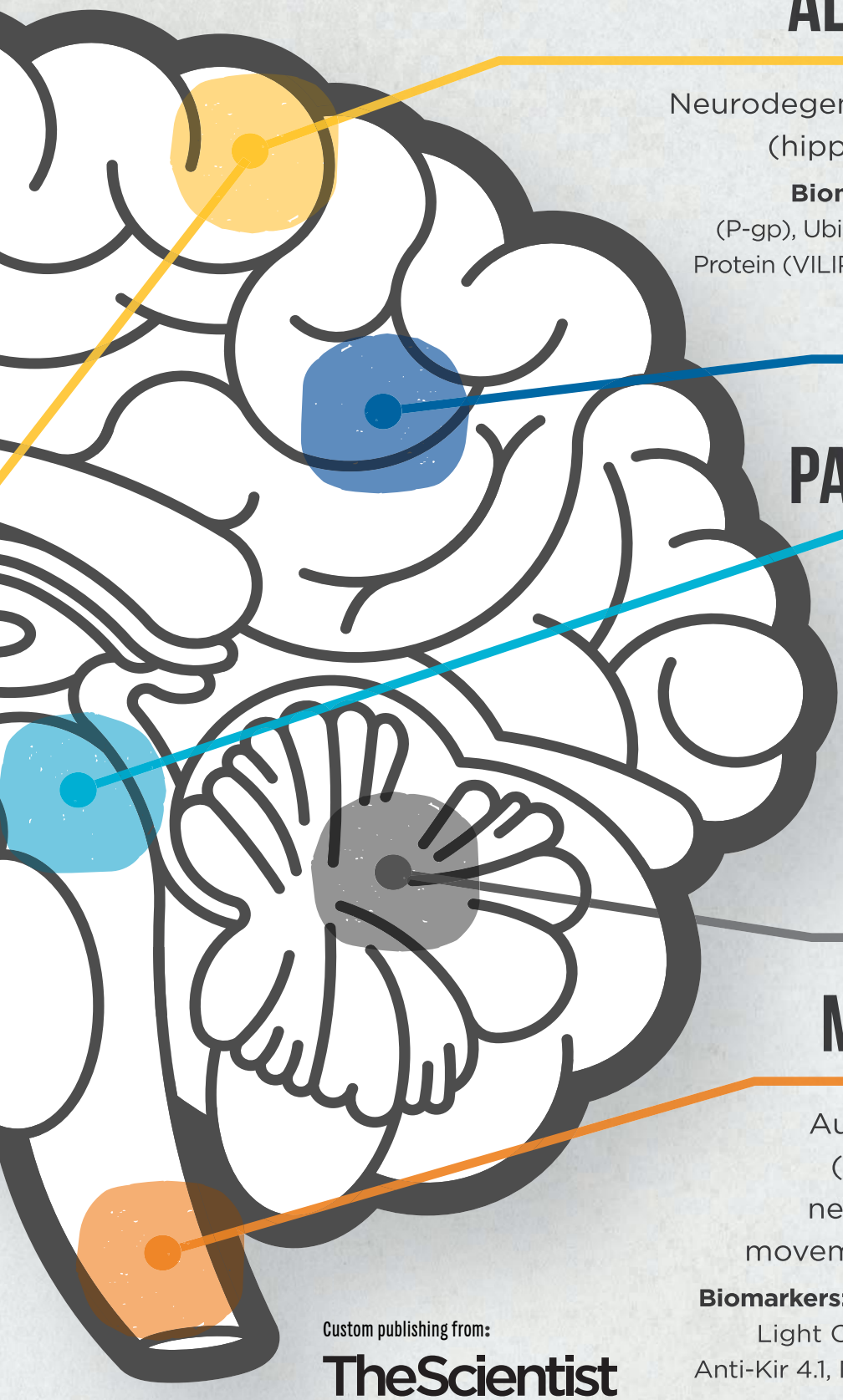
Biomarkers: Tau and its phosphorylated states, GFAP, S100β, Neuron-specific Enolase, Chitinase 3-like-1, Ubiquitin Carboxyl-terminal Hydrolase Enzyme L1, IL-1beta, TNF-alpha, IL-6 [8, 9, 10, 11]

SCHIZOPHRENIA

Biologically based disorder leading to cognitive dysfunction (prefrontal cortex)

Biomarkers: Prolactin, Resistin, Testosterone, Insulin, Platelet-derived growth factor (PDGF), IL-8, IL-1RA, IL-18 [15]

Dobson et al., "Cerebrospinal fluid and urinary biomarkers in multiple sclerosis," *Acta Neurol Scand,* doi:10.1111/ane.12119, 2013. 13. A. D'Ambrosio et al., "Peripheral blood biomarkers in multiple sclerosis," *Autoimmunity Rev,* doi:10.1016/j.autrev.2015.07.014, 2015. 14. S. Halbgebauer et al., "Detection of intrathecal immunoglobulin G synthesis by capillary isoelectric focusing immunoassay in oligoclonal band negative multiple sclerosis," *J Neurol,* doi:10.1007/s00415-016-8094-3, 2016. 15. M.K. Chan et al., "Applications of blood-based protein biomarker strategies in the study of psychiatric disorders," *Progress Neurobiol,* doi:10.1016/j.pneurobio.2014.08.002, 2014. 16. T. Urup et al., "Angiotensinogen and HLA class II predict bevacizumab response in recurrent glioblastoma patients," *Mol Oncol,* doi:10.1016/j.molonc.2016.05.005, 2016. 17. S. Ohtaki et al., "ACTC1 as an invasion and prognosis marker in glioma," *J Neurosurg,* doi:10.3171/2016.1.JNS152075, published online April 15, 2016. 18. G. Cheng, "Circulating miRNAs: Roles in cancer diagnosis, prognosis, and therapy," *Advanced Drug Delivery Rev,* doi:10.1016/j.addr.2014.09.001, 2015. 19. F. Saletta et al., "Molecular profiling of childhood cancer: Biomarkers and novel therapies," *BBA Clinical,* doi:10.1016/j.bbacli.2014.06.003, 2014. 20. R. Gulino et al., "MicroRNAs and pediatric tumors: Future perspectives," *Acta Histochemica,* doi:10.1016/j.acthis.2015.02.007, 2015. 21. M.D. Russell et al., "Biomarkers of pediatric brain tumors," *Front Pediatr,* doi:10.3389/fped.2013.00007, 2013. 22. L. Wang et al., "The potential biomarkers of drug addiction: Proteomic and metabolomics challenges," *Biomarkers,* doi:10.1080/1354750X.2016.1201530, 2016.



ALZHEIMER'S DISEASE

Neurodegeneration leads to memory deficits (hippocampus) and dementia (cortex)

Biomarkers: Tau, Amyloid-β 42, P-glycoprotein (P-gp), Ubiquitin, Apolipoprotein E (ApoE), Visinin-like Protein (VILIP-1), Chitinase 3-like-1 (YKL-40), microRNAs [1, 2, 3, 4]

PARKINSON'S DISEASE

Neurodegeneration in the brain stem (locus coeruleus and substantia nigra) lead to tremor, instability, and dementia

Biomarkers: DJ-1, Synapsin 1 (Syn 1), phosphorylated Syn 1, α-Synuclein, β-Glucocerebrosidase, Uric acid [5, 6, 7*]

MULTIPLE SCLEROSIS

Autoimmune degradation of myelin (white matter) leads to secondary neurodegeneration and progressive movement disorder, leading to paralysis

Biomarkers: Oligoclonal Bands (IgG/M), Kappa Free Light Chains, microRNAs, CXXL13, MOG-IgG & Anti-Kir 4.1, Microtubule-associated protein 2 (MAP2) [12, 13, 14]

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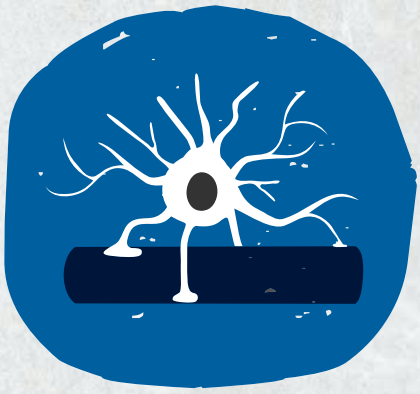
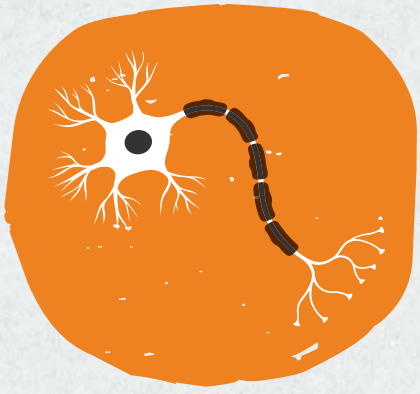


Rapidly progressive, astrocyte-derived brain tumor (cerebral hemispheres)

Biomarkers:
Angiotensinogen, HLA Class II, Alpha cardiac muscle 1 (ACTC1), microRNAs [16, 17, 18]

MEDULLOBLASTOMA



High-grade brain tumor with mixed cell types (cerebellum)

Biomarkers:
ERBB2, microRNAs, Follistatin-like Protein 5 (FSTL5), miR-495, Prostaglandin D2 Synthase (PGD2S), Polysialylated-Neural Cell Adhesion Molecule (PSA-NCAM) [19, 20, 21]

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Notebook

SEPTEMBER 2016

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Silent Screams

Bournemouth University neuropsychologist Simon Thompson is an expert on yawning. In 2011, he posited that high levels of the stress hormone cortisol may be a direct cause of yawning. "We believe that cortisol levels rise sufficiently to trigger a yawn for the purpose of lowering brain temperature," he says.

The idea became known as the Thompson cortisol hypothesis (*Interact J Med Res*, 1:e4, 2012). Linking yawning with fatigue, cortisol, and brain temperature, the hypothesis is ideally suited to being tested on people with multiple sclerosis (MS). Patients with the neurodegenerative disorder often report frequent yawning. They also suffer from fatigue, which has been associated with elevated brain temperature (*Neurology*, 86:P2.172, 2016).

Up to now, experiments to test the hypothesis have only been conducted on healthy subjects. So, as a big yawner who has lived with MS for 10 years, I couldn't miss an opportunity to get a firsthand glimpse of Thompson's experiments—and to be his first MS guinea pig.

A couple of months ago, I found myself alone in a small, windowless booth on the leafy Bournemouth campus in Poole, U.K., southwest of London. Three electrodes stuck to my beard along my jawline recorded electrical signals from my facial muscles while I sat in a chair in front of a computer. Following a quick spit into a test tube to measure my baseline cortisol levels, the experiment began. On the screen, a woman repeatedly yawned. Normally, this kind of image would trigger a yawn immediately, but for some reason, I resisted. And then, right at the end of the 3-minute video, a yawn of epic proportions escaped my mouth.

YAAAAAAAAWN: Might the relationship between yawning frequency and cortisol levels be a hallmark of diseases such as multiple sclerosis?

Thompson rushed into the room holding a small plastic tube to spit into. He explained that he planned to compare my cortisol levels from saliva samples taken before and after the experiment. But I couldn't spit. Suddenly I was in the midst of a yawning frenzy that felt like it would never end. Eventually, the waves of yawning subsided long enough for me to spit into the tube, and the test was over.

After squirreling away my sample next to my pre-test spit in a bag to send to the U.S. for analysis, Thompson showed me how he tries to coax a yawn from participants whose minds or bodies stubbornly refuse to yawn in response to the video of the woman. First he tries a series of pictures of yawners, from babies to the elderly, and if that doesn't draw out a yawn, he has participants read a few paragraphs of dry technical text all about yawning.

We've underestimated the reason for yawning by saying that it's just to get oxygen into your system.

—Simon Thompson
Bournemouth University

All of these tricks worked well on me, but apparently do not on everyone: of 82 healthy volunteers, about half didn't yawn at all (*J Neurol Neurosci*, 6:1-15, 2015).

What the data did clearly show was a significant difference between yawners and non-yawners. The cortisol levels of yawners rose significantly more than those of non-yawners. In addition, the yawners had higher resting levels of cortisol and higher electrical activity in their facial muscles than those who didn't yawn.

Other researchers are also turning up evidence supporting elements of Thompson's cortisol hypothesis of yawning. Brain temperature rises during bouts of fatigue; people yawn when they're fatigued; people also have high levels of cortisol when they're fatigued; and high levels of cortisol have been recorded in clinical conditions associated with fatigue, such as Cushing's syndrome. "We've had support for different parts of the loop but not directly between cortisol and yawning," Thompson says. "So it's just linking that all together."

The field of yawning research is still rife with competing theories and contradictory evidence, however. "I think that [Thompson's] hypothesis is true but only forms a very little part of the puzzle," says yawning expert Olivier Walusinski. "Cortisol is one among many other neuromediators involved in this behavior."

Thompson is determined to bring the cortisol hypothesis closer to a full-blown theory. His most recent study used functional magnetic resonance imaging (fMRI) on 13 healthy French volunteers to illuminate how cortisol varies during mental and physical tasks, and how cortisol acts on specific brain regions such as the hypothalamus (*J Neurol Neurosci*, 7:92, 2016). Thompson's next experiment will also use MRI, but the participants will all have MS. "It would be good to get a picture of whether people with MS have completely different cortisol fluctuations from people who don't," he says.

What about better-established theories of yawning, like the idea that a big morning yawn stretches the rib muscles to allow the lungs to get more oxygen? Might MS sufferers just require more air in their lungs? "We're not saying that yawning is not to do with oxygen," Thompson says. "We're saying we've underestimated the reason for yawning by saying that it's just to get oxygen into your system."

Central to his argument is fatigue. Whether the result of just having woken up or of having MS, fatigue means lower oxygen saturation in the blood and a higher brain temperature. Thompson reasons that, far from competing with the oxygen explanation, his work expands upon it. Similar to his idea that yawning lowers brain temperature, taking oxygen in lowers body temperature. "So it all could be linked," he says.

Ultimately, Thompson hopes to develop a diagnostic for MS and other neurological diseases based on the connection between cortisol and yawning. But that will take time and resources.

"We don't want to give false hope to people—so far, we've only done this on healthy people, and the potential for the clinical population is theoretical," Thompson says. "But we're confident something good will come out of it."

—Benjamin Skuse

Sixth Sense

Thinner than plastic wrap and lighter than a feather, electronic skin, also known as smart skin or imperceptible electronics, detects information about the internal and external environments. Such technology has been in development for wearable medical instruments, health monitors, prosthetics with sensory feedback, and even robotic skin. Now, scientists are expanding electronic skin into the realm of the once-impossible: endowing humans with a sixth sense.

"There have been many kinds of physiological and/or electrophysiological sensors for wearable electronics," Dae-Hyeong Kim of Seoul National University told *The Scientist* in an email. In 2014, for example, Kim and his colleagues developed a smart prosthetic skin that could sense pressure, temperature, and humidity and was equipped with stretchable electrode arrays for nerve stimulation (*Nat Commun*, 5:5747). But while sensors exist that can duplicate or enhance human senses, no one has yet developed a smart skin to detect a new type of stimuli. No one, that is, until Denys Makarov of the Helmholtz-Zentrum Dresden-Rossendorf in Germany, set his sights on sensing magnetic fields, which people have no natural ability to detect.

"It would truly be a sixth-sense technology," says Makarov. "Artificial magnetoreception is something that extends the five natural senses of humans to something that is unavailable."

To create a smart skin with magnetic field–sensing capabilities, Makarov, then at the Leibniz Institute for Solid State and Materials Research Dresden, and his colleagues added magnetic field–sensitive sensors to 1-µm–thick polymeric foils. They then laminated the ultrathin foils to an elastic material to create a stretchable, flexible, skin-like device that could detect magnetic fields of 1 millitesla or less—several orders of magnitude weaker than a typical refrigerator magnet (*Nat Commun*, 6:6080, 2015). For the purposes of this proof-of-concept experiment, the strength of the magnetic field detected was displayed on a series of LED lights. "The signal-to-noise is very good—it's a high-performance magnetic field sensor," Makarov says. "You can put it on a joint, on the knee or the finger, and it will not

change performance as you are bending your knee."

"The sensitivity of the magnetic sensor is very good, and its ultrathin nature provides extreme deformability," says Kim. "The potential applications are quite wide."

But there's a long way to go, Makarov notes. "This is very much a starting point." One important step will be to upgrade the readout method. While LED lights are relatively easy to work with, there are more-elegant options, Makarov says. The signal could be transmitted wirelessly to a smartphone, for example, which could be set up to vibrate in response to certain levels of magnetic fields.

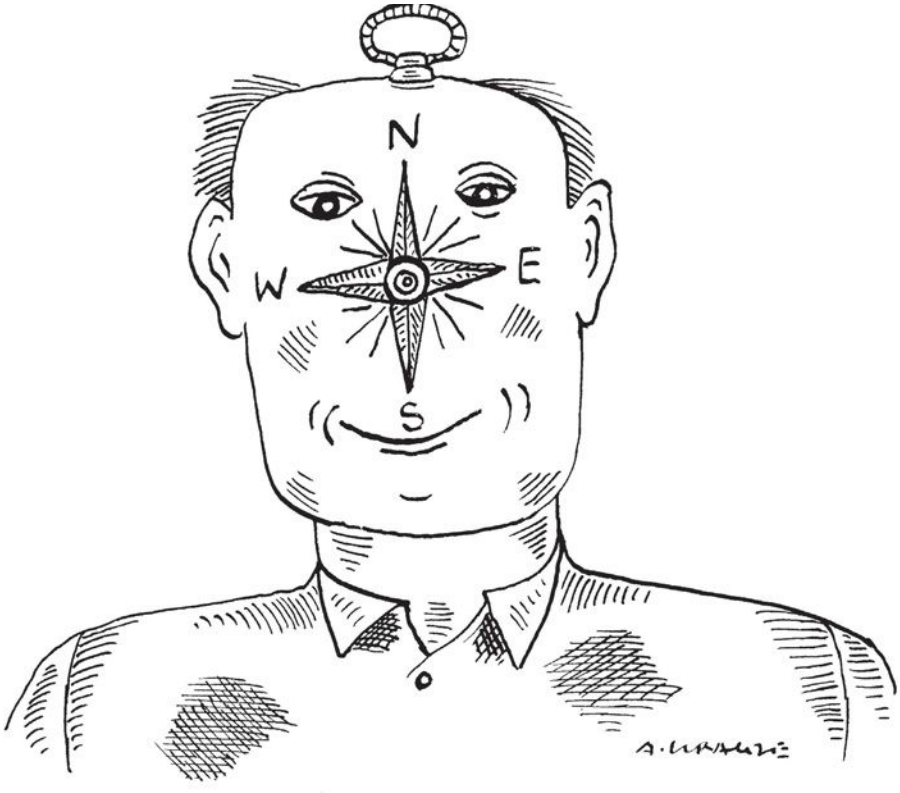
Another improvement Makarov would like to make is to integrate the magnetoreceptive technology with other smart-skin sensors, such as those for touch and temperature. Makarov's team is currently working to do just that, and he is expanding his lab to support the effort. "You will have touch sensors, temperature sensors, magnetic-field sensors combined on a single foil," he says.

Such multifunctionality will be key to making smart prosthetics, but Haixia "Alice" Zhang, a microelectronics professor at Peking University, suspects that magnetoreceptive features may be more readily adopted for robotics applications. "Magnetoreception is very important for smart sensing," Zhang, told *The Scientist* in an email. In March, she described a smart-skin device with sensitive touch detection (*ACS Nano*, 10:4083-91).

Makarov is hopeful magnetoreceptive skin could be used to create an artificial proprioceptive sense. With a magnet placed in your belt, for example, magnetic-field sensors in the smart skin on your hand could provide you with feedback about your hand's location in space. "It provides the possibility to monitor displacements of your body parts," he says. "In principle, you can rebuild the proprioceptive system with magnetic-field sensors."

Makarov also muses about the possibility of such magnetoreceptive capabilities being used as wearable navigational devices. But that would require a more powerful sensor; to realize artificial magnetoception, the technology must be sensitive enough to detect the Earth's magnetic field, as many birds and other species can (see "Senses Census" on page 43). With the current sensor, "we are speaking about fractions of millitesla, compared to the magnetic field of the earth which is tens of microtesla," Makarov says. "There is still maybe two orders of magnitude in field to go down."

—Jef Akst



ANDRZEJ KRAUZE

Harboring Hornbills

For Tajik Tachang, a member of the indigenous Nyishi tribe that lives in the state of Arunachal Pradesh in northeastern India, the typical day begins at six o'clock in the morning. He and his companions walk ancestral trails through patches of jungle surrounding the Pakke Tiger Reserve (PTR) in the undulating foothills of the eastern Himalayas. The tribesmen are looking for nests in the hollows of false hemp trees (*Tetrameles nudiflora*), but they're not seeking to kill the birds that use these nests, as did their fathers and grandfathers. They want to save them.

"Our group quietly watches their activities from a safe distance," Tajik says. "When we do find a nest, we mark the tree in red, with the initials of the species that has nested there for the season—GH [for great hornbill], OPH [oriental pied hornbill], etc." This not only enables Tajik and his compatriots to maintain a record of the nests for the season, but also serves as a warning to fellow villagers against tree felling or other human activity within a 100-meter radius of the site.

In the lush, green wilderness of the PTR and its surrounding jungles, the dense canopy of trees casts persistent twilight upon the forest floor, even during the middle of the day. These tropical rainforests are an integral part of the eastern Himalayan biodiversity hotspot and a favorite haunt of at least four species of hornbills—the great hornbill, the wreathed hornbill, the rufous-necked hornbill, and the oriental pied hornbill. Tall trees such as the false hemp, with large hollows in

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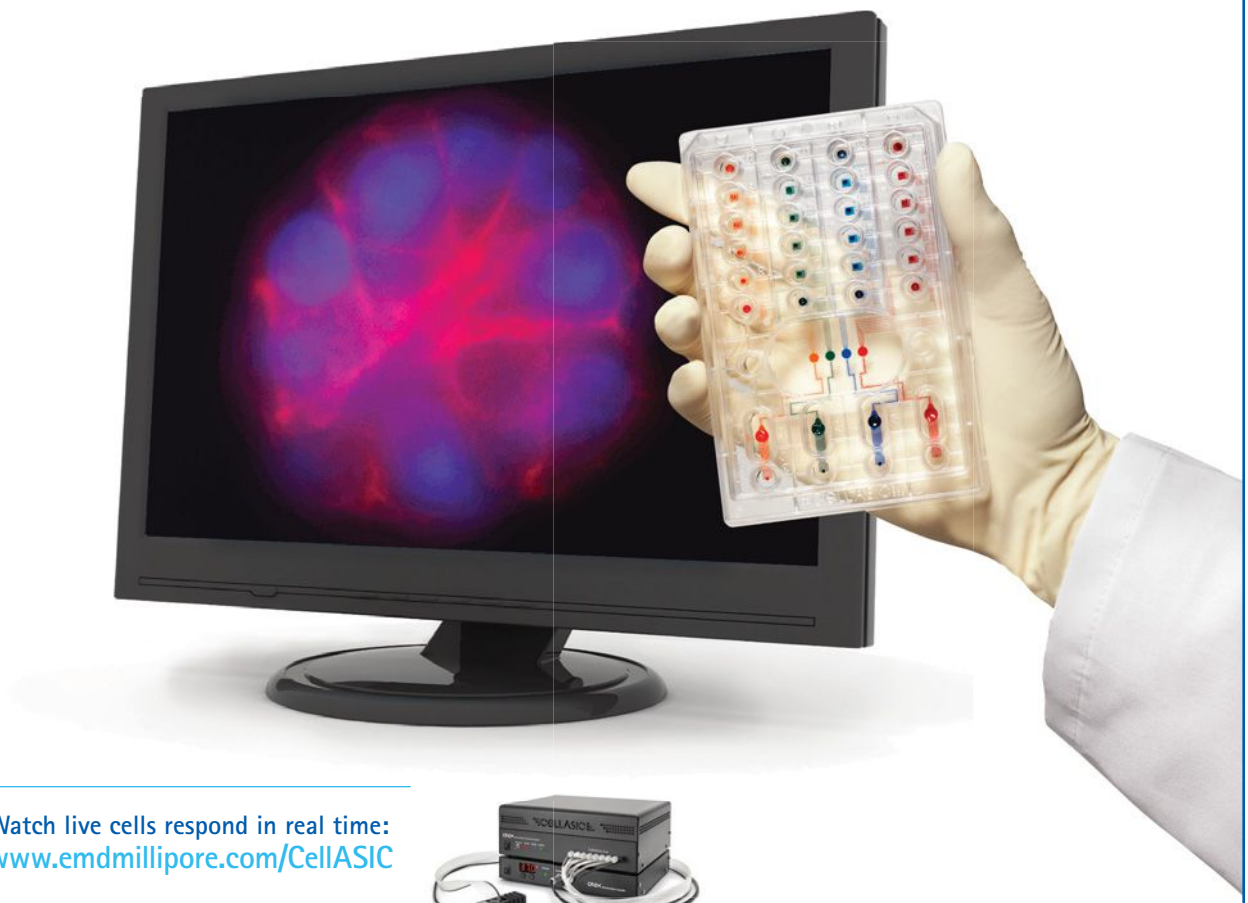

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QUARRY NO MORE: A male wreathed hornbill perches at the entrance to his nest, ready to supply food to its occupants.

their trunks and branches, are ideal for perching and nesting, while plants such as figs and tropical nutmeg contribute to the hornbills' frugivorous diet. The birds are slow-breeding, tending to fledge only one or two chicks per year, and they mate for life.

The forests fringing the southeastern part of the reserve are also the ancestral home of the Nyishi community. For generations, Nyishis hunted hornbills, named for their distinctive oversized beaks. The tribespeople harvested the bill and other parts of the bird's body for decorating traditional garb. For example, men traditionally donned *pudum*, or the Nyishi headgear, prominently displaying a hornbill's characteristic casque (an ornamental growth on the upper mandible of its beak). Nyishi people also shot hornbills for their meat and fat—the latter believed to cure body aches. To add to the birds' woes, their nesting trees made excellent timber. But today, the tribe's people have transformed themselves into conservationists, protecting the nests and habitat of the threatened hornbills.

"It was not difficult to monitor and conserve [hornbill species] within the reserve due to our stringent protection mechanisms," says Tana Tapi, the field director of PTR. "But in the forests outside of PTR, hunting and habitat loss emerged as major threats to their survival."

The loss of hornbill nesting trees also led to intense inter- and intraspecies competition for nesting sites. For example, there were occasions when wreathed hornbill pairs got evicted from their nest cavities by the much larger great hornbills, says Tapi.

NEST PROTECTOR: Nyishi tribesman Tajik Tachang stands in front of a tree harboring a great hornbill nest he monitors.

COURTESY OF NATURE CONSERVATION FOUNDATION; VIJAY TACHANG

As a member of the Nyishi community, Tapi had unique insight into tribal traditions. "Hunting was prevalent in every Nyishi family," he says. "The village headmen used muzzle-loaded [locally made] guns to shoot down birds and animals." But Tapi's perseverance enabled him to win over his fellow tribesmen, and he eventually convinced them to surrender their weapons and the hunting of hornbills. Tapi says that his task was not easy, but one day about seven years ago, the Nyishi tribesmen deposited nearly 67 guns and 14 steel traps in his office, heralding a new beginning.

The Nyishis' commitment to protect hornbills achieved a major milestone in 2012, with the launch of the Hornbill Nest Adoption Program (HNAP), which seeks to conserve forests outside the PTR for protection of the species and their nesting trees. Initiated by the Arunachal Pradesh Department of Environment and Forests and the Nature Conservation Foundation (NCF), an organization based in Mysore and Bangalore, the program carved a niche for itself in conservation history due to the intimate involvement of the local Nyishi community.

The Nyishi council of elders, or Ghora Aabhe, from adjoining villages joined with the Forests Department and NCF to support the initiative. Most of these village headmen, former hunters of the birds, swore to become their protectors, and village youths and tribe members followed their lead.

In addition to nest cataloging and monitoring, the program incorporated an innovative feature—nest adoption by people living in the Indian metropolises of New Delhi, Mumbai, Pune, Bengaluru, and other cities. Foster parents were assigned to hornbill families and were asked to donate an annual "adoption fee," which was used to sustain Nyishi nest protectors and to fund community welfare and development activities in local villages.

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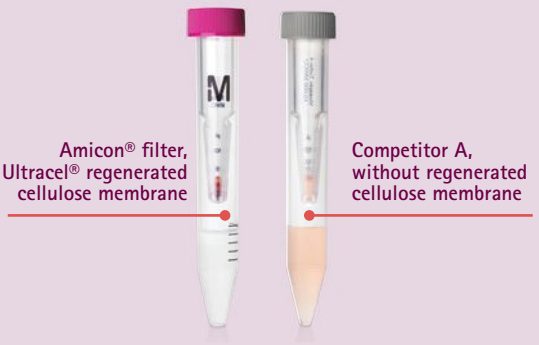

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Four years ago, HNAP began with just three Nyishi nest protectors. But today, 16 of them, along with other community members from 14 villages, participate in the program. There are currently 36 nests under their surveillance, and 60 chicks have fledged successfully under the Nyishis' watchful eye. There are an additional 41 nests within the PTR.

To date, HNAP has attracted 165 urban donors, among whom adoption fees range from $125 to $735. The effort has also received support from the Rotterdam Zoo in the Netherlands and the Greater Vancouver Zoo in Canada. This year, the Nyishi community won the India Biodiversity Award, a joint initiative of India's Ministry of Environment, Forest and Climate Change and the United Nations Development Program (UNDP).

All of this success and recognition gives Takam Nabum, chairman of the Ghora Aabhe Society, reasons to be proud. He displays the glittering plaque and the UNDP award in his office in the Nyishi village of Mobuso. "The program has not only given a new lease of life to our state bird, but to our community as well," he says. "The sustainable financial benefits received motivate us to protect the bird. Then, awards and recognitions such as these inspire us further."

Even changes to the Nyishis' traditional dress reflect their pledge to protect their one-time quarry. Men in the tribe have replaced actual hornbill casques used on their headgear with fiberglass or wooden facsimiles.

Aparajita Datta, an NCF wildlife biologist, has been recording an increasing number of hornbill fledglings leaving nests in July and August for the past decade—a hopeful sign for the threatened species. She says the Nyishi community has played an integral role in the protecting the birds and their habitat. "No long-term conservation effort can flourish without the active participation and involvement of forest communities," Datta says.

—Moushumi Basu

Prelude to a Sip

A stationary Carolina sphinx moth (*Manduca sexta*) is the Cinderella of the animal kingdom. The hummingbird-size insect has dull, dark wings that are mottled like charred wood, and a plump body reminiscent of a small breakfast sausage. Casual observers of *M. sexta* often see little else.

"They say, 'Oh, it doesn't look so nice. It's just grey.' But as soon as [the moths] start flying, they're completely impressed," says Danny Kessler, a pollination ecologist at the Max Planck Institute of Chemical Ecology in Germany. "They change their minds completely."

Hawkmoths, the group to which *M. sexta* belongs, whir their wings like hummingbirds as they flit between flowers, hovering to drink nectar. *M. sexta*'s proboscis, longer than its 2-inch body, stays unfurled, a straw ready to sip.

Kessler studies the interaction between the Carolina sphinx moth, whose

larvae are known as tobacco hornworms, and its preferred food source, the coyote tobacco plant (*Nicotiana attenuata*), to better understand how insect behavior affects a plant's reproductive success. *M. sexta* adults drink nectar from tobacco's skinny, white, trumpet-shape flowers, foraging from them at night and pollinating them in the process. Scientists have known for decades that the moth uses its antennae to detect the flowers' scent—even from several miles away, Kessler says.

But Kessler wondered, if the moth is so sensitive, couldn't plants cheat to exploit that sensitivity? As the moth drew in closer, it would become enveloped in a cloud of odor that could potentially overwhelm the insect's sense of smell, Kessler reasoned. A scentless mutant plant, nestled among redolent wild-type ones, then might be able to lure a pollinator without having to expend any energy in attracting it.

In 2007, Kessler and his team set up field experiments in Utah to find out. The group used RNA interference on the tobacco plant to inhibit production of the chemical it uses to attract pollinators: benzyl acetone (BA). (To humans, BA smells like strawberries.)

To be in tune with a Carolina sphinx moth's circadian rhythm, the team conducted the experiments at night. But the dark conditions made direct observations of moth-plant contacts difficult. Instead, the team counted the seeds from each plant as a proxy for hawkmoth visits. As they expected, plants without BA developed fewer seeds (*Science*, 321:1200-02, 2008; *eLife*, 4:e07641, 2015).

"If you don't produce flower scent, you're invisible to this hawkmoth at nighttime," Kessler says. "This is what I assumed [it meant]—what everyone assumed, actually."

But that assumption turned out to be wrong. Kessler and his team conducted follow-up experiments in an indoor wind tunnel about 7 feet long and 3 feet high by 3 feet wide. Here, they could blow a flower's odor downwind to one moth at a time and observe its response more easily. The scientists saw that the scentless flowers were not invisible to the hawkmoths. They visited the scentless flowers just as frequently as the scented ones (*eLife*, 5:e15039, 2016). "It was a total surprise," Kessler says.

To find out what was going on, Kessler and his team used a 3-D video tracking system to document the moth's behavior with scented and unscented flowers. And when they timed the moths' visits, they saw their answer: even though the moths visited

STEALTHY SNIFFER: Carolina sphinx moths may use olfactory receptors in their proboscises to determine the presence of an odor molecule produced by a plant.

DANNY KESSLER, MAX PLANCK INSTITUTE OF CHEMICAL ECOLOGY

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both kinds of flowers equally frequently, their visits to the unscented flowers were briefer—about half as long.

Kessler and his team suspected the moth might be using its proboscis to discriminate flowers by smell. To determine how *M. sexta* was sniffing with its proboscis, the researchers looked in the organ for the presence of olfactory receptors. One—the olfactory co-receptor, Orco—was present at the tip of the proboscis.

The scent is like a motivation. It's just like if you stay in front of a bakery, and you go in because it smells like fresh bread.

—Danny Kessler
Max Planck Institute of Chemical Ecology

"That was the smoking gun," says Robert Raguso, a chemical ecologist at Cornell University who was not involved in the study. "My first reaction when I saw the paper was to be very excited and a little jealous."

Twenty years ago, Raguso hypothesized *M. sexta* might have tiny odor-sensing hairs called sensilla on its proboscis. But, searching for them, he and others had come up empty-handed.

To experimentally confirm that the moth uses its proboscis to smell, Kessler's team needed a way of excluding information from the antennae, which also house olfactory receptors. "Some [designs of] experiments are not so nice, like you cut off the antennae," Kessler says. But such a manipulation renders moths unable to fly and likely changes their behavior in other ways.

So Kessler and his colleagues designed a Y-shape maze for the proboscis experiment instead. The moth could insert its proboscis at the entrance, which was covered by a fake flower. Then it had a choice: probe slightly to the left, where the air was laced with BA, or probe slightly to the right, where the air lacked BA. A vacuum applied at the entrance to the Y-maze prevented the air from leaking outside, precluding the moth's antennae from catching a whiff.

It worked. The moths vigorously checked both chambers with their proboscises, but devoted more time to exploring the side with BA.

"The scent is like a motivation. It's just like if you stay in front of a bakery, and you go in because it smells like fresh bread," Kessler says. "If it didn't, you might just pass by."

A Carolina sphinx moth won't spend as much time poking and prodding a flower that doesn't smell. In turn, that flower will have a smaller chance of being pollinated. These dynamics showed that the plants "can't cheat," he says: how inviting they smell affects their reproductive success.

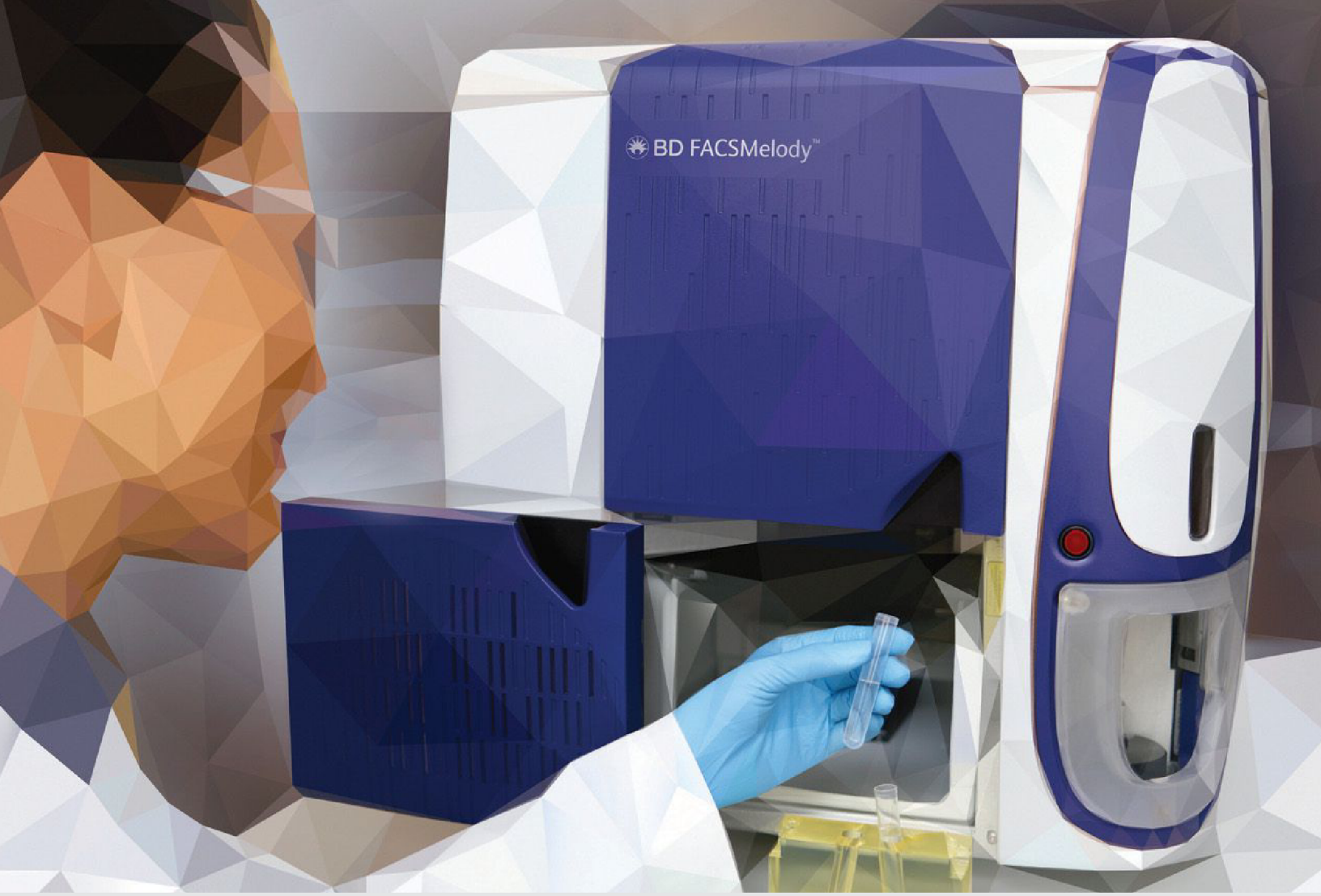
Kessler's lab focuses on the plant's ecology, but Kessler's collaborator Markus Knaden, who studies insect senses at the Max Planck Institute, has garnered some insight into why smell might be crucial to a moth's fitness. "From a distance, the moth should know whether this is a good flower to go to or not," he says. "The moth needs to make a good decision, because flight is very energy-demanding."

The work has made a splash. "I think it's a fantastic blend of understanding from both the perspective of the plant and the perspective of the pollinator," says Jeff Riffell, a chemical ecologist at the University of Washington in Seattle.

Raguso agrees with that assessment. "The rare quality of this paper, and some of the others by these authors, is that they make the extra effort to make a connection between physiological and behavioral mechanisms, and evolutionary and ecological phenomena," Raguso says. That's vital because it could help plant ecologists and insect biologists talk more, he says, which will help both groups to better understand pollination. Much of agriculture relies on this ecosystem service—a dance with not one, but two partners.

For Kessler, this work may be just the beginning of understanding what Carolina sphinx moths smell. "Most likely they have other receptors that can sense chemical compounds," he says. "And there are many chemical compounds in nectar."

—Alison F. Takemura



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Fixing Science's Human Bias

It's time to accelerate the conversation about why the research community is still not diverse.

BY EDWARD COURCHAINE AND SARAH SMAGA

"We out here. We've been here. We ain't leaving. We are loved." These words rang through the Yale University campus twice this past year: first in the fall, when a series of racially charged incidents sparked protests demanding that the school affirm its commitment to diversity; and again in the spring, when after months of "listening sessions" the university and its governing body, the Yale Corporation, announced that John C. Calhoun Residential College would not be renamed, meaning the college would continue to honor the ardent racist.

Yale students, faculty, and staff are not alone in their fight to increase diversity in academia and, specifically, in the sciences. When second graders are asked to draw a scientist, the results are overwhelmingly outdated: old, white, and male. These caricatures reflect the cultural notion that women and minorities don't belong in science. Despite the fact that many researchers genuinely want this view to change, this image is ingrained in our collective consciousness. The problem may lie in the widespread—but false—belief that the research community is purely meritocratic, that all young scientists have equal opportunity to be successful in their careers. We disagree. Only with an honest view of today's biased world can we begin to overcome the obsolete stereotype.

Social scientists, authors, and artists have repeatedly documented the extensive challenges that come with identifying as anything other than a heterosexual white male. When students are contacting prospective professors to do research projects, those with "white-sounding" names receive more replies (*J Appl Psychol*, 100:1678-712, 2015). In undergraduate biology classes, male students underestimate the academic performance of their female peers, making males the de facto "stars" of the classroom. And one study found that, although introductory biology classes were 60 percent female, only 40 percent of the discussion came from female voices (*CBE Life Sci Educ*, 13:478-92, 2014).

These problems persist through school and into the job search. In reviewing applications for a lab manager position, faculty considered an applicant with a male name more competent, hirable, and worth a larger salary than an applicant with a female name, even when the resumes they read were otherwise identical (*PNAS*, 109:16474-79, 2012). By the time they defend their theses, white men are less likely to change their preferences to non–tenure track positions than their peers, who often seek other career options. And at the postdoctoral level, a stage during which mentorship is crucial, elite male faculty tend to train fewer women.

Finally, those women, minorities, and members of the LGBT community who do make it into research careers continue to face an uphill climb. Black researchers are less likely to receive a National Institutes of Health (NIH) R01 than their peers (*Science*, 333:1015-19, 2011). Nearly half of female scientists of color report having been mistaken for a custodial worker or staff member. And at any stage of a woman's career, she will likely deal with harassment from her supervisors or peers. Such bias and exclusion are made worse by personal insecurities and self-doubt. "Social identity threat"—the fear of fulfilling nega-

tive stereotypes about a group or failing to fulfill positive ones—causes stress, reduces performance, and prevents women and minority groups in STEM from achieving their full potential.

While these examples deal with classical notions of race and gender, every facet of an individual's identity, including sexual orientation, gender identity, disability, and socioeconomic status, can make pursuing a career in science challenging.

Social scientists, authors, and artists have repeatedly documented the extensive challenges that come with identifying as anything other than a heterosexual white male.

The good news is that there are initiatives that are taking steps in the right direction. Since 1995, the Science, Technology and Research Scholars (STARS) program at Yale University has supported underprivileged and underrepresented students by facilitating mentorship, networking, research opportunities, and professional development. On a larger scale, the American Astronomical Society and its parent organization the American Institute of Physics have developed resources and outreach to improve the status of minorities in astronomy, and other professional societies are tackling the issue as well. Even more broadly, federal agencies have had long-standing initiatives to increase the diversity of the research pipeline, funding undergraduate lab experiences and educational programs for women and minorities. Confronted with the funding gap for minority researchers, the NIH has begun to look critically at the grant review process, and has acknowledged the importance of recognizing where implicit bias may be harming certain applicants.

But such efforts are proving insufficient in shifting the trend lines. As last fall's protests at Yale and this spring's faculty letter demonstrate, there is a demand to further interrogate entrenched inequality in and around academia. We echo that demand for accountability. The university's failure to rename Calhoun College is a clear statement that inclusion is not yet prioritized by the upper levels of campus administration.

At an even higher institutional level, we feel that funding agencies should take a hard stance against scientists who are found guilty of creating a hostile environment for their colleagues. A scientific career is challenging enough without the added barriers of prejudice, harassment, or indifference. Mentorship is built on trust, and scientists already place a great deal of trust in one another to produce honest and reliable data. Funding agencies should make it clear that holistic and inclusive mentorship should be equally valued.

Most important, however, is to remember what individuals can do to solve this problem. We feel strongly that anyone who wishes to become a scientist should have that opportunity. We support the efforts currently underway at Yale and elsewhere, but the wheels of these institutions turn slowly. Change must begin today, and it starts with us. We need to remember that everyone carries their identity into the lab, classroom, and field; for some, this is a heavy burden. We must acknowledge the inequality that surrounds us, and how our own unconscious bias contributes to it. We must then confront our own subjectivity and engage in conversations about diversity, rather than ignoring it or intellectualizing it. ■

Edward Courchaine and Sarah Smaga are graduate students in molecular biophysics and biochemistry at Yale University. They are members of the Yale Science Diplomats, an organization of graduate students and postdocs working to improve science communication and advocacy. The Science Diplomats, particularly Kenneth Buck, Richard Crouse, Bryan Leland, Amanda Pellowe, and Savannah Thais, researched and contributed to this article in collaboration with Women in Science at Yale, the Yale Graduate Chapter of oSTEM, and the Yale League of Black Scientists.

Acquiring Extra Senses

Animals' diverse sensory abilities will guide a technology-based revolution that gives humans perception beyond our natural senses.

BY BERND FRITZSCH

Vision, hearing, taste, smell, and touch: these are the five major senses humans are accustomed to. Our understanding of the world has been shaped by the information we are accessing with these senses. But while these are the only senses humans perceive consciously, they are not the only senses that we have. For example, the semicircular canals of the inner ear contribute to our sense of balance. Similarly, we know when our legs are stretched out or flexed because receptors inform about stretch and load on our muscle fibers and tendons. (See "Proprioception: The Sense Within" on page 36.) We also receive sensory feedback on the filling of our bladders and stomachs. Such internal senses are essential for daily life, and we are rarely aware of them as we are of visual or auditory stimuli.

Outside of humans, species across the animal kingdom harbor different—sometimes more powerful—sensory capabilities. Some animals can see infrared or ultraviolet, for example, and many species hear pitches well out of the range of human hearing. Some snakes are sensitive to heat, "seeing" the temperature of their environment. Many fish and salamanders can sense small electric discharges generated when muscle fibers contract, and some insects, birds, and mammals appear to use the Earth's magnetic field to orient and navigate. (See "Senses Census" on page 43.)

As we continue to learn about the diverse sensory capabilities that exist in nature and to develop technologies that enable detection of a broad range of sensory input, the logical next step is to put new senses into old (human) brains. Devices that replace lost senses already exist. Cochlear or vestibular implants convert auditory and balance input into nervous impulses sent to the user's brain, and analogous optical devices that can give sight back to the blind are close to coming online. (See "The Bionic Eye," *The Scientist*, October 2014.)

Devices that provide humans with senses outside of the traditional five are on the horizon. The brain has proven extraordinarily plastic. It can, for example, interpret sound stimuli even if the signal reaches areas of the brain dedicated for image processing. Initial experimental work was done in animals, rewiring the brain's pathways for processing sound, vision, etc. However, it now appears that such sensory crosstalk can happen naturally. For example, some blind people describe that they "see" around them, using sound (produced by their stick or tongue) in a manner similar to that of an echolocating animal. Thus, we have reason to

believe that we can integrate detection technology with human biology in a way that allows us to at least subconsciously perceive and process stimuli outside of humans' natural capabilities.

Imagine this: infrared information is directly fed into your visual cortex. You can now "see" warm-blooded animals during the night, much like a snake hunting a mouse. You might even be able to tell who around you has a fever by simply glimpsing their infrared temperature. Imagine seeing ultraviolet light as added color, or using polarized light to help you orient yourself in an unknown area, the way an ant does. Imagine having a dog's sense of smell, or the sense of ultrasound hearing that would enable you to listen to bats. Imagine being equipped with sensors to detect magnetic or electric fields.

Such technology is not far off. The bionic eyes being developed could easily have expanded wavelength ranges, covering infrared and ultraviolet. Cochlear implants could be tuned to expand the range to ultra- and infrasound to hear bat and elephant communications. Emerging "smart skin" technologies offer touch and temperature senses—to furnish sensation to prosthetic limbs, for example—and we could soon add magnetoreception to our array of sensory modalities. (See "Sixth Sense" on page 17.)

As we continue to learn about the diverse sensory capabilities that exist in nature and to develop technologies that enable detection of a broad range of sensory input, the logical next step is to put new senses into old (human) brains.

As these technologies continue to advance, researchers will expand our world beyond the limits of the traditional five senses. In the more distant future, we may even perceive radio signals that permanently connect us with larger networks, thus allowing us to tap into the multitude of sensors beyond those in and on our bodies. Even sensors too large to be incorporated into the human body—think of the vast laser arrays needed to detect disturbances in gravitational waves—could be fed into our consciousness. Then we'd truly have transgressed the boundaries imposed on our worldview by the limited sensory capacity of our species. ■

Bernd Fritzsch is a member of the German National Academy of Sciences and director of the Aging Mind and Brain Initiative at the University of Iowa in Iowa City. His research aims to retain nerve function for use with cochlear and vestibular implants.

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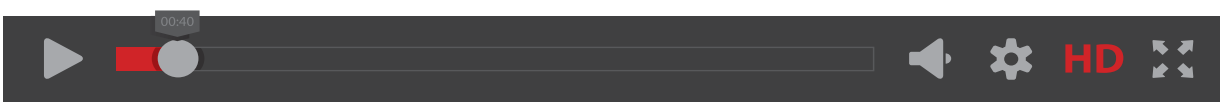

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Promoting Protein Partnerships

Scientists generate new protein-protein interactions at an impressive PACE.

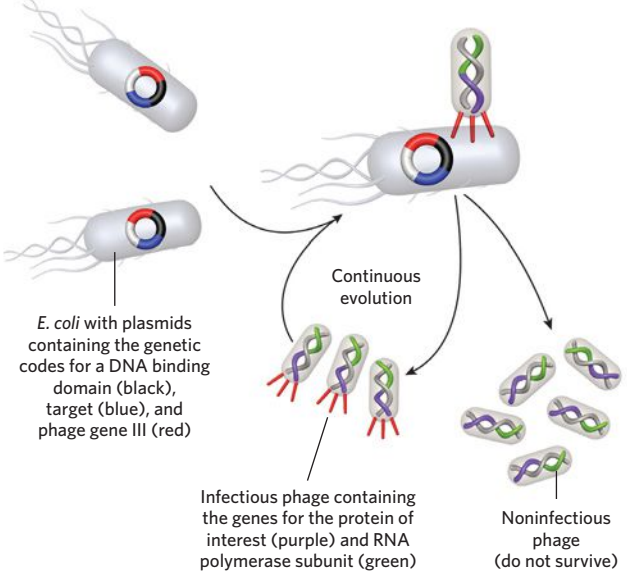
BY RUTH WILLIAMS

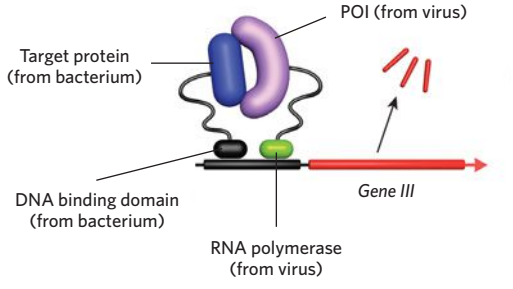
Since scientists began directing the evolution of proteins to obtain various desirable outcomes, the tools and techniques used to accomplish these goals have themselves evolved. One recent development, for example, is phage-assisted continuous evolution (PACE), which can generate desired protein variants in a fraction of the time it takes using traditional stepwise evolution methods.

And now PACE, too, has evolved. Early examples of PACE were largely used to evolve DNA-binding proteins, which was all well and good, says Greg Weiss of the University of California, Irvine, but the latest incarnation of the technique—protein-binding PACE—is "easily the coolest demonstration of PACE to date." The ability to evolve novel protein-protein interactions, Weiss explains, "brings the technology into the realm of . . . the therapeutics industry, diagnostics development—a whole bunch of fields."

The basic principle behind any PACE approach, says Harvard's David Liu, who first developed the technique in 2011, is that bacteriophage viruses and the *E. coli* they infect must be engineered so that the virus's survival depends on the particular interaction the researchers are trying to evolve. In the case of protein-binding PACE, the protein of interest (POI), which is expressed by the virus, must partner with the target protein—expressed in the bacterium—to drive expression of an essential virus gene. Put simply, the viruses need to quickly evolve the POI's ability to bind to the target, or die.

Liu and colleagues, in partnership with Monsanto, have used protein-binding PACE to generate a new variant of a bacterial toxin that binds to a receptor in the insect pest *Trichoplusia ni*, also known as the cabbage looper, and in so doing have created an insecticide hundreds of times more potent than the wild-type toxin, to which many pests have become resistant. Engineering the toxin-receptor system took a lot of work, says Liu, but "mercifully, all of that development greatly benefits future applications . . . without requiring us to reinvent the wheel." (*Nature*, 533:58-63, 2016) ■





A PERFECT MATCH: To evolve a strong binding affinity between a protein of interest (POI) and a desired target, the gene for the POI (fused to an RNA polymerase subunit) is first encoded into the genome of a bacteriophage lacking a gene (*gene III*) critical for robust infection of bacteria. These POI-containing viruses are then cultured with *E. coli* that contain *gene III* as well as the POI's desired target (left).

Interaction between the POI and target results in recruitment of the *E. coli* RNA polymerase to the *gene III* promoter (black), which drives transcription (above). Thus, only those viruses whose POI evolves a strong binding affinity for the target will be able to drive *gene III* expression, continuously infect the *E. coli*, and survive.

AT A GLANCE

TECHNIQUE	HOW IT WORKS	EASE OF SET UP
Cell-surface display	A library of bacteria or yeast contains variants of a protein of interest (POI) displayed on cell surfaces. Cells with target-bound POIs are isolated and the POI gene is further mutated to improve binding affinity.	Relatively easy
Protein-binding PACE	Bacteriophages lacking an essential gene (*gene III*) are engineered to express the POI. *E. coli* are engineered to contain *gene III* and the POI's target. Upon bacteriophage infection of *E. coli*, only viruses producing a robust POI-target interaction generate gene III and thus viable virions.	Necessitates large quantities of media—approximately 6 liters/day for 3 weeks—together with complex plumbing to enable correct culture media flow rates

Proprioception: The Sense Within

Knowing where our bodies are in space is critical for the control of our movements and for our sense of self.

BY UWE PROSKE AND SIMON GANDEVIA

In 1971, at the age of 19, Ian Waterman suffered a bout of severe viral gastroenteritis. The illness triggered an autoimmune response that stole his ability to gauge where his limbs were in relation to their environment. As described by Columbia University neurologist Jonathan Cole, Waterman was not paralyzed; his limbs moved, but he had no control over them. He felt disembodied, as if he was floating in air.[1,2]

The five basic senses—sight, hearing, smell, taste, and touch—enable us to perceive the world around us. But what about sensations generated by the actions of our own bodies? As Waterman's case demonstrates, the ability to sense our bodies is critical for telling us where we are in our surroundings as well as for the execution of normal movements. Sometimes referred to as the "sixth sense," proprioception includes the sense of position and movement of our limbs, the senses of muscle force and effort, and the sense of balance. These senses, triggered by our everyday activities, allow us to carry out our tasks successfully, without thinking; absent feedback from proprioceptors, we, like Waterman, would be lost.

We remain largely unaware of the actions of the sense organs responsible for generating our proprioceptive senses. Have you ever stood in a darkened room and tried to touch the tip of your nose with your index finger? Most of us can do that with uncanny accuracy without even trying. But if we cannot see it, how do we know where our arm is as it travels through the air aiming for the nose? And how do we know where our nose is? To make things even more bizarre, if the biceps muscle of the arm touching the nose is vibrated, it generates the sensation that the arm is lengthening and the nose is beginning to grow. All of this is the subject of proprioception.

Research on proprioception has lagged behind work on the five basic senses, perhaps because it is a sense we are largely unaware of. However, during the last 50 years neuroscientists have taken advantage of new stimulation and imaging techniques to achieve further insight into this elusive yet essential sense, acquiring new knowledge both at the receptor level and on the central processing of proprioceptive information.

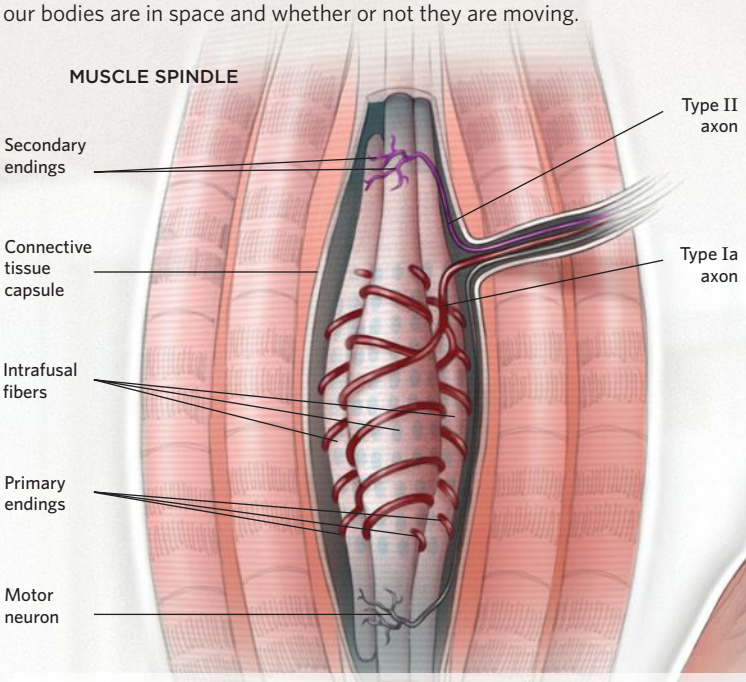
Sensing limb position and movement

Reflections on how we sense our body's movements date back to Galen in the 2nd century CE. But it wasn't until the early 1800s that specific ideas were formulated about the mechanisms underlying proprioception. German physiologists proposed that there was no need for any peripheral sense organs in proprioception; rather, they believed that neurons in the brain driving muscle contractions sent copies of their signals to adjacent sensory areas to generate the required sensation. It was called a "sensation of innervation."[3] At the turn of the 20th century, English neurophysiologist Charles Sherrington challenged this idea, on the grounds that we were aware of the positions of our limbs even when they lay relaxed, unmoving.[4] Sherrington believed that there were sensory receptors in peripheral tissues that signaled position and movement. Today, elements of both sets of ideas contribute to the accepted view.

The most obvious place to locate a sense organ that signals position and movement of a limb is in the joints, and for many years it was believed that joint receptors were the principal proprioceptors. Recordings of responses of central neurons during joint movements supported this idea.[5] But there were other possi-

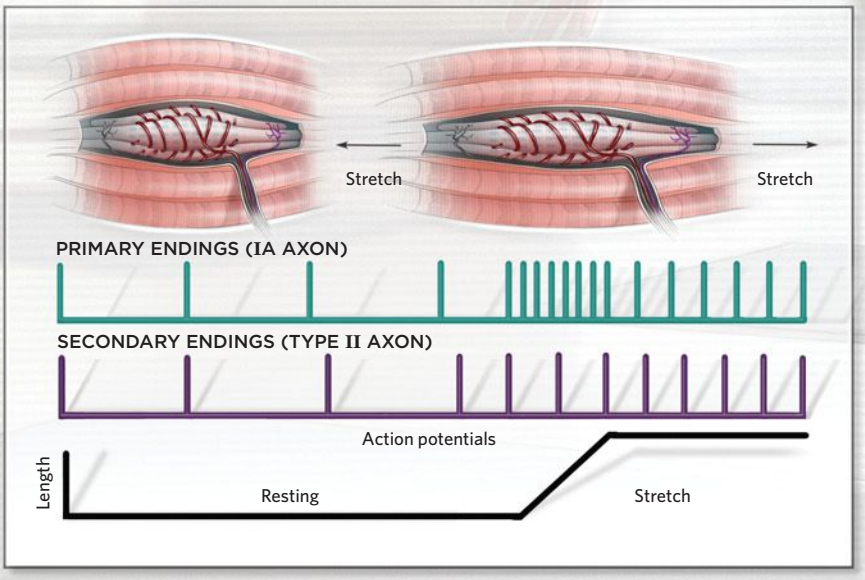
PROPRIOCEPTIVE RECEPTORS

There are several different types of proprioceptors, two of which are the muscle spindle and the tendon organ. Feedback from these sensors provides information about where our bodies are in space and whether or not they are moving.

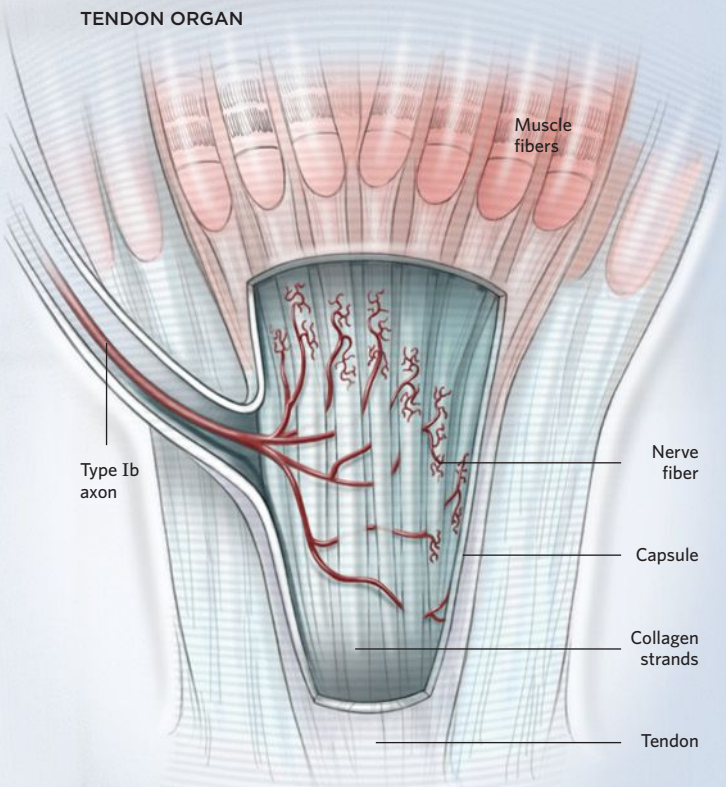


MUSCLE SPINDLES

Scattered throughout skeletal muscle, the muscle spindles are composed of connective tissue capsules containing bundles of specialized muscle fibers called intrafusal fibers. Wrapped around the middle of these fibers are the spiral terminals of a large sensory neuron, the type Ia axon. To one side of these spiral terminals, collectively called the primary endings, are the secondary endings, the terminals of a smaller sensory neuron, the type II axon. Both types of nerve endings serve as stretch sensors that send feedback about muscle contraction and length changes to the brain.

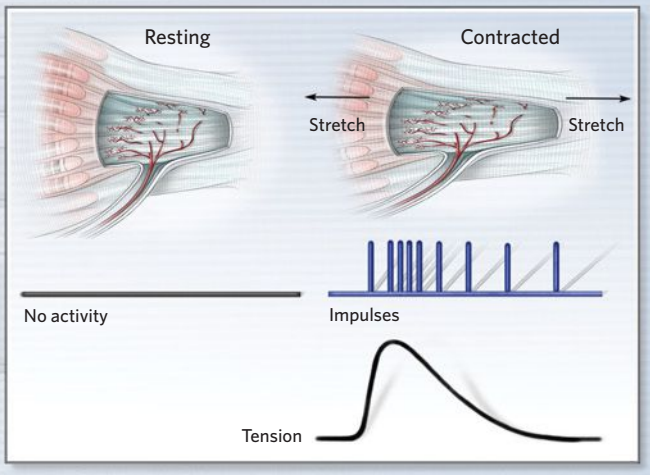


At rest, muscle spindles generate a trickle of nerve impulses. Stretching the muscle raises the number of nerve impulses from both the spindle's primary and secondary endings. Impulses in the primary endings signal both the rate of change in muscle length and the length change itself. Primary endings are therefore both movement and position sensors. This sensitivity to the rate of the stretch makes the primary endings responsive to muscle vibration. The secondary endings respond only to the length change, making them position sensors; they are vibration-insensitive.



TENDON ORGANS

Tendons attach muscles to bones. At the junction between muscle fibers and tendons lie the tendon organs, small bundles of tendon strands enclosed within a connective tissue capsule, similar to that in muscle spindles. The axon of a large sensory neuron, the type Ib axon, penetrates the capsule and terminates on the collagen strands. Each strand is attached to a single muscle fiber. In a typical tendon organ there are 10–20 innervated tendon strands with attached muscle fibers.



Like muscle spindles, tendon organs are stretch sensors. They are particularly sensitive to muscle contraction. In the resting state, tendon organs are silent. When the muscle fiber contracts, it pulls on the tendon strand and stretches it. This stretches the nerve ending of the Ib axon to generate nerve impulses. During a contraction, as muscle tension rises and then falls, the pattern of impulses increases and then decreases in frequency and number.

bilities. When the forearm is rotating about the elbow joint, there is movement not only at the joint; muscles inserting into the joint—the elbow flexors and extensors—are stretched and shortened as well. In a landmark series of experiments in 1972, Guy Goodwin and colleagues at the University of Oxford provided evidence that receptors in muscles, not in joints, were the most likely candidates for generating our sense of limb position and movement.[6]

Goodwin's team showed that if the biceps muscle of one arm of a blindfolded subject was vibrated, the subject perceived the arm as extending, even though it had not moved at all. The subject indicated the illusion by tracking the movement with their other arm. The authors argued that vibration had stimulated muscle spindles, stretch-sensitive capsules found in most of our skeletal muscles. The response to vibration mimicked spindle activity generated by muscle stretch, leading to the illusion of a stretching biceps, that is, extension of the arm. Vibration of the triceps muscle led to sensations of the arm moving into flexion—that is, the illusion that the triceps was being stretched. Importantly, vibrating the elbow joint did not produce any sensations of movement or displaced position, so the illusion could not be attributed to responses of skin or joint receptors.

Muscle spindles are unique in that they have two kinds of sensory nerve endings: the primary ending responds to both stretch of a muscle and the rate of stretch; the secondary ending responds only to the stretch. Earlier animal experiments had shown that the primary endings are especially sensitive to muscle vibration, while the secondary endings are vibration-insensitive. In 1973, Ian McCloskey at the University of New South Wales in Australia showed that the illusion of arm extension was largest with vibration frequencies of 80–100 Hz; when the frequency was lowered, the illusion, predominantly of movement, morphed into one of displaced position.[7] On the basis of these findings, he proposed that there were two senses: the sense of limb movement, generated largely by the primary endings of muscle spindles, and the sense of limb position, generated by both the primary and secondary endings.

Since then, the vibration illusion has been demonstrated many times at a variety of different joints, essentially confirming Goodwin's initial findings. And in 1986, J.C. Gilhodes and colleagues of Centre National de la Recherche Scientifique (CNRS) in France further showed that if the two antagonist muscle groups acting at the elbow joint—the flexors and extensors—were both vibrated at the same time, there was no vibration illusion.[8] If the frequency of vibration of one antagonist was lowered, an illusion gradually began to emerge, its size being directly proportional to the difference in vibration frequencies applied to the two muscles. These observations suggest that the brain is not processing signals from each muscle in isolation, but compares signals coming from the antagonist muscle groups and computes arm position from their difference.

In 2014, one of us (U.P.) and colleagues proposed that for this kind of arm-position matching task, it is not only the difference in signals from the antagonists that matters, but, in addition, the brain computes the difference in signals coming from both arms; when the difference is small, the arms are closely aligned.[9] Supporting

evidence for that view came the same year from Naoyuki Hakuta and colleagues of Showa University School of Medicine in Japan who showed that the size of the vibration illusion in one arm could be halved by vibrating the equivalent muscle of the other arm.[10] It seems that the brain is constantly monitoring the movements of our arms relative to one another, most likely to be able to accurately align them for tasks such as the manipulation of objects and tools.

Matching and pointing

The vibration illusion used a limb position–matching task: subjects are asked to indicate the sensation generated in one arm by tracking it with their other arm. It is, in fact, a sensation-matching task. But in everyday life, we don't go around matching the perceived positions of our limbs. If asked where we think our limbs are, we point to them.

Earlier this year, Anthony Tsay and colleagues at Monash University in Australia once again tested the vibration illusion, but this time, rather than tracking the illusion with the other arm, they asked subjects to point to the perceived position of the vibrated arm that remained hidden from view. Surprisingly, when the experiment was carried out in this way, subjects did not indicate any illusory displacement of their arm during vibration.[11] Yet when Tsay and his team used the more traditional matching task, the same subjects demonstrated normal vibration illusions. Furthermore, characteristic position errors seen in a matching task following a muscle contraction that have been attributed to muscle spindles, were no longer present in a pointing task. Taken together, these findings, suggest that in a pointing task the muscle spindles no longer play the dominant role of position sensors that they do in matching tasks; the source of the position signal changes depending on the nature of the task.

What, then, might be the position sensor in this pointing task? One possibility is skin receptors. In a matching task from one of our groups (S.G.'s), rhythmic stretching of the skin overlying a muscle can generate illusions of limb movement.[12] When Tsay and colleagues tested this hypothesis, they did not find any evidence that skin stretch receptors contributed to position sense in a pointing task. Another candidate is the sensory nerve endings in the joints—as had been originally hypothesized during the first half of the 20th century. There is evidence from our experiments measuring movement-detection thresholds that joints can contribute a signal, at least for the fingers.[13] The role of joints has not been studied in position matching and pointing tasks, however. This is a challenge for the future.

The body model

During an arm-matching task, the brain uses the difference in signal strength from the two arms to determine their relative positions. But in a pointing task, because we are determining the position of only one arm, the two-arm signal-difference mechanism cannot be used to indicate the position of the hidden arm.

So how is position sense generated in a pointing task? Tsay and colleagues postulate that in pointing tasks, the position signal coming from the hidden arm accesses a map of the body located in the brain to determine arm position. In a 2010 study, Matthew Longo and Patrick Haggard of University College London asked subjects to place one hand under a table, out of sight, while with their other hand they pointed to the perceived positions of different landmarks on the hidden hand, such as the fingertips and knuckles. When responses were plotted on a map, they revealed a distorted shape, squatter and wider than the actual hand.[14] The authors proposed that the brain uses information coming from the hand, including proprioceptive inputs, and combines them with a central map, or body model, to determine location of the landmarks. The distortions in shape resembled those seen in sensory maps drawn on the human cortex many years earlier by neurosurgeons Wilder Penfield and Edwin Boldrey[15]—the famed homunculus, which represents differences in the cortical innervation density of the body's various parts. The distortions in the perceived shape of the hand described by Longo and Haggard may be related to the density of receptors on the surface of the hand, but it remains unclear how the information provided by the distorted proprioceptive maps is used to locate the position of the limbs in space.

In addition to knowing where our limbs are in space, at least two other sensations contribute to our physical self-awareness: a sense of force and a sense of effort or heaviness.

When subjects were shown drawings of differently shaped hands, they were able to correctly select the one that was closest to the true shape of their hand. So while a body model generated by proprioceptive inputs showed characteristic distortions, another map called the body image—probably based on remembered visual information—provided a more accurate representation.

There are a number of serious, debilitating conditions associated with disturbances to the body image. These include eating disorders such as anorexia nervosa; conditions in which the patient denies that a part of their body actually belongs to them; and out-of-body experiences, where the patient believes that their body is no longer under their own control. Indeed, our very sense of self is believed to be generated in association with the central processing of proprioceptive information. Another related and well-known phenomenon is phantom limb, where an amputated limb is perceived to continue to exist.

Our body image is labile and can be modified. Simultaneous touching of a hidden hand and a visible rubber hand lying near it leads the rubber hand to be adopted as part of the body.[16] As we move about during everyday activities, there must be a continuous updating of the map, based on incoming movement information. This was first recognized many years ago[17] and emphasizes the key link between the proprioceptive sensory system and the motor system.

While we have learned a lot in recent years about the peripheral signals responsible for the senses of limb position and movement, the picture continues to evolve. We are beginning to recognize that the source of the signals can change, depending on the task undertaken. Yet we still know relatively little about the central processing of the incoming information. How do we derive the metrics of body parts, for example, or process constantly changing spatial signals during ongoing body movements? This is an area where we should focus future research efforts.

The senses of effort, force, and heaviness

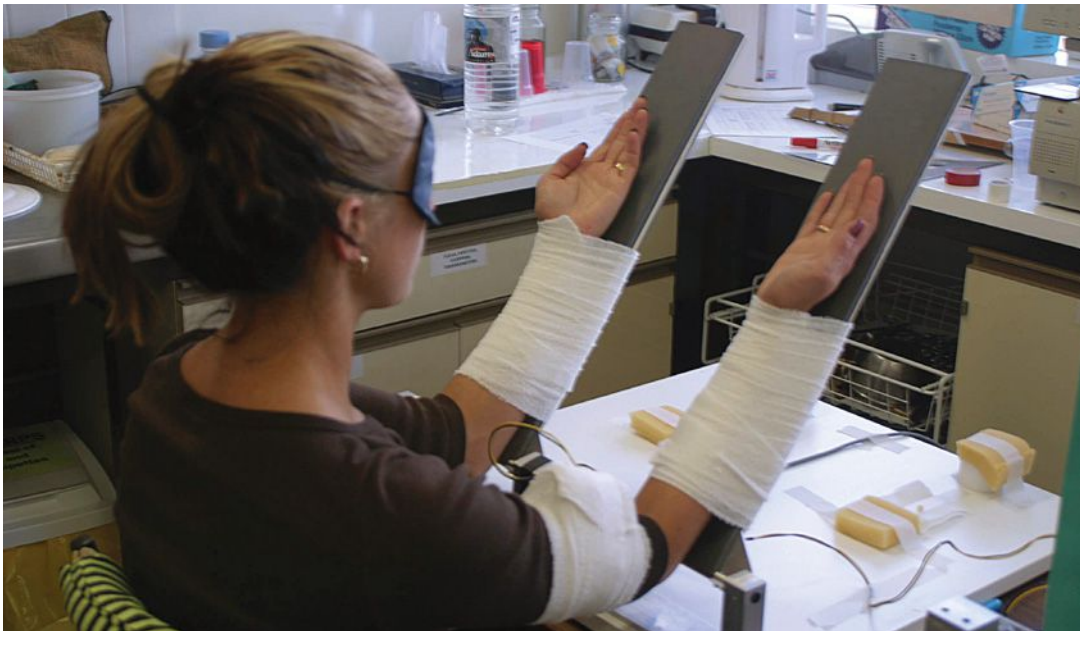
In addition to knowing where our limbs are in space, at least two other sensations contribute to our physical self-awareness: a sense of force and a sense of effort or heaviness. When we are asked to compare the heaviness of two objects of nearly the same weight, we typically juggle them up and down in our hands before making our judgment. It suggests that our sense of heaviness is closely allied to the sense of movement.

If our muscles are infused with a muscle relaxant, whatever we are holding, and even our limbs themselves, feel much heavier. According to work from one of our labs (S.G.'s), this is a disturbance in the sense of effort triggered by muscle weakness.[18] In response to this weakness, our motor neurons fire at an increased rate to generate the required level of muscle force, and this higher firing rate results in a greater perceived effort, giving the impression of increased weight. Similarly, whenever our muscles become weaker as a result of fatigue from exercise, the motor neurons increase their firing rate to compensate for the loss in force. That is why our limbs feel like lead at the end of vigorous exercise.

In a simplified view, the sense of effort is produced by impulses in the motor cortex that both travel down the spinal cord to the lower motor neurons to trigger muscle contraction, and relay back to sensory areas of the brain, where the sensation of effort is generated. However, there is evidence from fatigue experiments, in which magnetic brain stimulation was used to mimic motor commands, that the sense of effort is generated somewhere upstream of the motor cortex and that the effort-force relationship undergoes constant adjustment.[19] Furthermore, if the sensory and motor neurons supplying a limb are blocked and an attempt is made to try to move the paralyzed, anesthetised limb, this can generate sensations of changed limb position and movement in the absence of any actual movement, S.G.'s group showed.[20] So the sense of effort is linked in some way not only to the sense of force, but also to the senses of position and movement.

As well as having a centrally generated sense of effort, we are able to sense muscle force from the action of receptors specifically designed as tension sensors, the tendon organs. At each end of a muscle is a tendon, which anchors the muscle to bone; at the junction between tendon and muscle fibers lies a population of sensors called the Golgi tendon organs. Each consists of a large sensory axon that terminates on the tendon strands that connect at one end to the tendon proper and at the other to each of 10–20 individual muscle fibers. Each muscle fiber belongs to a different motor unit. The tendon organ will respond to the contraction of a single motor unit. Contraction of the whole muscle will engage a population of tendon organs, which send their impulses to the cerebral cortex with information about the amount of force exerted. So whenever we contract our muscles we have a centrally generated sense of effort accompanied by a sense of muscle force arising in our tendon organs.

In an experiment aimed to show this, subjects were asked to compare the stiffness of a series of compression springs.[21] The sub-



COURTESY OF UWE PROSKE

MATCHING GAME: Volunteers are blindfolded and their arms are strapped to two hinged paddles. Experimenters move one arm to a test position and ask the subjects to use their other arm to match the position of the first. In a different experimental setup (not pictured), only one arm is strapped in, and study participants indicate the position of the arm by moving the empty paddle to align with the perceived position of the hidden arm. Position errors from these two scenarios suggest that different receptors operating at the elbow joint and its attached muscles are responsible for the subjects' assessments of arm position.

jects pressed a spring with one hand and used their other hand to select from a range of springs one with matching stiffness. Under control conditions, subjects were quite accurate in their choice. When the muscles of one hand were weakened by infusing a muscle relaxant, even though subjects now complained that their weakened hand required much more effort to compress the springs, they were still surprisingly accurate in choosing a spring of matching stiffness. But when subjects were instructed to match efforts, not forces, they made large errors in their comparison of spring stiffness. It seems we have the ability to selectively choose between our senses of effort and of muscle force, depending on the nature of the task.

Our "sixth sense" not only enables us to control the movements we make, but provides us with the ability to perceive ourselves moving in space and acting in relation to our surroundings.

Researchers have recently proposed that force signals of peripheral origin arise in both tendon organs and muscle spindles. If a muscle is progressively paralyzed, at the onset of the paralysis lifted objects feel heavier. As the paralysis deepens, paradoxically, lifted objects become lighter again. This result has been attributed to the action of muscle spindles.[22] When the muscle starts to weaken from paralysis, the spindles remain unparalyzed, and their signals remain strong, contributing to the sense of increased heaviness. When the paralysis gets deep enough, the intrafusal fibers of spindles become paralyzed as well, leading to a reduction of the spindle signal, and as a result, the object feels less heavy than before. Thus, in addition to a centrally generated sense of effort, we have a peripherally generated sense of force or of heaviness, which arises from signals in both muscle spindles and tendon organs.

Getting a grip

Over the many months after he suffered his proprioceptive loss, Waterman gradually learned to move again. At first, just standing stably was difficult. Using vision and a conscious will to move, Waterman, now in his mid-60s, is able to slowly combine muscle actions to achieve desired movements, such as lifting a cup of coffee. The more complicated the movement, the harder he has to think. Seeing his body and trunk are of critical importance. In the dark he remains completely helpless.

Remarkably, Waterman is also able to compare the heaviness of objects of identical size as well as the rest of us who have an intact proprioceptive system—provided his eyes are open. It seems that he judges the heaviness of objects by observing the speed and extent of their movement when he lifts them.

Waterman's unique case emphasizes the importance of proprioception in our daily lives. Our "sixth sense" not only enables us to control the movements we make, but provides us with our sense of self, the awareness of our body and its movements as we navigate through our surroundings. As we unravel the neural mechanisms that underlie proprioception, we are learning more about how the brain processes sensory information. And that will ultimately lead us to a better understanding of ourselves. ■

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Senses Census

From detecting gravity and the Earth's magnetic field to feeling heat and the movement of water around them, animals can do more than just see, smell, touch, taste, and hear.

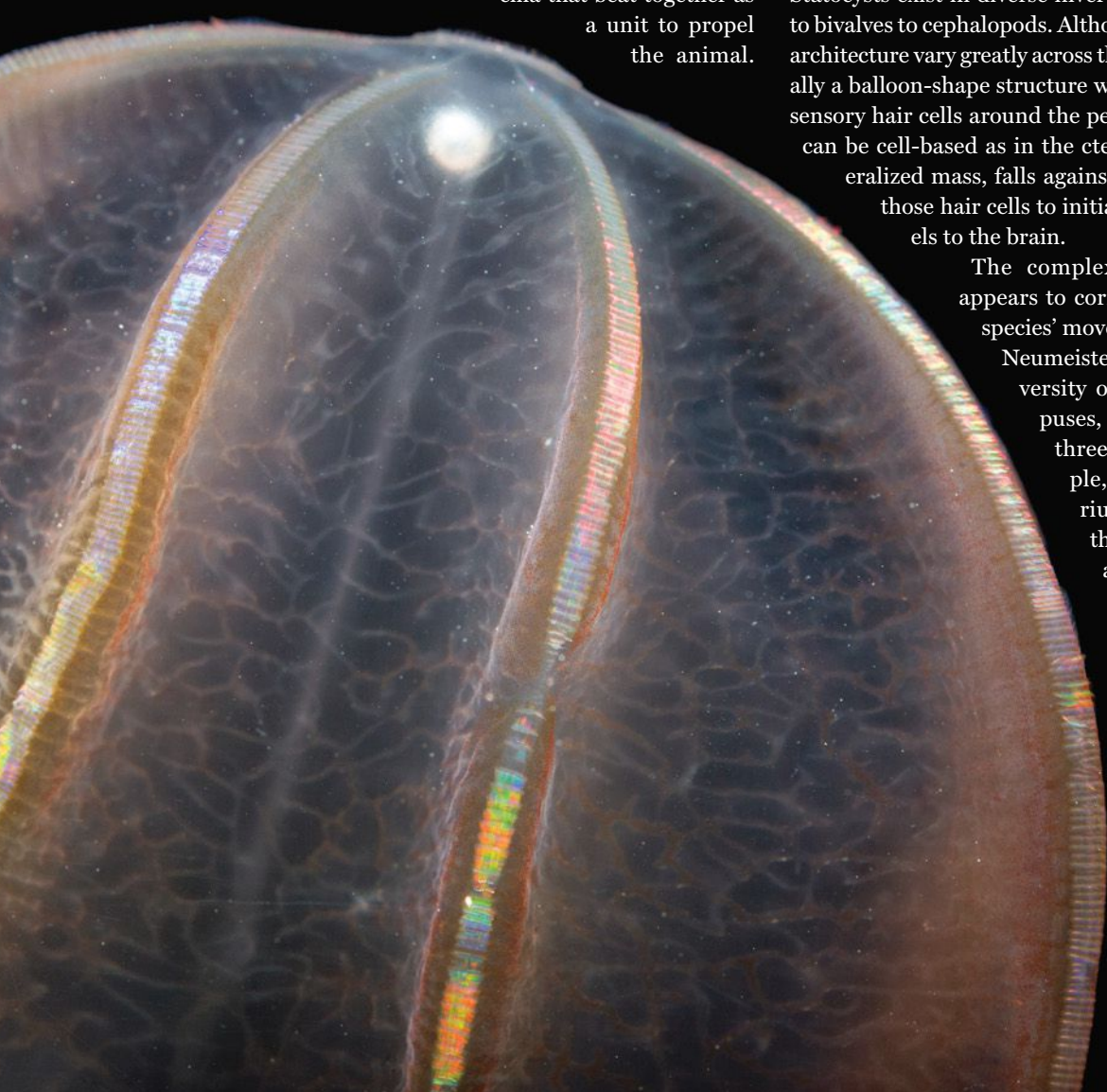
BY *THE SCIENTIST* STAFF

Growing up, we learn that there are five senses: sight, smell, touch, taste, and hearing. For the past five years, *The Scientist* has taken deep dives into each of those senses, explorations that revealed diverse mechanisms of perception and the impressive range of these senses in humans and diverse other animals. But as any biologist knows, there are more than just five senses, and it's difficult to put a number on how many others there are. Humans' vestibular sense, for example, detects gravity and balance through special organs in the bony labyrinth of the inner ear. Receptors in our muscles and joints inform our sense of body position. (See "Proprioception: The Sense Within" on page 36.) And around the animal kingdom, numerous other sense organs aid the perception of their worlds.

DETECTING GRAVITY AND MOTION

The ability to detect gravity and the body's motion may be one of the most ancient senses. In vertebrates, the complex vestibular system handles this task via the otolith organs and semicircular canals of the inner ear. Invertebrates rely on a simpler structure known as a statocyst to sense their own movement and body position relative to the Earth's gravitational pull. Even comb jellies (ctenophores), which may have been the first multicellular animals to evolve, have a rudimentary statocyst—essentially, a weight resting on four springs that bend when the organism tilts in the water.

The comb jelly's single statocyst sits at the animal's uppermost tip, under a transparent dome of fused cilia. A mass of cells called lithocytes, each containing a large, membrane-bound concretion of minerals, forms a statolith, which sits atop four columns called balancers, each made up of 150–200 sensory cilia. As the organism tilts, the statolith falls towards the Earth's core, bending the balancers. Each balancer is linked to two rows of the ctenophore's eight comb plates, from which extend hundreds of thousands of cilia that beat together as a unit to propel the animal.



As the balancers bend, they adjust the frequency of ciliary beating in their associated comb plates. "They're the pacemakers for the beating of the locomotor cilia," says Sidney Tamm, a researcher at the Marine Biological Laboratory in Woods Hole, Massachusetts, who has detailed the structure and function of the ctenophore statocyst (*Biol Bull,* 227:7-18, 2014; *Biol Bull,* 229:173-84, 2015).

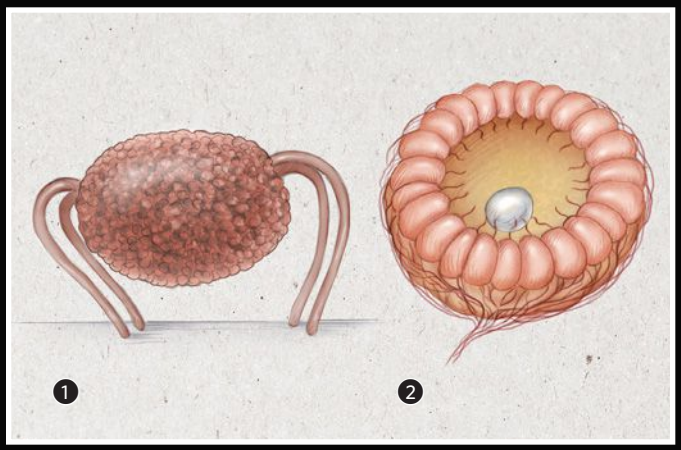
The complexity of the statocyst system appears to correlate with the complexity of a species' movement and behavior.

—Heike Neumeister, City University of New York

Sensing gravity's pull and the subsequent ciliary response is entirely mechanical, Tamm notes—no nerves are involved in ctenophore statocyst function. Most other animals with statocyst sensing, on the other hand, do employ a nervous system. Statocysts exist in diverse invertebrate species, from flatworms to bivalves to cephalopods. Although the details of the statocyst's architecture vary greatly across these different groups, it is generally a balloon-shape structure with a statolith in the center and sensory hair cells around the perimeter. As the statolith, which can be cell-based as in the ctenophore or a noncellular mineralized mass, falls against one side of the sac, it triggers those hair cells to initiate a nervous impulse that travels to the brain.

The complexity of the statocyst system appears to correlate with the complexity of a species' movement and behavior, says Heike Neumeister, a researcher at the City University of New York. Squids and octopuses, which move rapidly around in three-dimensional space, for example, have highly adapted equilibrium receptor organs. Likewise, the nautilus, whose relatives were among the first animals to leave the bottom of the ocean and begin swimming and employing buoyancy, has a fairly advanced system. Each of its two statocysts is able to detect not only gravity, like the ctenophore's, but angular accelerations as well, like those of octopuses, squids, and cuttlefishes (*Phil Trans R Soc Lond B,* 352:1565-88, 1997). "[Nautilus] statocysts are an intermediate state of

A BALANCING ACT: Ctenophore statocysts (1), consist of a statolith composed of lithocyte cells and four compound cilia called balancers that serve as the statolith's legs. As the animal tilts in the water, the statolith falls to the side, bending the balancers and triggering a mechanical signal to adjust the frequency of ciliary beating along the ctenophore's eight comb plates. Other invertebrates have a more complex statocyst, in which a sphere of sensory hair cells detects the movement of a statolith floating within it (2). When the statolith falls against a hair cell, it triggers an electrical impulse that sends the information to the animal's central nervous system.

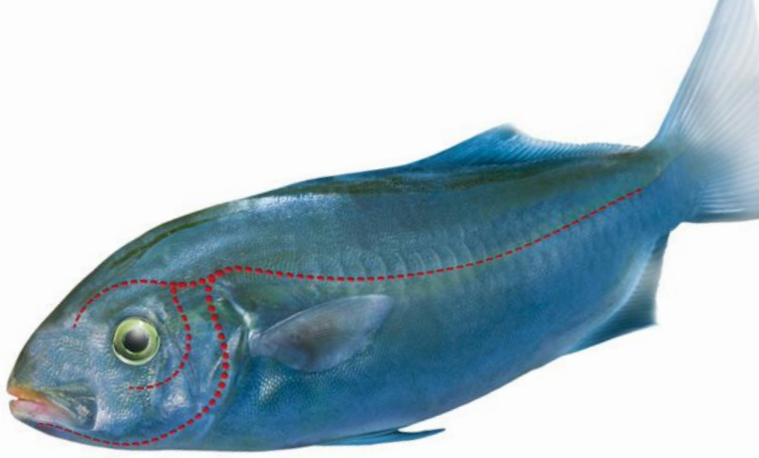


evolution between simpler mollusks and modern cephalopods," says Neumeister.

These sensory systems may be damaged by the man-made noise now resonating throughout the world's oceans. Michel André, a bioacoustics researcher at the Polytechnic University of Catalonia in Barcelona, Spain, started looking into the effects of noise pollution on cephalopods after the number of giant squid washing ashore along the west coast of Spain shot up in 2001 and then again in 2003. "The postmortem analysis couldn't reveal the causes of the death," recalls André. Nearby, however, researchers were conducting ocean seismic surveys, using pulses of high-intensity, low-frequency sound to map the ocean floor. Although, these animals don't have ears, André and others wondered if that noise might be affecting the squids' sense of balance.

Sure enough, exposing squid, octopuses, and cuttlefish to low-frequency sound, which caused the animals' whole bodies to vibrate, universally resulted in damage to their statocysts. Hair cells were ruptured or missing; the statocysts themselves sometimes had lesions or holes; even the associated nerve fibers suffered damage. As a result, the animals became disoriented, often floating to the water's surface (*Front Ecol Environ*, doi:10.1890/100124, 2011). "They eventually died because they were not eating," says André. "I don't think that [anyone thought] that animals who could not hear would be suffering from acoustic trauma. . . . This is something we have to be concerned about."

—Jef Akst

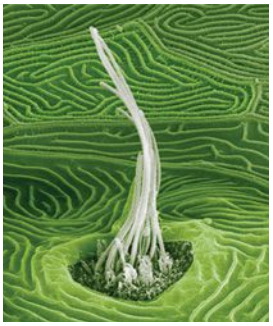


FEELING THE FLOW

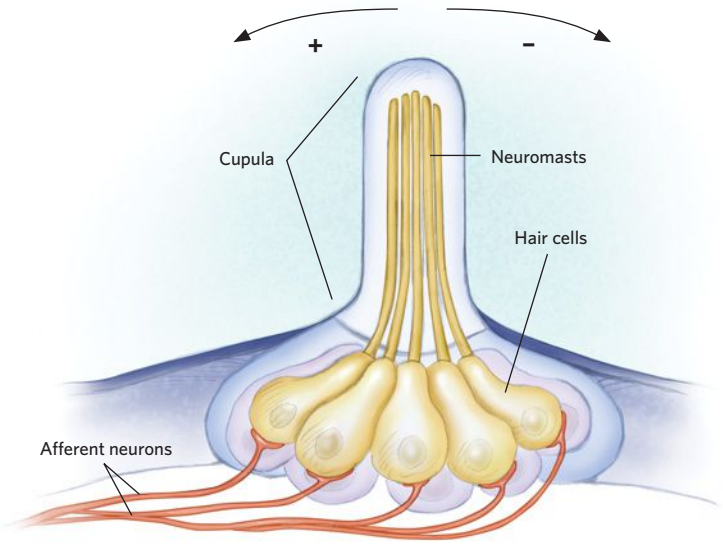
Light, sound, and odors travel through water very differently than they do in air. Accordingly, aquatic animals have sensory systems tuned to their fluid medium—most notably, the lateral line system. Observable as distinct pores that run along the flanks and dot the heads of more than 30,000 fish species, the lateral line is composed of mechanoreceptors called neuromasts—clusters of hair cells not unlike those found in the mammalian ear and vestibular system—that relay information about the velocity and acceleration of water flow.

"If you live underwater, the water is often moving with respect to your body, and it's carrying the environment with it," says University of California, Irvine, biologist Matt McHenry, who studies the lateral line sense in fish. "To have some sense of where it's going and how fast it's going seems pretty fundamental. It makes a lot of sense that they would be tuned in to flow." Despite more than 100 years of research on the lateral line, however, many questions remain about its structure and function, how the sense relays information to the nervous system, and how it affects fish behavior.

Transparent zebrafish larvae, whose surface lateral line structures can be observed without the need for dissection, are starting to yield answers. Using a high-power microscope, Jimmy Liao of the University of Florida's Whitney Laboratory for Marine Science and his colleagues attach tiny glass probes to individual neuromasts and stimulate the mechanoreceptors with controlled vibrations. "We're able to tickle an individual neuromast and record from the neuron that innervates that specific cluster," he says. With this system, Liao's team has found that a neuromast's response to different water velocities depends on its position in space (*J Neurophysiol*, 112:1329-39, 2014). "If you bend [a neuromast] halfway and then give it a velocity, that's very different than just giving it the velocity in its normal configuration," Liao says.



NEUROMASTS: Clusters of hair cells project from the surface of a larval zebrafish's skin to sense the water's movement. (Cupula has been removed.)



HAIRLINE: Modified epithelial cells called hair cells—similar to those in the mammalian inner ear—are the work horses of the lateral line in fishes. Hair cells connect to afferent neurons and are grouped together into structures called neuromasts whose hairs are covered by a jelly-like secretion called the cupula. When moving water or vibrations trigger neuromasts, which sit inside pores on the head, body, and tail of the fish, hair cells stimulate neurons to relay information about velocity or acceleration to sensory ganglia distributed through the fish's body.

Liao and his collaborators have also determined that the sensors stimulate sensory neurons in a nonlinear fashion—that is, with increasing velocity, the nervous response only increases up to a certain point, then levels off (*J Neurophysiol*, 113:657-68, 2015). And the researchers have traced the nervous connections from neuromasts found on the flank of a fish's body to specific locations within the posterior lateral line ganglion, a group of nerve cells outside the brain. Tail neuromasts are connected to afferent neurons found in the center of the ganglion, Liao says, while neuromasts closer to the head contact neurons on its periphery.

When it comes to the specific role of lateral line sensing in fish behavior, however, the research is still somewhat murky. "We have a very crude understanding for what behaviors depend on this sense," says McHenry. "At a receptor level, I think we have a pretty good handle for what kind of information they're extracting, but in real-world applications it's not clear why that's useful a lot of the time."

One challenge is isolating sensory information detected by the lateral line from information detected by other fish senses, specifically vision, says Sheryl Coombs, an emeritus professor at Bowling Green State University who has spent decades studying the links between the lateral line sense and fish behavior. "Most behaviors rely on animals integrating information across the senses," she says. "It's difficult sometimes to pick apart the role of the lateral line because the senses act together in complementary ways, often."

To get around this problem, Coombs has studied nocturnal fish and species that live in complete darkness, such as the Mexican blind cave fish (*Astyanax mexicanus*), which often lacks eyes altogether. In this species, Coombs has found that the fish may use their lateral line sense to construct rudimentary maps of their surroundings. "They're basically 'listening'—for lack of a better word—to their own flow field that they create by moving through the water," she says. "They create the flow, and then they're listening to distortions in that flow created by the presence of the obstacle. It's sort of analogous to echolocation in the sense that animals are producing a sound and they're listening to how the sound bounces back." —Bob Grant

MAGNETORECEPTION

Mollusks, insects, birds, and some mammals are able to sense Earth's magnetic field, but how they do so remains a mystery. In the last couple of decades, "most of the research [has focused] on proteins and genetics in the various animals, speculating on possible means of magnetoreception," says Roswitha Wiltschko, who—along with her husband, Wolfgang Wiltschko—ran a magnetoreception lab at Goethe University Frankfurt, Germany, until she retired in 2012.

Although the details are still unclear, most magnetoreception researchers have converged upon two key mechanisms: one based on magnetite, an iron oxide found in magnetotactic bacteria, mollusk teeth, and bird beaks; and the other on cryptochromes, blue-light photoreceptors first identified in *Arabidopsis* that are known to mediate a variety of light-related responses in plants and animals.

Once we have found magnetoreception structures reliably, we can start trying to understand how they convert the magnetic field into a neural response.

—Roswitha Winklhofer
Goethe University Frankfurt

In 2001, Michael Winklhofer, then at Ludwig Maximilian University of Munich, and colleagues reported their identification of magnetite in the beaks of homing pigeons (*Eur J Mineral*, 13:659-69). A year earlier, Klaus Schulten of the University of Illinois at Urbana-Champaign and colleagues proposed that cryptochromes in the bird eye might also play a role in avian magnetoreception (*Biophys J*, 78:707-18, 2000). Specifically, the authors suggested that photoactivated cryptochromes form a pair of charged radicals, which are thought to affect a bird's sensitivity to light. Schulten and his colleagues speculated that Earth's magnetic fields could somehow affect these cryptochrome reactions in a way that would alter the bird's visual system, providing information about its orientation. (See "A Sense of Mystery," *The Scientist*, August 2013.)

Over the years, support for this idea has emerged. In 2007, Henrik Mouritsen

of the University of Oldenburg, Germany, and colleagues showed that blue light–exposed avian cryptochrome 1a indeed forms long-lived radical pairs (*PLOS ONE,* 2:e1106). And this April, Peter Hore of the University of Oxford and colleagues published a computer-based modeling study showing that light-dependent chemical reactions in cryptochrome proteins in the eyes of migratory birds could "account for the high precision with which birds are able to detect the direction of the Earth's magnetic field," the authors wrote (*PNAS,* 113:4634-39, 2016).

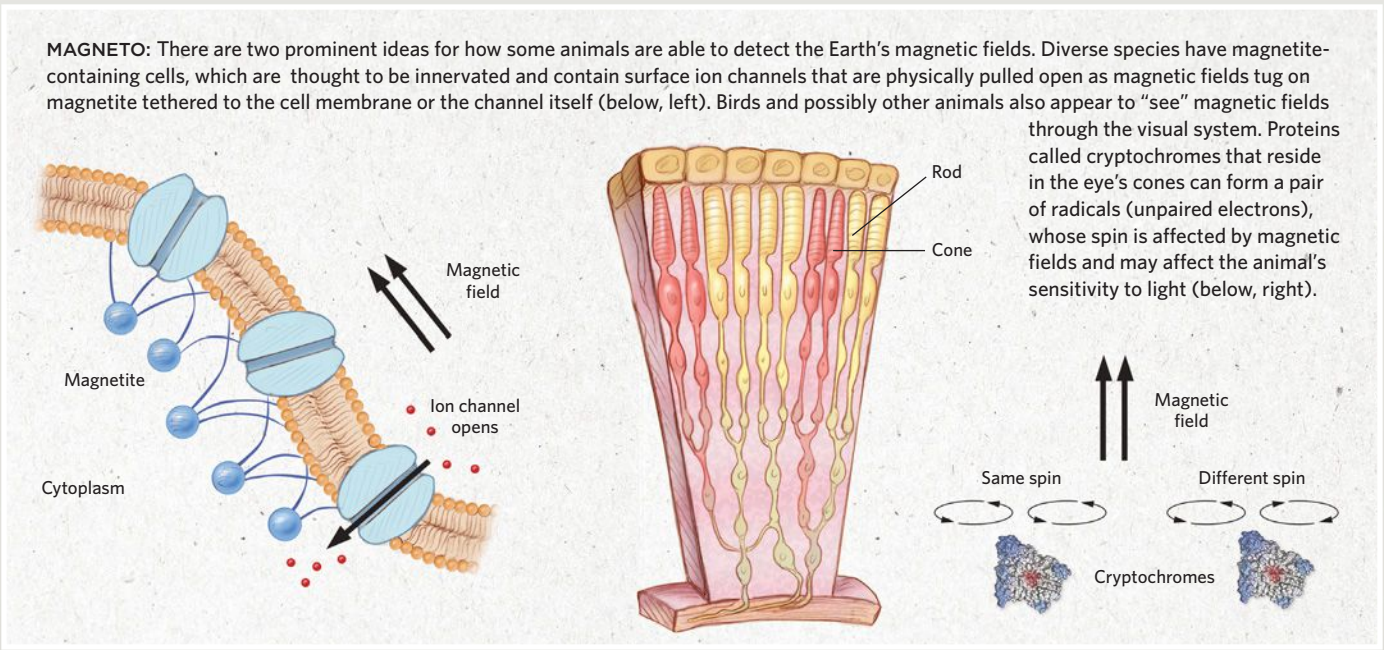
Birds seem to use both the magnetite and the radical pair/cryptochrome–based mechanisms. Cryptochrome-based orientation has also been reported in *Drosophila* and cockroaches, and researchers have found evidence of magnetite-based navigation in animals from mollusks to honeybees. And there may be other components of magnetoreception still to discover, as scientists continue their search for magnetic sensory structures across the animal kingdom. Late last year, for example, biophysicist Can Xie of Peking University in Beijing and colleagues identified a *Drosophila* protein, dubbed MagR, that—when bound to photosensitive Cry—has a permanent magnetic moment, the researchers reported, meaning it spontaneously aligns with magnetic fields (*Nat Mater,* 15:217-26, 2015). The MagR/Cry complex, the researchers noted, exhibits properties of both magnetite-based and photochemical magnetoreception. (See "Biological Compass," *The Scientist,* November 2015). The study was met with skepticism, however, and the results have yet to be independently verified.

In addition to mechanism, questions remain about the function of magnetoreceptive capabilities. "Once we have found [magnetoreception structures] reliably, we can start trying to understand how they convert the magnetic field into a neural response, and at the brain level, how are the single responses processed and integrated with other navigational information to tell the animal where it is and where to go," says Winklhofer.

In the mid-1990s, for example, Wiltschko and her husband Wolfgang demonstrated that migratory birds called silvereyes (*Zosterops lateralis*) reacted to a strong magnetic pulse by shifting their orientations 90° clockwise, returning to their original headings around a week later (*Experientia,* 50:697-700, 1994). Magnetic field manipulations can also affect *Drosophila* navigation, John Phillips, now of Virginia Tech, has shown (*J Comp Physiol A,* 172:303-08, 1993). And Richard Holland, now of Bangor University, U.K., and colleagues showed in the mid-2000s that experimentally shifting the Earth's magnetic field altered homing behavior in *Eptesicus fuscus* bats (*Nature,* 444:702, 2006).

"Some animals use their magnetic sense for long-distance navigation, some for magnetic alignment or orientation, and some animals may have the capability to sense the magnetic field but do nothing," says Xie. Or, at least, nothing that has yet been recognized by researchers.

—Tracy Vence



MAGNETO: There are two prominent ideas for how some animals are able to detect the Earth's magnetic fields. Diverse species have magnetite-containing cells, which are thought to be innervated and contain surface ion channels that are physically pulled open as magnetic fields tug on magnetite tethered to the cell membrane or the channel itself (below, left). Birds and possibly other animals also appear to "see" magnetic fields through the visual system. Proteins called cryptochromes that reside in the eye's cones can form a pair of radicals (unpaired electrons), whose spin is affected by magnetic fields and may affect the animal's sensitivity to light (below, right).

THERMORECEPTION

Many animals are able to sense heat in the environment, but vampire bats and several types of snakes are the only vertebrates known to have highly specialized systems for doing so. Humans and other mammals sense external temperature with heat-sensitive nerve fibers, but pit vipers, boa constrictors, and pythons have evolved organs in their faces that the animals use to detect infrared (IR) energy emitted by prey and to select ecological niches. And vampire bats have IR receptors on their noses that let them home in on the most blood-laden veins in their prey.

"Infrared sense is basically a souped-up [version] of thermoreception in humans," says David Julius, a professor and chair of the physiology department at the University of California, San Francisco (UCSF), who studies this sense in snakes. The difference is, snakes and vampire bats "have a very specialized anatomical apparatus to measure heat," he says.

These IR-sensing apparatuses, known as pit organs, have evolved at least twice in the snake world—once in the ancient family that includes pythons and boas (family Boidae) and once in the pit vipers (subfamily Crotalinae), which includes rattlesnakes. Pythons and boas have three or more simple pits between scales on their upper and sometimes lower lips; each pit consists of a membrane that is lined with heat-sensitive receptors innervated by the trigeminal nerve. Pit vipers, by contrast, typically have one large, deep pit on either side of their heads, and the structure is more complex, lined with a richly vascularized membrane covering an air-filled chamber that directs heat onto the IR-sensitive tissue. This geometry maximizes heat absorption, Julius notes, and also ensures efficient cooling of the pit, which reduces thermal afterimages.

In 2010, Julius and Elena Gracheva, now at Yale University, identified the heat-sensitive ion channel TRPA1 (transient receptor potential cation channel A1) that triggers the trigeminal nerve signal in both groups of snakes (*Nature,* 464:1006-11). The same channels in humans are activated by chemical irritants such as mustard oil or by acid, and the resulting signal is similar to those produced by wounds on the skin, Gracheva says. In snakes, these channels have mutated to become sensitive to heat as well.

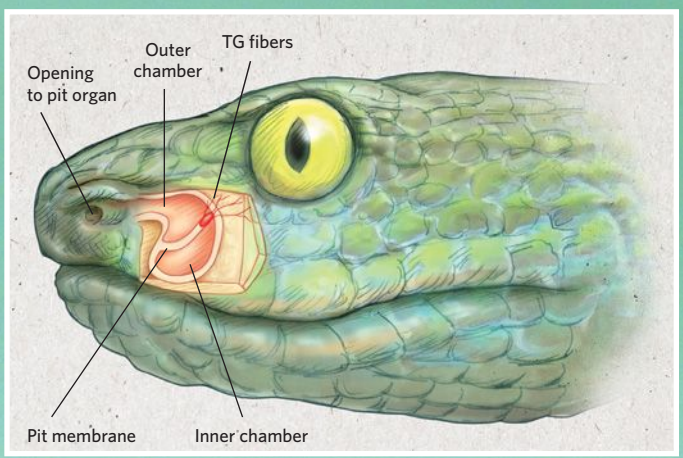
Vampire bats—which, true to their name, feed on the blood of other creatures—are the only mammals known to have a highly developed infrared sense. Like snakes, the bats have an innervated epithelial pit, which is located in a membrane on the bats' noses. In 2011, Julius, Gracheva, and their colleagues identified the key heat-sensitive ion channel in vampire bats as TRPV1 (*Nature,* 476:88-91). In humans, this channel is normally triggered by temperatures above 43 °C, but in the bats, it is activated at 30 °C, the researchers found.

More than 30 years ago biologists Peter Hartline, now of New England Biolabs in Ipswich, Massachusetts, and Eric Newman, now at the University of Minnesota, found that information from the snake pit organ activates a brain region called the optic tectum (known in mammals as the superior colliculus), which is known to process visual input (*Science,* 213:789-91, 1981). The pit organ appears to act like a pinhole camera for infrared light, producing an IR image, Newman says. However, it's impossible to know whether snakes actually "see" in infrared.

"Unfortunately we don't have a sensory map [of the brain] in snakes or vampire bats," Gracheva agrees. "I don't think we have enough data to say [these animals] can superimpose a sensory picture onto the visual picture, though it definitely would make sense."

—Tanya Lewis

HEAT SENSE: Pit organs consist of a large, hollow, air-filled outer chamber and a smaller inner chamber separated by a membrane embedded with heat-sensitive receptors. The receptors are innervated by the trigeminal ganglia (TG), which transmit the infrared signals to the brain.



The number of taxa that are now effectively known to detect weak electric fields is increasing.

—Shaun Collin, University of Western Australia

ELECTRORECEPTION

Sharks and other fish are well known for their ability to detect electric fields, with some species able to sense fields as weak as a few nanovolts per centimeter—several million times more sensitive than humans. But it turns out that they aren't the only ones. In recent years, evidence for electroreception has been accumulating all over the animal kingdom: in monotremes (such as the platypus), crayfish, dolphins, and, most recently, bees.

"The number of taxa that are now effectively known to detect weak electric fields is increasing," says Shaun Collin of the University of Western Australia, "although some of these we don't know very much about yet, and for some we only have evidence of a behavioral response."

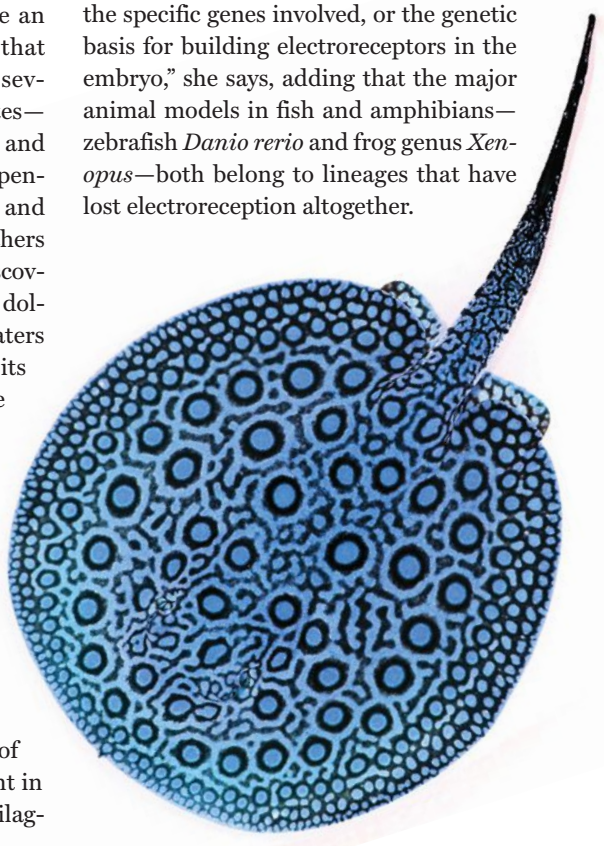
First formally described in the middle of the last century in weakly electric fish (*J Exp Biol,* 35:451-86, 1958), electroreception operates most effectively over less than half a meter in water—a more conductive medium than air. The sense is most frequently employed by aquatic or semi-aquatic animals to find prey in environments where other senses are less reliable—in murky or turbid water, for example, or where food can bury itself in sediment. Such "electrolocation" is usually passive, relying on bioelectric fields generated by the nerves and muscles of other animals, but some species, such as knifefish, measure distortions in electric fields that they themselves generate.

Researchers have also documented other functions of electroreception. "Especially in the stingray family, it is used in social communication," says Collin. "The opposite sex can use it to assess whether there's a potential for mating, and discriminate that opportunity from something that could turn into predation." And some baby sharks appear to use electroreception for predator aversion. According to research by Collin's group, electric fields trigger a "freeze" response in bamboo sharks while they're still in egg sacs (*PLOS ONE,* doi:10.1371/journal.pone.0052551, 2013).

Electroreception is thought to be an ancestral trait among vertebrates that has subsequently been lost from several lineages (including the amniotes—the group comprising reptiles, birds, and mammals), and then re-evolved independently at least twice in teleost fish and once in monotremes. In 2011, researchers added cetaceans to that list, after discovering electroreception in the Guiana dolphin, a resident of murky coastal waters around South America that evolved its electroreceptors from what used to be whiskers (*Proc R Soc B,* doi:10.1098/rspb.2011.1127).

Most electroreceptors consist of modified hair cells with voltage-sensitive protein channels, arranged in bundles that activate nerves leading to the brain. "The classic example is the ampullae of Lorenzini," says Collin. Described in 1678 by Italian anatomist Stefano Lorenzini, ampullae are extensions of the lateral line system that are present in dense clusters over the heads of cartilaginous fish such as sharks and rays. Each ampulla consists of a bundle of electrosensory cells at the end of a pore filled with a hydrogel that was recently shown to have the highest reported proton conductivity of any known biological material (*Sci Advances,* 2:e1600112, 2016).

But pinning down how any of these receptors operate at a molecular level remains a challenge, notes Clare Baker, a neuroscientist at the University of Cambridge. "We hardly know anything about the specific genes involved, or the genetic basis for building electroreceptors in the embryo," she says, adding that the major animal models in fish and amphibians—zebrafish *Danio rerio* and frog genus *Xenopus*—both belong to lineages that have lost electroreception altogether.

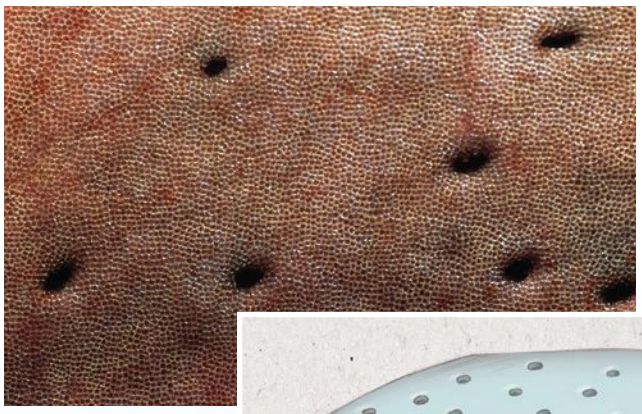


Baker's group has adopted the paddlefish, a relative of the sturgeon, as a model organism. Electrosensitivity in these animals, as in other primitive vertebrates such as the axolotl, depends on modified hair cells that develop as part of the ancestral lateral line system and are homologous to the ampullary organs of sharks. Fate-mapping experiments in these species have identified candidate genes for electroreceptor development (*Evol Dev*, 14:277-85, 2012), and Baker says future work will use gene-editing technologies such as CRISPR-Cas9 to get a better grip on these genes' functions.

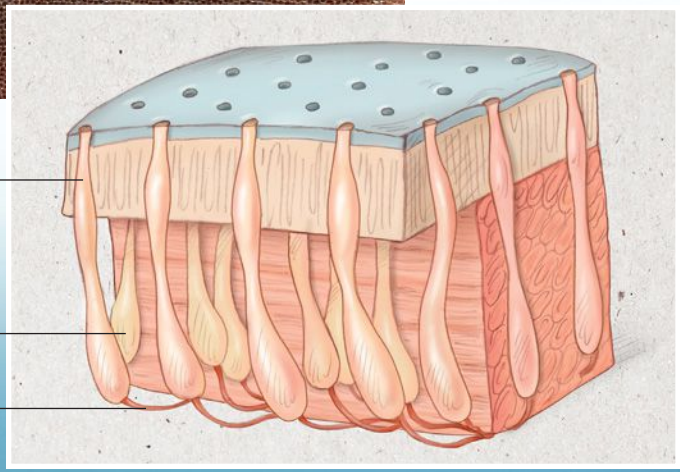
Meanwhile, the field is continuing to uncover surprises. In 2013, research from Daniel Robert's group at the University of Bristol showed that bumblebees are capable of detecting the weak electric fields generated by flowers, and use this information to discriminate between food sources of differing quality (*Science,* 340:66-69). And earlier this year, the same researchers identified bees' electrosensors as tiny hairs that move in the presence of electric fields (*PNAS,* 113:7261-65, 2016). "Electroreception provides another source of information," says Robert, who suspects that a flower's electric field may indicate to bees when nectar and pollen are available. "They're really good at learning where the resources are."

For Collin, the Bristol team's findings are indicative of how much more there is still to discover about electroreception. Even in large clades such as reptiles and birds, "there is circumstantial evidence that they might have electroreception, but there hasn't been anything concrete," he says. "There may well still be examples of functions we don't even know about."

—Catherine Offord

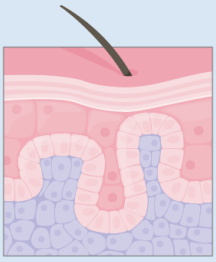
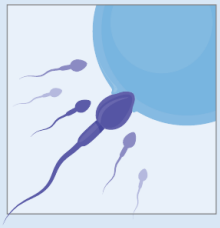
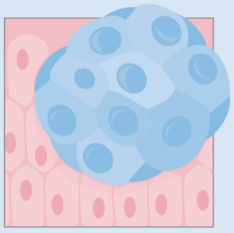
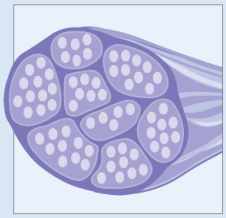


Ampullae of Lorenzini openings in great white shark skin



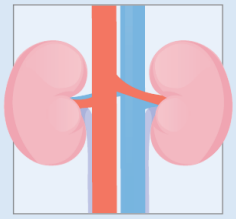
ELECTRIC SLIDE: Sharks and other cartilaginous fish have highly specialized electroreceptive organs called the ampullae of Lorenzini. These bundles of sensory cells, situated at the end of jelly-filled pores in the skin, detect electric fields in the water surrounding the fish and send signals to the brain.

SHARK: © RODRIGO FRISCIONE/GETTY IMAGES; SKIN: © GARY BELL/OCEANWIDEIMAGES.COM; ILLUSTRATION: © LAURIE O'KEEFE

Senses in Unlikely Places

Odor, taste, and light receptors are present in many different parts of the body, and they have surprisingly diverse functions.



BY SANDEEP RAVINDRAN

Dan Berkowitz might never have noticed the phenomenon if not for the new lights. In 2012, Johns Hopkins University's Berkowitz had just moved to a lab space where the lights were motion-activated, and his postdoc Gautam Sikka soon began to observe a curious response in the blood vessels he had isolated for study: whenever he walked in and the lights turned on, the vessels exerted less pressure on the force transducer the researchers had attached to constantly stream data.

A literature search revealed that the relaxation of blood vessels in response to light, called photorelaxation, had been described almost 50 years earlier, but the underlying mechanisms had never been fully elucidated. Berkowitz wondered if these effects were mediated by resident light-sensing pigments. If so, it wouldn't be the first time that a sensory receptor had been found outside of a sense organ.

The light, odor, and taste receptors located in our eyes, noses, and tongues flood our brain with information about the world around us. But these same sensory receptors are also present in unexpected places around the body, where they serve a surprising range of biological roles. In the last decade or so, researchers have found that the gut "tastes" parasites before initiating immune responses, and the kidneys "smell" fatty acids, regulating blood pressure in response. Sure enough, upon further investigation Berkowitz found that it was melanopsin, a light-sensing pigment that serves circadian entrainment and other nonvisual functions in the eye, that modulated the relaxation of blood vessels when the lab lights came on.

In contrast to the early days of the field, the idea of sensory receptors outside of sensory organs is no longer unusual. "They're all just chemoreceptors, and you can use them in lots of different contexts in physiologically different systems," says University of Colorado Denver neurobiologist Thomas Finger.

Now researchers are characterizing such sense receptors present in different tissues around the body and working to understand their functions, with the eventual goal of using these receptors for various diagnostic or therapeutic applications. Preliminary trials are underway to test therapeutic uses of light-induced vasodilation in humans, for example, and clinical trials will soon test whether a patient's taste receptors—both those in the mouth and those in the airway—could be used to diagnose and to treat respiratory infections, respectively. While many of the details of the receptors' activation and downstream signaling remain unclear, researchers are finally getting closer to making sense of what these receptors do outside of the classic sense organs. And labs are using modern genetic tools, such as arrays to detect gene expression or protein levels in different tissues, to pin them down.

"Now, people are looking for them," says Jennifer Pluznick of Johns Hopkins University. "If it comes up in an array, people are more likely to say, 'Oh, this could be interesting,' rather than, 'This is meaningless.'"

Follow that smell

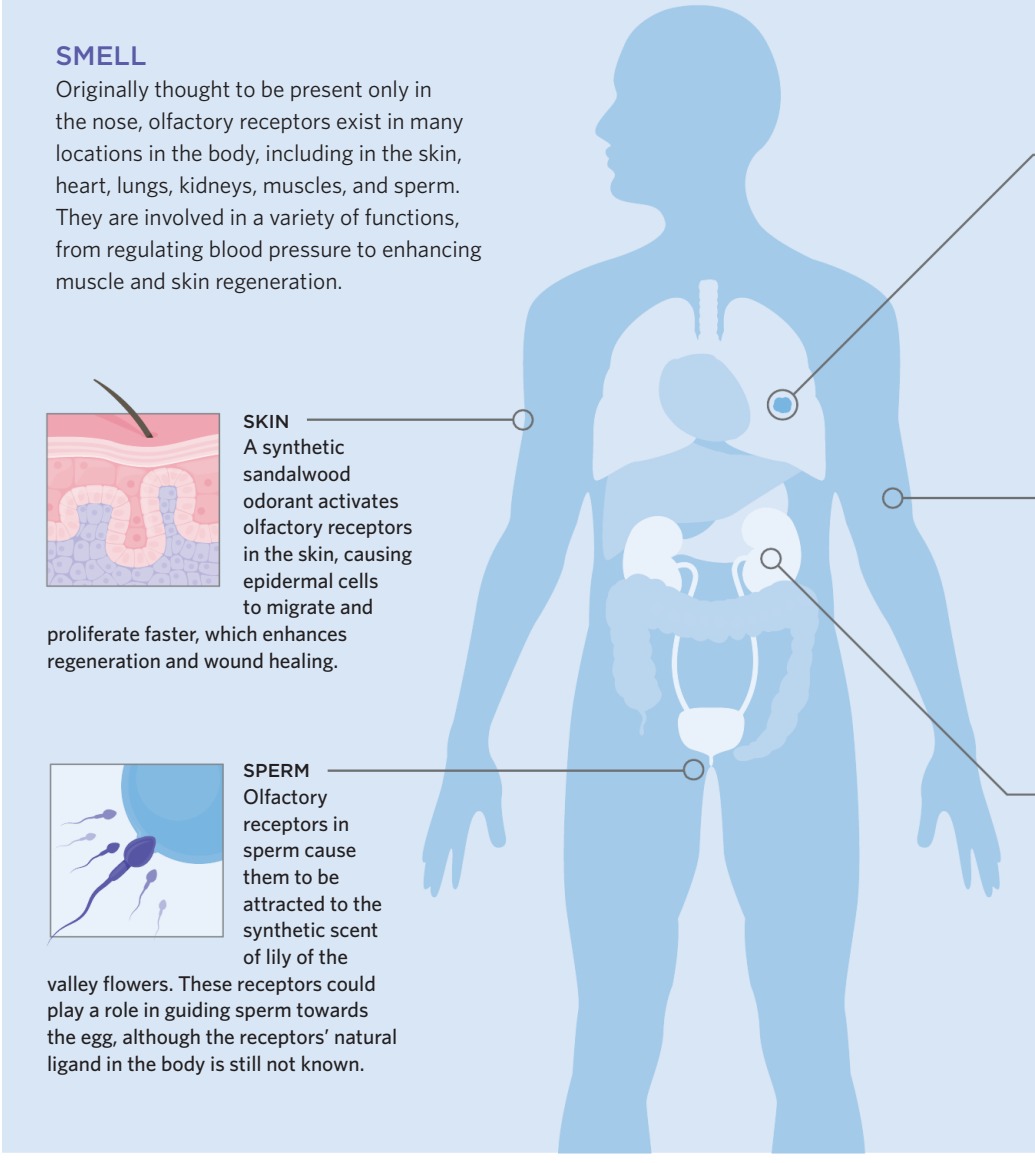
In 1991, Richard Axel and Linda Buck of Columbia University first described the family of genes that encode olfactory receptors, jump-starting the molecular study of olfaction.[1] The assumption at the time was that olfactory receptors were only expressed in the nose, but almost immediately, reports of receptor gene expression in other tissues began appearing in the literature. In studies published in 1992 and 1993, for example, researchers at the Université Libre de Bruxelles detected the expression of olfactory receptor genes in dog sperm.[2,3] But whether these receptors were functional was still an open question.

Intrigued, Hanns Hatt of Ruhr-University Bochum decided to look for these receptors in human sperm. "We could indeed show that such olfactory receptor genes are expressed in human sperm cells also, and a couple of olfactory receptor proteins could be detected," says Hatt. In 2003, Hatt and his colleagues showed that olfactory receptors in human sperm were functional and could be activated by an odor molecule, just like the receptors in the nose.[4] Still, the findings were met with resistance from the field, Hatt says. "At the beginning it was really hard to convince my scientific colleagues that these olfactory receptors are not expressed exclusively in the nose."

Over the next decade, Hatt's team and others continued to identify olfactory receptors in a variety of human tissues, including the lungs, liver, skin, heart, and intestines. In fact, they are some of the most highly expressed genes in many tissues. "One can be sure that these receptors must have enormous importance for the cell," Hatt says.

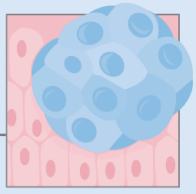
Now, the looming question is: What are these receptors doing? "The big problem is that, to study the function of olfactory receptor proteins, one has to know how you can activate the receptor," says Hatt. Humans have some 350 types of functional olfactory receptors (mice and rats have about 1,000), and researchers have only identified the activating odor molecules for 10 percent to 20 percent of them. To find out what activates a particular receptor, Hatt expresses its gene in a human cell line and exposes the cells to a panel of a few hundred to a few thousand different odor molecules, typically commercially available artificial scents such as those used for perfumes or other cosmetic products.

Using this technique, Hatt successfully identified a scent molecule that activates the olfactory receptors in sperm—a synthetic odorant that smells like lily of the valley flowers—which has allowed him to conduct functional studies. The sperm swim toward the synthetic odorant and speed up as the odorant concentration increases.[4] Hatt suggests that these olfactory receptors could be guiding sperm as they swim towards the egg, though investigators are still testing this hypothesis and trying to identify the receptor's natural ligand in the body.

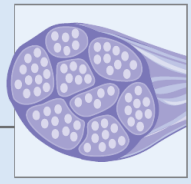


Now people are looking for them. If it comes up in an array, people are more likely to say, "Oh, this could be interesting," rather than, "This is meaningless."

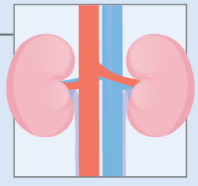
—Jennifer Pluznick, Johns Hopkins University



CANCER
Olfactory receptors are highly expressed in many different types of cancer cells, and stimulating these receptors can cause tumors to shrink in cell culture.

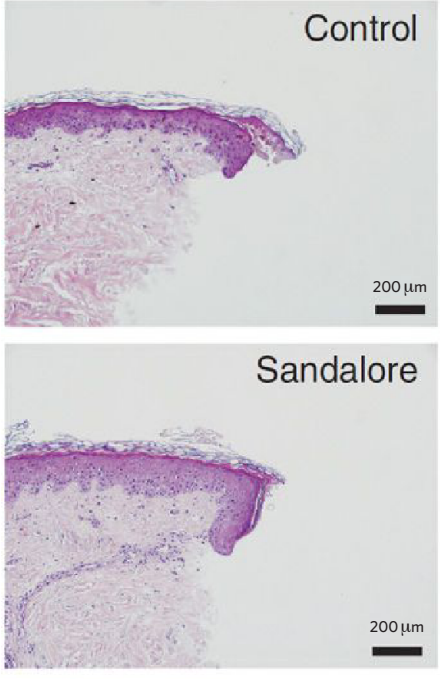


MUSCLE
The same olfactory receptor found in sperm is also found in the muscles of mice, where it directs muscle migration by attracting muscle cells toward a particular scent. Overexpressing this receptor improves regeneration, and without it muscle fibers are more prone to injury.



KIDNEY
Short-chain fatty acids produced by gut bacteria can activate an olfactory receptor found in mouse kidney cells, resulting in changes in blood pressure. This receptor may act in conjunction with a nonolfactory receptor to buffer against swings in blood pressure as fatty-acid levels fluctuate.

FOLLOW THAT SMELL: Treating human skin cells with an artificial sandalwood scent called sandalore activates an olfactory receptor that causes increased migration and proliferation, improving wound healing. The receptor's natural ligand is unknown.



Hatt also found that an artificial sandalwood scent called sandalore activates an olfactory receptor in skin. Activating this receptor stimulated skin cells to migrate and proliferate more rapidly, leading to faster regeneration and wound healing.[5] Again, the receptor's natural ligand—likely a chemical or hormone with a similar structure to sandalore—remains to be determined.

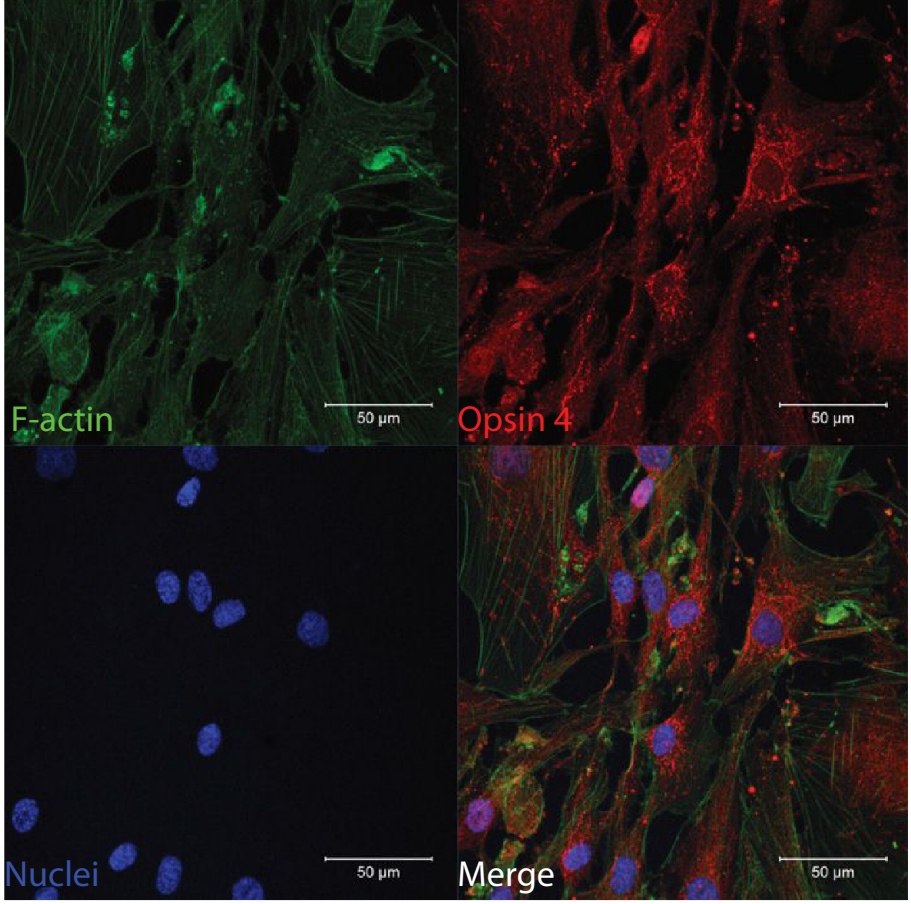
Some olfactory receptors have similar functions in different tissues. In 2009, Emory University's Grace Pavlath was studying how muscle cells fuse to form multinucleated fibers when she noticed high expression levels for the same olfactory receptor Hatt had found in sperm. She found that this olfactory receptor attracts muscle cells toward a particular scent, thus directing muscle migration. "It makes absolute sense that you would use it as a 'tractor beam' to tell cells where to go," Pavlath says. Without this receptor, muscle fibers in mice are more prone to injury and regenerate poorly, whereas overexpressing this receptor improves regeneration.[6,7] Finding the receptor's natural ligand "would allow you potentially to design drugs that could activate this receptor and enhance muscle regeneration," says Pavlath.

In a few cases, researchers may have identified the natural ligands responsible for activating olfactory receptors around the body. In the kidney, for example, Johns Hopkins University's Pluznick found that certain short-chain fatty acids produced by gut bacteria can activate olfactory receptor 78 (Olfr78), which in mice triggers changes in blood pressure. When researchers injected mice lacking the gene for Olfr78 with short-chain fatty acids, the animals' blood pressure dropped, suggesting that Olfr78 by itself normally increases blood pressure in response to the compounds. But blood pressure regulation is complicated, and Pluznick found another, nonolfactory receptor called Gpr41 that decreased blood pressure in response to short-chain fatty acids and had a stronger effect than Olfr78.[8] Pluznick suggests that the two receptors might act together to produce a buffering effect that protects against wild swings in blood pressure as fatty-acid levels fluctuate.

"There are hints in the literature that things that you would expect to increase short-chain fatty production are associated with changes in blood pressure," says Pluznick. Gut bacteria produce short-chain fatty acids when the microbes digest foods rich in fiber, for example, and eating more fiber has been associated with lower blood pressure. One could imagine, then, that eating fibrous foods, or ingesting probiotics, might help regulate blood pressure, Pluznick adds. "That's obviously a long-term dream goal, but I think there's some potential excitement there."

Misplaced olfactory receptors may also prove useful in cancer treatment. Hatt has identified olfactory receptors expressed very highly in a number of different types of cancer cells, and stimulating those receptors caused tumors to shrink in vitro.[9] Although much more research is needed to be able to translate those findings into a cancer therapy, Hatt is hopeful that the field is headed in that direction. "I think it will be a huge new family of possible targets for diagnosis and therapy for different diseases," he says.

BUSSE ET AL., *J INVEST DERMATOL*, 134:2823-32, 2014.



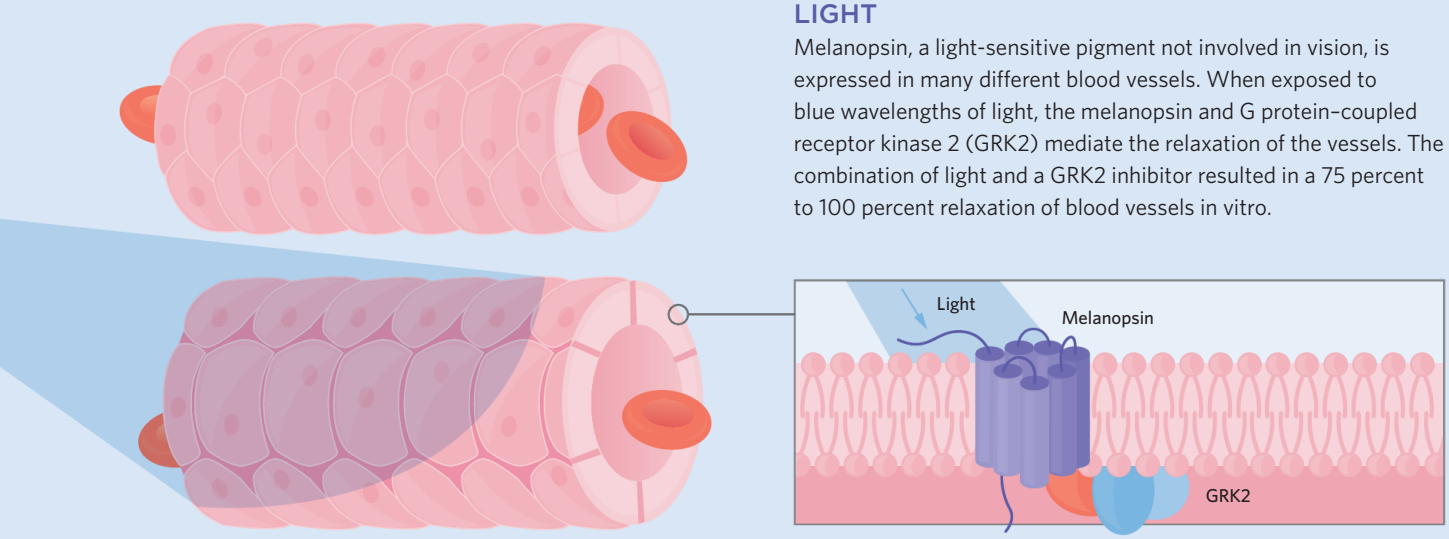
LIGHTING UP THE BLOOD: Human aortic smooth muscle cells produce melanopsin (Opsin 4), a photopigment that mediates a relaxation of the vasculature in response to blue light.

Light relaxation

In the late 1990s, when the University of Virginia's Ignacio Provencio first started looking for melanopsin—hitherto found only in amphibian eyes—in mammals, "it was largely considered a quixotic endeavor by most people in the vision field," he says. It wasn't until 2000, when he documented the expression of melanopsin in the human and mouse retina (specifically, in the cells of the inner retina), that it became clear that the opsins in rods and cones weren't the only light-sensitive photopigments in mammalian eyes.[10] Since then, melanopsin has been implicated in a number of light-induced phenomena in mammals, including regulation of the circadian clock, constriction of the pupil in response to light, and effects on alertness, learning, and metabolism.

"We're still at that discovery stage where we're trying to identify what are these other effects of light," says Provencio.

It was only after Berkowitz switched lab spaces that it became clear to him that melanopsin wasn't limited to the retina. After Sikka observed the relaxation of blood vessels when the automatic lights switched on, Berkowitz and his team began searching for expression of the melanopsin gene *Opn4* and found that it was "quite ubiquitous throughout blood vessels," he says. Using *Opn4* knockout mice and pharmacological inhibitors, the researchers confirmed that melanopsin indeed mediated the relaxation of blood vessels in response to light.[11] This photorelaxation effect was specific to the blue wavelengths of light, consistent with the pigment's absorption spectrum. Berkowitz's team also discovered that photorelaxation was regulated by G protein–coupled receptor kinase 2 (GRK2). While exposure to light alone could cause a 20 percent to 25 percent relaxation of blood vessels, coupling light with a GRK2 inhibitor resulted in a 75 percent to 100 percent relaxation.



But what is the function of this light sensitivity? Shining blue light on the tail arteries of mice, which are close enough to the surface for light to penetrate, decreased tail artery blood pressure and increased blood flow in the tail. Most blood vessels are deep inside the body, however, where they wouldn't be exposed to light. "It might be that this is a vestige of evolution," Berkowitz speculates. Alternatively, there might be other things besides light that activate melanopsin in vivo. It's also possible that there could be some as-yet-unknown metabolic processes that generate light within the body, says Berkowitz. "It's a little far-fetched, but it's possible."

Regardless of its physiological role, Berkowitz is trying to harness light-induced photorelaxation to treat vascular diseases, such as Raynaud's phenomenon. Patients with Raynaud's experience an extreme constriction of the blood vessels in their fingers and toes in response to cold, limiting circulation and causing these extremities to feel numb or painful. Patients could potentially wear gloves that emit blue light to improve their peripheral blood flow, Berkowitz suggests. This could "induce some relief for these patients that have terrible cold and pain and can't pick up a glass of cold soda," he says.

In addition, light therapy could help newborns who suffer from pulmonary hypertension—high blood pressure in the arteries of their lungs and hearts. Current drug treatments have various side effects, and "light might be a safe potential alternative," says Berkowitz, who is currently developing techniques to sidestep the fact that blue light doesn't penetrate very deep into the body. "Hopefully, if that technology works, we can leverage it to other diseases in which vasoconstriction is a significant problem," he says. "Any kind of disease process in which blood vessel constriction is a problem, whether it's diabetes, peripheral vascular disease, [or] coronary artery disease, could potentially be treated using light-based therapy."

A taste for pathogens

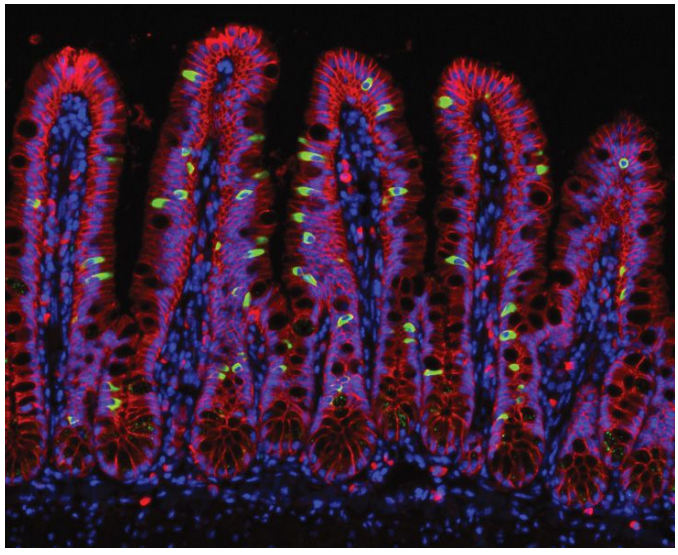
Yet another type of sensory machinery that is spread throughout the body is taste receptors, which in the mouth allow us to enjoy a rich palette of sweet, bitter, salty, sour, and umami flavors. "They're all over the place," says University of Colorado's Finger. "They're in the gut; they're in the testes; they're in sperm. . . . The challenge is to find out what their real role in vivo is." (See "Matters of Taste," *The Scientist,* November 2011.)

Some taste receptors in the gut appear to detect nutrients from food. Others are present in mouse testes and sperm, and knocking them out renders mice infertile, although the mechanism is unknown. A number of recent reports also indicate a role for taste receptors in our body's immune reaction to certain bacteria and parasites.

Finger initially studied the chemical senses of fish, which have sensory cells in their skin that are biochemically and structurally similar to the cells in our taste buds. In 2003, he and his colleagues showed that these cells, called solitary chemosensory cells, were also present in mammalian noses.[12] The researchers identified solitary chemosensory cells expressing bitter taste receptors in the mouse upper respiratory tract and showed that molecules produced by gram-negative bacteria to communicate with each other activated these receptors and stimulated the secretion of inflammatory peptides to initiate an innate immune response.[13]

Since Finger's study, bitter taste receptors have been identified in solitary chemosensory cells of the human upper airway as well, and Noam Cohen of the University of Pennsylvania and the Monell Chemical Senses Center has found that sweet taste receptors are also expressed in those cells, and that the two play complementary roles in innate immunity.[14] Activating sweet receptors with glucose or sucrose inhibits the bitter receptors on the same cell. Cohen found that the normal low level of glucose in the airway was sufficient to inhibit bitter receptors and prevent the secretion of antimicrobial peptides. Bacteria in the airway feed on glucose, so levels of the sugar are "basically an indirect measurement of how much bacteria are around," says Cohen—"the more bacteria, the less glucose." He suspects that as bacteria increase, glucose levels drop, freeing up bitter receptors to respond to bacterial compounds. Some patients with chronic sinusitis or diabetes have elevated glucose levels in their airway, which could help explain their susceptibility to respiratory infections, Cohen speculates.

"It's a really, really cool story of how the sweet and bitter receptors in this one cell, the solitary chemosensory cells, are like yin and yang," says Cohen. "The sweet receptor is measuring how much glucose is sitting in the airway. As long as the sweet receptor is being stimulated, it inhibits activation of the bitter receptor."



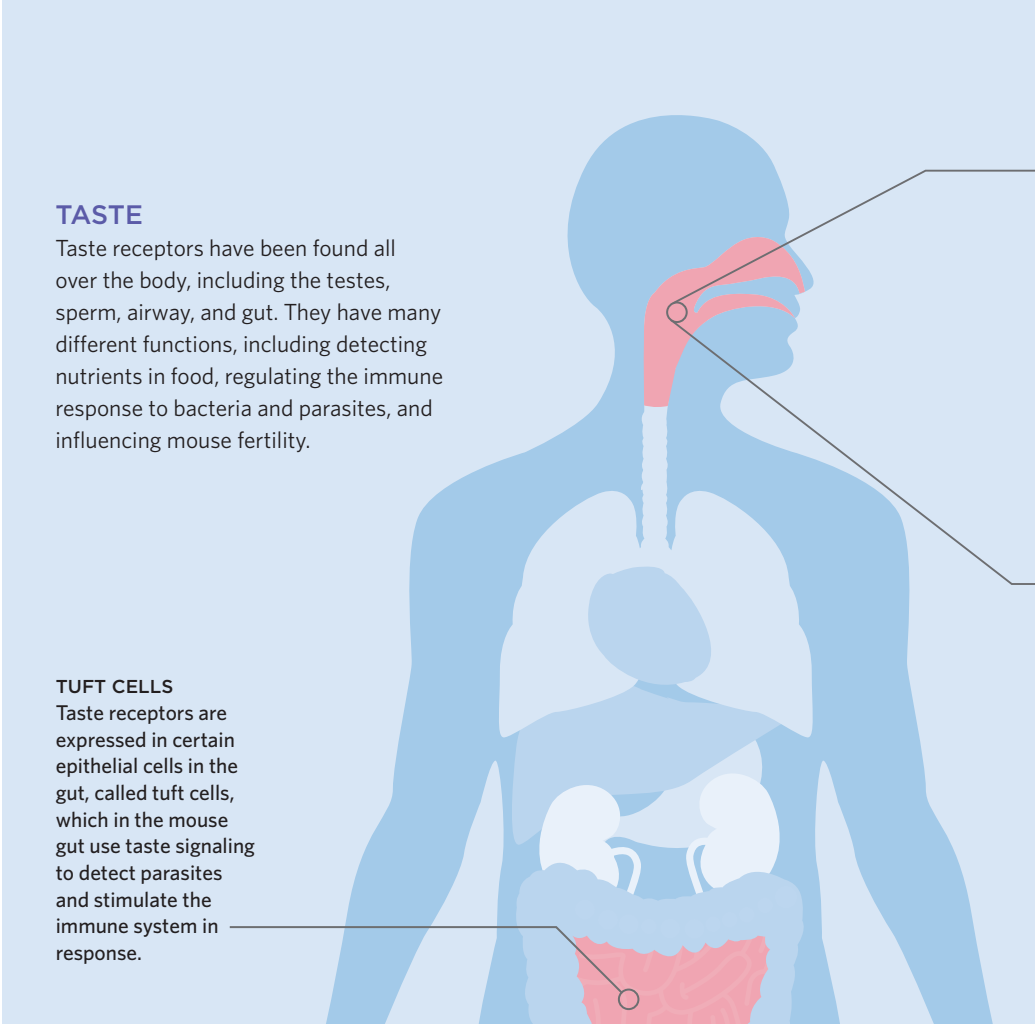
A GUT FEELING: Sweet and bitter taste receptors expressed in tuft cells (green) in the small intestine's epithelium (red) detect parasites and stimulate the immune system in response. (Nuclei stained in blue.)

MICHAEL HOWITT

Other upper respiratory cells have hairlike protrusions called motile cilia that serve as motors to drive mucus out of the airway, carrying bacteria and irritants with it. A team at the University of Iowa discovered that these cells also express bitter taste receptors on the cilia that can sense and respond to bacterial signaling molecules.[15] Just like the bitter receptors found in the solitary chemoreceptor cells, Cohen and colleagues found, these ciliated cells' taste receptors can stimulate the innate immune system upon binding to bacterial compounds. They also respond by increasing the speed with which the cilia beat.[16]

Taste receptors' response to bacterial signals occurs within seconds or minutes, making them a first line of defense against airway pathogens, Cohen says. As a result, the type of receptors we have may affect how we react to upper respiratory infections. Cohen studied T2R38, a bitter taste receptor in ciliated cells, that is quite variable in human populations. It's the same receptor that's thought to make some people "super-tasters," particularly sensitive to bitter compounds found in foods such as broccoli and brussels sprouts. Patients with the super-taster version of the receptor may be able to detect bacterial compounds at very low concentrations in the airway, Cohen suggests. The receptor "doesn't even let bacteria get a foothold to set up any kind of colonization or infection," he adds. Cohen and his colleagues found that patients with this version of T2R38 very rarely got gram-negative upper respiratory infections, and the type of receptor a patient possesses predicts the success of sinus surgery. When patients have chronic sinusitis that's severe enough to require surgery, Cohen found, those who are more sensitive to bitter compounds do much better post-surgery, with fewer subsequent infections and improved breathing and sleeping compared with those who are less sensitive.

Cohen suggests that having a patient taste a variety of nontoxic chemicals to rate their bitterness could serve as a cheap and rapid diagnostic tool. "Our ultimate goal is to use this to try to determine what potential pathogens an individual could be susceptible to," he says. Eventually, researchers may be able to take advantage of the taste receptors for therapeutic purposes, perhaps by squirting a bitter substance into the nose to stimulate an innate immune response. Cohen plans to start testing candidate therapeutic compounds in clinical trials this year. "If this works the way we think it might work, it might be the first line of therapy you get when you come in with some sort of infection," he says.



It's a really, really cool story of how the sweet and bitter receptors in this one cell, the solitary chemosensory cells, are like yin and yang.

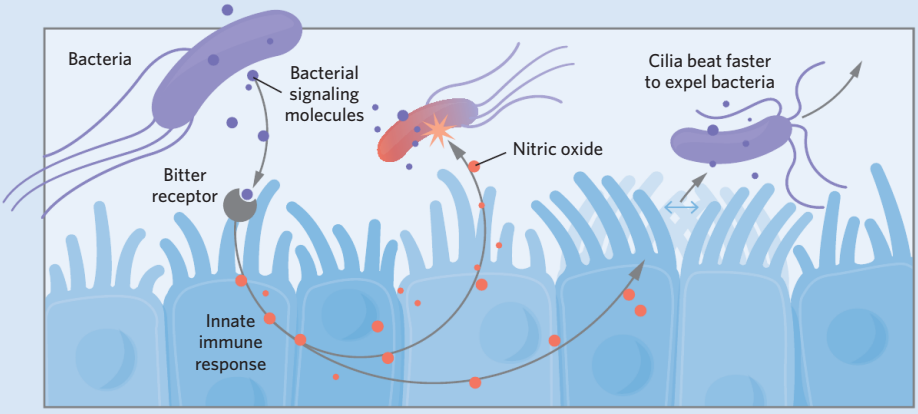
—Noam Cohen, University of Pennsylvania

Recent findings suggest that taste receptors may also play a critical role in immune responses in the gut. Taste receptors are expressed in certain epithelial cells in the gut, called tuft cells, and "the assumption was that this might have something to do with reacting to food," says Harvard University's Michael Howitt. But after seeing Finger's and Cohen's work, Howitt wondered if taste could also play a role in detecting microbes.

Sure enough, Howitt discovered that taste signaling by tuft cells can be activated by single-celled and roundworm parasites in the mouse gut.[17] "If you think about the intestine almost as a medieval castle, the tuft cells are like the little guards posted on the various towers," he says. "If they detect, through taste, the parasites, then they sound the alarm to the immune system." Although the exact receptors involved remain to be discovered, Howitt found that without key taste signaling molecules, the immune system never reacts, and the gut struggles to get rid of the parasites. The findings raise the possibility "of taste not only as a way to discriminate between different foods, but that it might be broadly con-

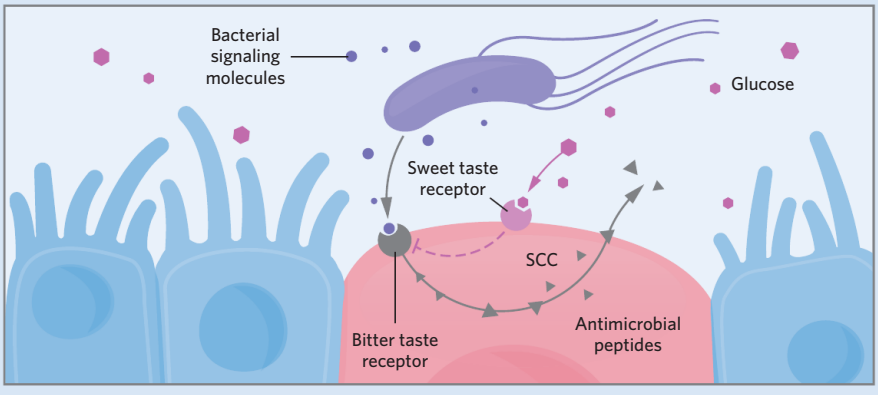
CILIATED CELLS

Bitter taste receptors are also expressed by upper respiratory cells containing hair-like protrusions called cilia. When these bitter receptors sense bacterial signaling molecules, the cells release nitric oxide that kills the bacteria. They also increase the speed with which the cilia beat to expel bacteria from the airway.



SOLITARY CHEMOSENSORY CELLS

Solitary chemosensory cells (SCCs) in the upper respiratory tract express both bitter and sweet taste receptors. When bitter receptors in mice are activated by bacterial signaling molecules, they stimulate the secretion of antimicrobial peptides. Sweet receptors inhibit these bitter taste receptors unless glucose levels drop, which is a sign of increased bacterial growth.



served in detecting a whole suite of different microbes," Howitt says.

"I think it's a very interesting couple of stories panning out about the protective uses of these receptors in taste-like cells in the gut and in the airways," says Robert Margolskee of the Monell Center. And activating taste receptors to stimulate a person's immune cells could hold some benefits over other treatments, says Margolskee—namely, "you wouldn't expect to get drug-resistant bacteria."

Many questions remain about the sensory receptors that have been identified throughout the body—in particular, what activates them and what physiological functions they serve. But with potential therapeutic applications on the horizon, "it is a field that is growing enormously," says Ruhr-University Bochum's Hatt. "Twenty years ago, no one was really excited by olfaction or taste. . . . That has changed dramatically." ■

Sandeep Ravindran is a freelance science writer living in New York City.

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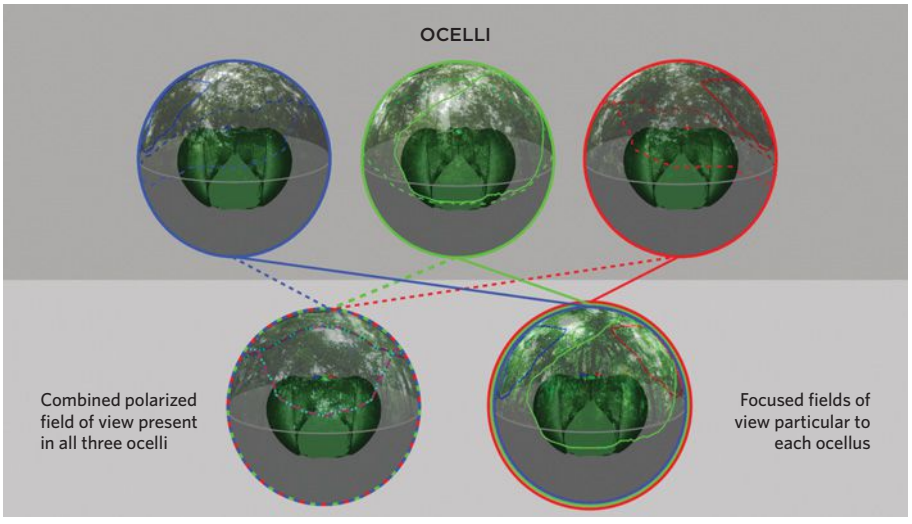
The Literature

EDITOR'S CHOICE IN PHYSIOLOGY

Double Vision

THE PAPER

G.J. Taylor et al., "The dual function of orchid bee ocelli as revealed by X-ray microtomography," *Curr Biol*, doi:10.1016/j.cub.2016.03.038, 2016.



THREE IN ONE: The three ocelli (top row), or simple eyes, of an orchid bee contain two fields of view. The polarized-light field of view is shared among all three ocelli to provide a trinocular view of the sky (dashed lines in bottom row, left), which potentially acts as a navigational aid as the bee flies through its dense, canopy-covered rainforest habitat. The solid lines represent each ocellus's second, unique field of view produced by the perception of focused light.

For neuroscientist Emily Baird, a researcher who describes her passions as "animals, behavior, flying, and robots," bees have always offered a fascinating study system. But in recent years, Baird, the head of a research team at Lund University in Sweden, has become particularly interested in how flight mechanisms are molded by the environments in which they operate.

While honeybees and bumblebees fly through relatively open habitats, for instance, decelerating to land on appealing flowers, other species, such as orchid bees (*Euglossa imperialis*), hurtle through the cluttered and ill-lit undergrowth of tropical rainforests. "I was interested in understanding how a bee that lives in the rainforest manages to detect and avoid obstacles while flying at really high speeds," Baird says, adding that this question essentially boils down to, "How do they really use vision?"

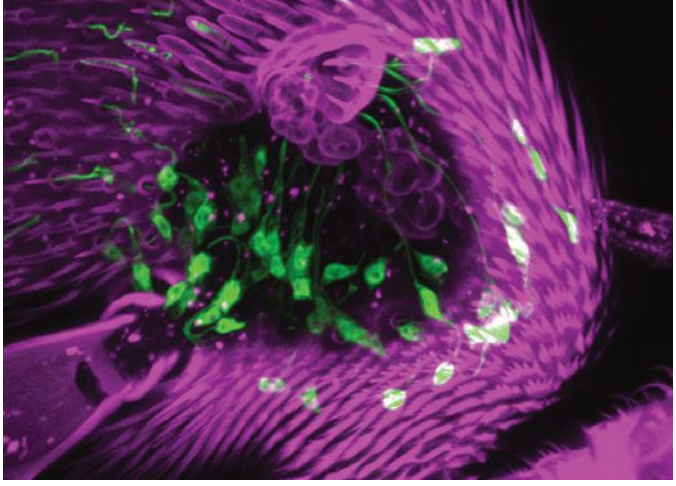
In addition to two large compound eyes, bees and many other flying insects have three simple eyes, or ocelli, located on the tops of their heads. These structures have traditionally been thought of as flight stabilizers, which allow insects to orient their bodies relative to the horizon. But much of the understanding of the ocelli's structure comes from traditional microscopy—a technique that involves slicing up samples. "You lose resolution in one direction," says Baird, "so you can't really make good three-dimensional models of the eyes. And you also don't know how they're sitting in the head."

To understand the visual inputs an orchid bee uses during flight, Baird and her colleagues tried another technique, X-ray micro-computed tomography, to model light reception by the ocelli in three dimensions. To their surprise, they discovered that each simple eye has not one, but two visual fields. "These eyes essentially have at least two different areas in them that are looking at different parts of the world," Baird explains.

One area, aimed at the horizon, receives focused light that likely aids flight stabilization. But another area receives unfocused light from above. Taking a closer look, the researchers found that the photoreceptors in this area of each ocellus are sensitive to the orientation of polarized light—a potential navigational cue. "As long as you can see the sky and are sensitive to polarized light, as many insects are, then you can actually use this as a compass reference," Baird says. "We predict that these eyes are acting like polarized-light analyzers."

"It's a very nice paper," says neuroscientist Holger Krapp of Imperial College London. "The function of the ocelli was not entirely understood in many different insects. . . . This paper suggests really that there are two different aspects of visual information that can be used in different contexts." He adds that behavioral experiments could provide supporting evidence that orchid bees really use light polarization to navigate during flight.

The Lund team already has behavioral experiments underway in orchid and other bees, says Baird, although separating information from the compound and simple eyes will be challenging. "We know that part of the large compound eyes are also sensitive to polarized light," says Baird. "So it's a bit of a mystery why they have this extra area of polarization sensitivity. That's something we have to continue researching to understand." —Catherine Offord



DAMPNESS DETECTORS: Neurons expressing receptors responsive to humidity glow green in a structure of the *D. melanogaster* antenna.

CELL & MOLECULAR BIOLOGY

Humid-o-Meter

THE PAPER

A. Enjin et al., "Humidity sensing in *Drosophila*," *Curr Biol*, doi:10.1016/j.cub.2016.03.049, 2016.

MUGGY MYSTIQUE

Scientists have known for decades that insects can sense their environment's humidity. Fruit flies, for instance, have distinct relative humidity (RH) preferences: a recent study led by Marco Gallio of Northwestern University showed that a species from the Sonoran desert seeks out drier conditions, whereas an afrotropical species likes it muggy. The next step was to find out how the flies detect RH.

SULTRY BEHAVIOR

Humidity sensing is thought to occur in the antennae, so the team looked for ionotropic receptors expressed there whose function was unknown. Using mutants and RNAi to disrupt the functioning of any of three such receptors—IR25a, IR93a, and IR40a—the scientists could abolish humidity preferences in their flies. While IR25a and IR93a helped fruit flies respond to temperature as well as RH, disruption of IR40a only affected humidity responses.

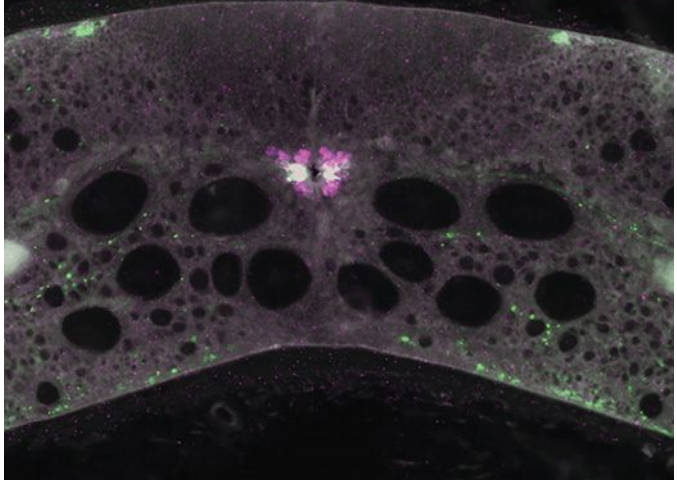
MODEL OF PERCEPTION

The receptors are present on neurons tucked into poreless structures called sensilla located in an invagination of each antenna. Gallio suspects that sensilla change shape in response to RH. Mechanosensory neurons might then detect that signal, enabling the fly to respond to dampness.

TWO SENSES

Jing Wang of the University of California, San Diego, says the results suggest insects might integrate temperature and humidity perception to assess their surroundings. Both humidity- and temperature-sensing neurons reside in sensilla, and "because [the neurons] share the same environment, they have the potential to interact," Wang says.

—Alison F. Takemura



NEUTRALIZERS: Cells in the lamprey spinal cord have PKD2L1 receptors (pink) that detect alkaline pH and produce somatostatin (green) to lower it.

NEUROSCIENCE

Base Line

THE PAPER

E. Jalalvand et al., "The spinal cord has an intrinsic system for the control of pH," *Curr Biol*, 26:1346-51, 2016.

pH SWINGS

Bodies like to keep their pH close to 7.4, whether that means hyperventilating to make the blood alkaline, or burning energy, shifting to anaerobic metabolism, and producing lactate to make the blood acidic. The lungs and kidneys can regulate pH changes systemically, but they may not act quickly on a local scale. Because even small pH changes can dramatically affect the nervous system, a study led by Sten Grillner of Karolinska Institute in Sweden looked for a mechanism for pH homeostasis in the spinal cord.

CHANNELING CHANGE

Using the lamprey as a model system, the researchers observed that a type of spinal canal neuron, called CSF-c, fired more rapidly when they bathed it with high pH (7.7) or low pH (7.1) media. They could suspend the elevated activity by blocking two ion channels: PKD2L1 channels, which stimulate neurons in alkaline conditions, or ASIC3 channels, which, the team showed previously, do the same in acidic states.

BREAK TIME

As the neurons fired, they released the hormone somatostatin, which inhibited the lamprey's locomotor network. These results suggest that, whichever direction pH deviates, "the response of the system is just to reduce activity as much as possible," Grillner says. The pH-regulating role of CSF-c neurons is likely conserved among animals, the authors suspect, given the presence of these neurons across vertebrate taxa.

LOCAL CONTROL

"It's an interesting finding because it adds a level of regulation to maintain homeostasis in the central nervous system," says Pierre Magistretti of King Abdullah University of Science and Technology in Saudi Arabia who was not involved in the work.

—Alison F. Takemura

LUND UNIVERSITY, ANDERS ENJIN; NOBEL INSTITUTE FOR NEUROPHYSIOLOGY, ELHAM JALALVAND

PROFILE

Sensing Time

Neurobiologist Dean Buonomano has focused his career on understanding how the brain tells time.

BY ANNA AZVOLINSKY

Dean Buonomano was among the first neuroscientists to begin to ask how the human brain encodes time. It's not an easy concept to grasp, Buonomano says, and for that reason many researchers overlook it. "The first field of modern science was probably geometry, which was formalized by Euclid around 300 B.C.," says the researcher, who is currently working on a popular-science book about time and the brain. "What's amazing about geometry is that there is absolutely no time involved; it's the study of things that never change. And there's a reason why it is one of the first science fields. Science is much easier if you can ignore time."

Buonomano was in grad school when he became enamored of the question of how we navigate through time. As a graduate student at the University of Texas (UT) Health Science Center at Houston, Buonomano collaborated with Michael Mauk after he heard Mauk's lecture on his studies of the neural circuits in the cerebellum. "He had really transformative ideas about how the cerebellum tells time and how the dynamics of neural circuits in the cerebellum might code for timing," says Buonomano. "His ideas were really something that directed the rest of my career."

"Over the past 10 years, the field has definitely embraced the intrinsic model—that all of the circuits in the brain can tell time—more and more, and that's been a really rewarding process to participate in."

Mauk and Buonomano modeled the way the cerebellum's circuits could respond to stimuli and showed that this type of neuronal network can differentiate between time intervals that differ by just tens of milliseconds. Such networks also have the ability to tune the timing of their responses, the two found. "My collaboration with him was absolutely formative for me," says Buonomano. "Mauk had this very influential notion that time is encoded in the changing patterns of neuronal activity."

Today, Buonomano's laboratory at the University of California, Los Angeles, uses computational modeling, in vitro electrophysiology, and human psychophysics experiments to explore how neurons and the brain as a whole perceive and respond to time. Here, Buonomano describes how he performed his first experiments on his little sister, bathed mice with antidandruff shampoo, and hypothesized that timing is so integral to brain function that all of our brain's circuits keep tabs on the clock.

BUONOMANO BEGINS

Young experimenter. Buonomano was born in Providence, Rhode Island, lived in Hamilton, Ontario, in Canada, and then, when he was seven, moved with his parents to São Paulo, Brazil. His father, a physicist and mathematician, had accepted a faculty position at the State University of Campinas. His younger sister was born two years later. "One of my initial interests in neurobiology was a result of my big-brother experience, of witnessing a young brain develop. I saw my sister go from a baby that's vulnerable and helpless to a child making sense of the buzzing, sometimes confusing sensory world we live in."

Buonomano's sibling became his first experimental subject: "I did little experiments on my sister, which made me appreciate how amazing the brain really is." He lovingly called her "dummy." "One day I was out front playing with my friends, and someone called someone else a dummy, and she came running out and asked who called her. I realized then that I should stop calling her that, but also that she didn't know any better, and that it's individual patterns and environment that help us make sense of who we are." After reading a *Scientific American* article about rapid eye movement (REM) sleep, Buonomano says, he would wait for his sister to fall asleep and then open her eyelids to see if her eyes were indeed moving back and forth.

Good old days. By high school, Buonomano knew he wanted to study neuroscience. "Not that I knew much about the brain, but I liked the idea that our personalities and our ability to learn and feel can be attributable to what's happening in the brain." He also developed an interest in computer programming. In 1982, Buonomano's father brought home one of the first available personal computers, the TRS-80. "It was a golden age of computers. These machines could do cool things, but didn't until you learned how to program and manipulate them," says Buonomano. His interests in time and timing were already evident: he programmed his TRS-80 to do reaction-time tests by asking how long it took his subjects (family and friends) to distinguish between a real word and a nonsense word.

Botany, schmotany. Buonomano studied biology at São Paulo State University, in Campinas. The classroom curriculum emphasized botany, which held little interest for him. "I did the absolute minimum work I had to." Instead, Buonomano sought out opportunities to do research. His first laboratory experience, as a freshman, was washing mice with antidan-

COURTESY OF DEAN BUONOMANO

DEAN BUONOMANO

Professor of Behavioral Neuroscience, Neurobiology
Brain Research Institute
University of California, Los Angeles

Greatest Hits

- Demonstrated long-term associative plasticity at synapses between sensory and motor neurons
- Using neuronal circuit modeling, demonstrated that neurons discriminate between time intervals and durations, providing evidence that individual neurons can tell time.
- With colleagues, developed the first neural network models of timing, and computational models now referred to as reservoir computing
- Along with colleagues, established that neurons are able to discriminate between short time intervals through perceptual learning, but that learning does not generalize to novel intervals
- Showed that short-term synaptic plasticity allows neural network models to distinguish temporal intervals and patterns
- Demonstrated that even cortical circuits in a dish can—in a sense—learn to keep time.
- Established that it is possible to tame chaos in recurrent neural network models by tuning the recurrent synaptic weights, which allows for the robust encoding of time and memories.

druff shampoo. "The principal investigator was testing whether metals in the shampoo were affecting the myelination process of peripheral nerves in mice." Then, in another lab, he worked with a mouse model of depression and learned pharmacology. He also found time to study computer programming, and created computational models based on the neuroscience papers he was reading.

BUONOMANO BLOOMS

A seminal year. During his senior year of university, Buonomano read what he thought were the latest papers on synaptic plasticity—those studying the neural circuits of *Aplysia californica*, a marine mollusk called a sea slug. The largest neurons in the *Aplysia* nervous system are roughly 100 times larger than those in mice, making it relatively easy to isolate them and to measure their activity. "I thought this was the most cutting-edge research at the time—understanding how synapses change with experience," says Buonomano. He didn't realize that four different laboratories studying rodents had reported synaptic long-term potentiation (LTP), the strengthening of the communication signal between two neurons after stimulation—a concept important in learning and memory. The "cutting-edge" papers he was reading in Brazil were actually about a year behind. "Nineteen-eighty-six was a transformative year in neuroscience," he says. Had he known about the LTP studies in mammals, he likely would have gone to a mouse neuroscience lab, says Buonomano.

Cellular learning. Wanting to do computational work in addition to experiments, Buonomano joined John Byrne's laboratory at UT Houston as a graduate student. Byrne was doing both synaptic plasticity studies and electrophysiology, using "very sophisticated computational modeling." There, Buonomano began to study neuronal plasticity in a part of the central nervous system of *Aplysia* called the pleural-pedal ganglia. Byrne's lab already had some evidence that a synapse between a motor and a sensory neuron becomes stronger if two pathways are activated at the same time: when an action potential in the sensory neuron is accompanied by activation of a facilitator pathway that feeds into the synapse, the result is a stronger postsynaptic potential in the motor neuron.

In work published in *Science* in 1990, Buonomano demonstrated that the synaptic plasticity of the pleural ganglion can be long-lived, lasting up to 24 hours. When he paired activation of the presynaptic, sensory neuron with a facilitator path-

way—in this case, neurons that release serotonin—the strength of the sensory-to-motor-neuron synapse was stronger 24 hours later. In other words, the *Aplysia* neurons were able to form long-term memories.

The element of time. Inspired by his work with Mauk, Buonomano hypothesized that short-term synaptic plasticity was a way for circuits to keep track of time. "All synapses basically exhibit short-term synaptic plasticity, but no one knew why," he says. Unlike long-term synaptic plasticity, which is thought to be the basis for memory formation, short-term synaptic plasticity "means every time the presynaptic neuron fires, that synapse might get a bit weaker or stronger but just for the next few hundred milliseconds," explains Buonomano. "It provides a little memory of what happened in the prior 100 or 200 milliseconds."

After earning his PhD, Buonomano headed to the University of California, San Francisco, to work under Michael Merzenich, who had recently shown that experience can remodel the mammalian cortex, providing evidence for brain plasticity in adults. When Buonomano added short-term synaptic plasticity to a computer model of the cortex that he had created, the neural circuitry was able to discriminate between time intervals. Different sets of neurons responded to shorter or longer intervals of 50, 100, or 200 milliseconds, for example. The work was published in *Science* in 1995. "The synaptic plasticity component allowed cortical circuits, in a sense, to tell time, which we need for speech or music or many other tasks."

Learning to hear. As a postdoc in Merzenich's lab, Buonomano also worked with human subjects. In 1996, he and another postdoc, Beverly Wright, found that people, when trained, could improve their recognition of specific time intervals between tones, from 50 to 500 milliseconds. "The interesting finding is that when someone improved their ability to recognize a 100-millisecond interval, they were no better at recognizing the other intervals," says Buonomano. "If we had a clock in our brain that told time, we should get better at recognizing all time intervals. What this suggested is that interval perceptual learning was interval specific."

Decoding time. In 1998, Buonomano joined the faculty at the University of California, Los Angeles, with a joint appointment in the departments of neurobiology and psychology. He continued to dissect how short-term synaptic plasticity relates to the brain's ability to process time. In 2000, he built a computational model that simulated the way neurons respond to time intervals. "This paper was the first to show, in a simple way, how you can make cells respond selectively to one interval or another using simple circuits and short-term synaptic plasticity," he says.

It's all relative. Buonomano argues that in sensing short, millisecond fractions of time, our brain is constantly influenced by the next time interval, analogous to new ripples in a pond that overlay existing ones. Time, he says, is encoded within the behavior of neural circuits. In 2007, he and his then graduate student Uma Karmarkar showed that it is relatively easy to confuse people on how long a split second lasts: the subject's perception depended on the placement of a third, distracting tone. The human study and accompanying modeling suggests that the brain doesn't rely on a specialized internal clock to register short intervals, but rather, uses the natural dynamics of synapses and neurons to tell time. "It is very difficult for us to time intervals independent of each other," Buonomano says. "Everything is encoded in the context of what just happened."

Keep it simple. Buonomano has been a big proponent of using simple in vitro systems to study the full complexity of the brain's billions of neurons and thousands of circuits. "I argue that if you look at circuits in your brain as little computational devices that can learn and pick up patterns, then one of the most valuable tools is cultures of mammalian cortical circuits that are set to learn and pick up patterns," he says. Buonomano has used such in vitro models—of mouse and rat brain slices—to ask whether these neural circuits are able to learn to tell time upon repeated exposure to intervals in the range of 50 to 500 milliseconds presented as light stimulation. In a 2010 study and in a paper in *Neuron* published this June, the lab demonstrated that the answer is yes. The neural circuits sense and adapt to the sensory world around them, learning to anticipate the timing of the light exposure and revealing the intrinsic ability to tell time. "These studies provide strong support for the notion that timing is a general computation that neurons and neural circuits evolved to do," says Buonomano.

BUONOMANO BUILDS

Two views of time. "In the field of timing, there are two general views," says Buonomano. "One is the 'dedicated model' that proposes a central clock in the brain, in the same way that a computer or smartphone has a timer chip whose job it is to tell time and govern the timing of other functions like the stopwatch or alarm clock. The second, the 'intrinsic model,' says that timing is such an important task that it doesn't make sense to have a dedicated part of the brain that tells time but that all of the circuits in the brain can tell time. . . . Over the past 10 years, the field has definitely embraced the intrinsic timing model more and more, and that's been a really rewarding process to participate in."

Keeping it small. "My philosophy in managing a lab has always been to keep it small. I have not had more than three or four people in the lab and, right or wrong, that's been a conscious decision from the start. That has helped me initially to continue to do experiments and, more recently, to continue doing computational and theoretical work. Staying active, I think, is a better way to do science, as opposed to too much grant writing. The advice many of us get is to have as big a lab as possible because you don't know which projects will work out. But, in a way, it's easier to run a smaller lab and to have fewer grants and to continue doing science yourself." ■

Katie Kindt: Sensory Sleuth

Acting Chief, Section on Sensory Cell Development and Function, National Institute on Deafness and Other Communication Disorders. Age: 38

BY KAREN ZUSI

As soon as she started her first real science class in seventh grade, Katie Kindt was hooked on genetics. "There was always something about genetics that made sense to me," she recalls. "It linked the unknown in the world to something that we could follow."

Kindt began doing research as an undergraduate at the University of Wisconsin–Eau Claire in the late 1990s, earning her bachelor's in molecular biology and biochemistry. In graduate school at the University of California, San Diego (UCSD), she discovered a passion for neuroscience during her lab rotations. "I've actually never taken a neuroscience class," Kindt says, but she completed her PhD studying mechanosensation in *Caenorhabditis elegans* under neuroscientist William Schafer.

In Schafer's lab, Kindt combined genetics with microscopy to study neuron formation and function, observing neural migration in real time. She discovered that dopamine modulates the response to touch in *C. elegans,* and that when the worms lack a D1-like dopamine receptor, their mechanoreceptors are less sensitive.[1] Kindt also demonstrated that TRPA-1, a member of the transient receptor potential ion channel family, is involved in mechanosensation in *C. elegans*.[2]

"Now, techniques of using optogenetic indicators are very widespread, throughout not just *C. elegans* but in all the mammalian systems as well," says Schafer, who is now at the Medical Research Council Laboratory of Molecular Biology in the U.K. "[Kindt] was one of the early pioneers in showing that this technique was useful as a way to look at neural activity in intact animals."

Kindt graduated from UCSD in 2006 and moved onto a postdoc at Oregon Health & Science University under sensory biologist Teresa Nicolson. There, she traded *C. elegans* for zebrafish. "I was interested in trying to take these imaging-based approaches into a vertebrate system," Kindt says. "I really liked the accessibility of the zebrafish auditory, vestibular, and lateral line systems. It's a microscopist's dream."

During her postdoc, Kindt found that while the lateral line hair cells are only capable of being stimulated in a specific direction in the adult zebrafish, this polarity is reversed during development.[3] Some of these results have also been shown in mouse auditory hair cells.

In 2013, Kindt joined the National Institutes of Health's National Institute on Deafness and Other Communication Disorders (NIDCD) as an investigator. She's recently started studying the lateral line to examine the coordinated activity of hair cells—which also sense sound in the inner ears of mammals—in vivo. "She's wonderful—she's a great colleague," says Doris Wu, a molecular biologist at NIDCD who was on the search committee that hired Kindt. "She's very bright and has an interesting niche of expertise and research thinking."

Kindt began incorporating CRISPR technology into her research when she set up her lab at NIDCD, knocking out whole classes of genes to identify novel proteins required for synapse formation and function in zebrafish. She tries to make her research as translatable as possible, to understand hair cell formation and regenerative processes that may someday help treat noise- and age-related hearing loss. "All hair cells have genes and proteins that are very well conserved," she explains.

Microscopy remains at the core of Kindt's research program. "I love all the imaging we're able to do," Kindt adds. "Looking at all the activity, all the different cells simultaneously, seeing all these things happen live—it's really amazing." ■

NIH/NIDCD

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Building a Blood-Brain Barrier

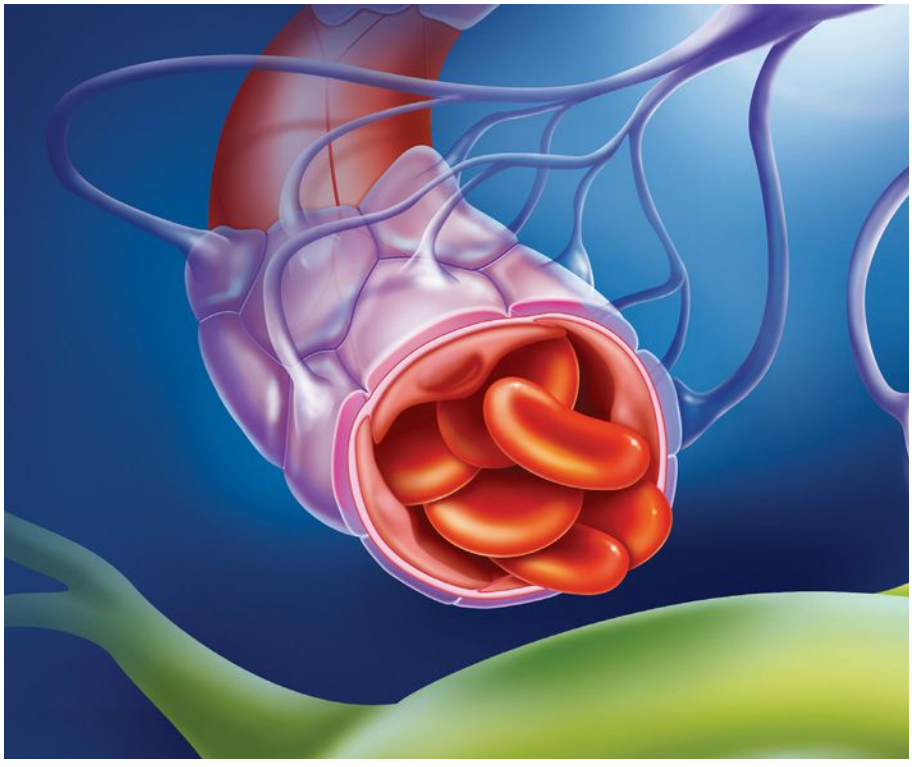
Choosing the right in vitro model of the blood-brain barrier requires wading through varied cell sources, cell types, and cell culture conditions.

BY JYOTI MADHUSOODANAN

A molecular force field protects the human brain. Aided by support cells such as astrocytes and pericytes, endothelial cells lining brain capillaries produce junction-forming proteins to create a barricade with a high electrical resistance, as well as a range of transporters and receptor proteins that keep molecules in the blood from crossing into the brain. This blood-brain barrier protects the brain and spinal cord from infections, toxins, and inflammation—but also blocks drugs from reaching injured or dysfunctional neurons, hampering efforts to treat brain injury or disease.

Since the early 1970s, researchers have tried to mimic this protective layer of cells in vitro, first attempting to isolate intact brain capillaries, and, later, working to isolate and culture endothelial cells from animal tissue in single-layer sheets. Such static, two-dimensional cultures are still widely used, particularly in screening new drug molecules. As cell culture techniques have advanced in the last decade, however, more-sophisticated models that better reflect the structure's physiological roles have begun to emerge. Some rely on human stem cells, while others incorporate multiple cell types, often from different animal sources, grown together in various arrangements. Microfluidic models with a variety of 3-D structures are also gaining in popularity as systems in which to study the functions of a range of cell types or biological processes such as metastasis.

"The greatest unmet need is a standardized model representing all the complexities of an intact brain endothelium that works in a high-throughput environment," says Birger Brodin, a professor of pharmacy at the University of Copenhagen in Denmark. "We have many good in vitro models, but we don't have a one-size-fits-all model."



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Sources of cells, their numbers, and how they are co-cultured can all vary, so picking the ideal in vitro model for a study depends on the research question at hand, Brodin adds. *The Scientist* spoke with experts about the myriad options and their varied uses.

How many cell types, and from where?

Endothelial cells grown in single-layer sheets naturally form tight junctions to create a barrier with high electrical resistance, but they have a major drawback: isolated endothelial cells gradually lose their barrier functions without the presence of other cell types that they normally interact with.

In 2007, Mária Deli of the Hungarian Academy of Sciences and colleagues found that a more anatomically correct model of the blood-brain barrier needed to include at least three cell types. For these models, rat endothelial cells and pericytes, which support the maintenance of capillaries throughout the body, were grown on the surface of a permeable membrane that formed a small well inside the larger well of a standard tissue culture plate. The team simultaneously cultured rat astrocytes, star-shape glial cells that perform various supporting and immune functions within the brain, on the base of the larger well (*Cell*

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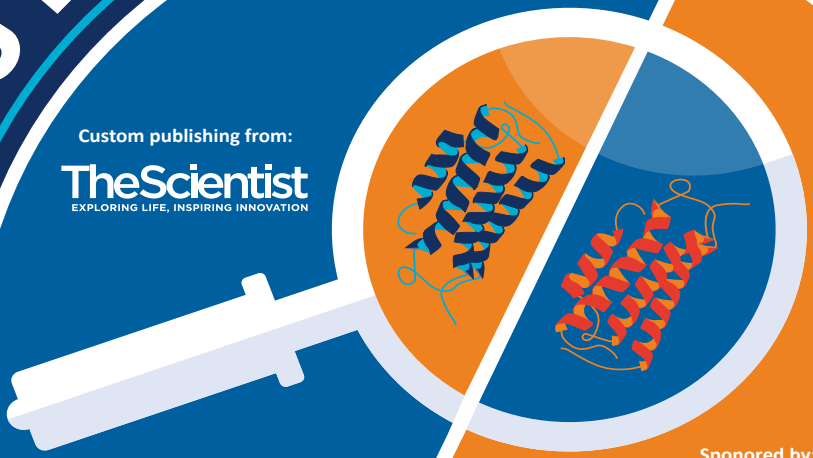

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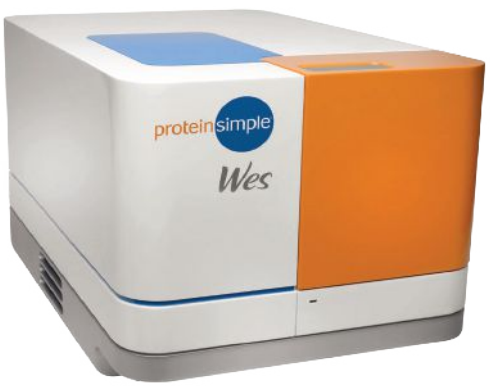

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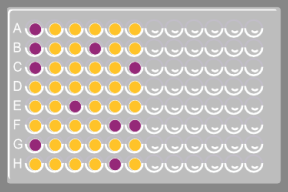
STANDARD PROTEIN ANALYSIS M



ELISA (Enzyme-linked immunosorbent assay) is a common
immunoassay for specifically detecting proteins in solution

DIRECT ELISA:

STEP 1:

Microwell plates are coated with antigen (overnight)

STEP 2:

Primary enzyme-linked antibody binds to the antigen (1 hr – overnight)

STEP 3:

The ELISA is developed by adding the enzyme's substrate, which changes color if a reaction occurs (30 sec – 5 mins)

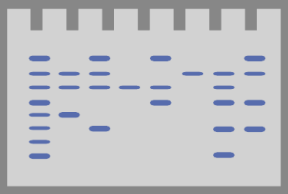
SANDWICH ELISA:

STEP 1:

Microwell plates are coated with antigen-specific antibody, which then binds to antigen (30 min – 2 hrs)

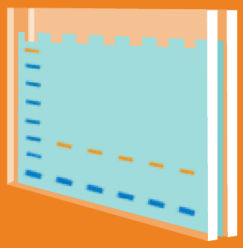
STEP 2:

Enzyme-linked secondary antibody binds to the antigen (30 mins – 2 hrs)



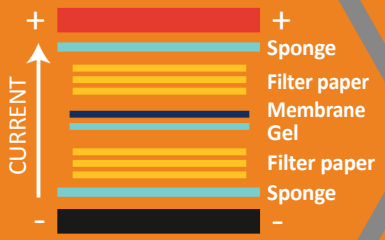
WESTERN BLOT (WB) is the standard method for as
protein size and relative abundance

STEP 1:



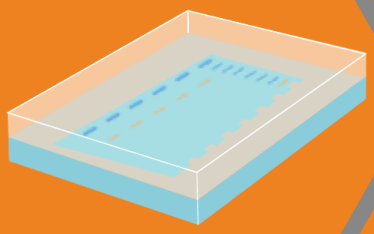
Protein sample (10 – 20 µL/lane) is electrophoretically separated on a sieving matrix or polymer

STEP 2:



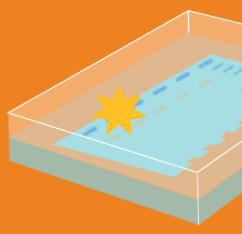
The proteins, separated by molecular weight, are transferred to a membrane (1 hr – overnight)

STEP 3:



Antibodies against the protein of interest are incubated with the blot (1 hr – overnight)

STEP 4:



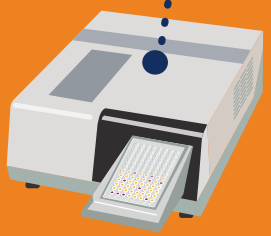
Substrate-conjugated secondary antibodies that recognize the primary antibod are incubated with the blot

METHODS

plate-based

A STANDARD ELISA CAN TAKE ANYWHERE FROM 8–36 HOURS PER PLATE

STEP 4:



Color change within each well is detected by spectrophotometry, and results are compared to a standard curve to determine concentration

ssessing

A STANDARD WESTERN BLOT WITH 10 –12 LANES CAN TAKE ANYWHERE FROM 7.5 – 39 HOURS (PER BLOT) AND REQUIRE 10-20 µL OF SAMPLE PER LANE

STEP 5:

The blot is processed with photographic film or imaged with a blot imaging system and data is analyzed to quantitate signal

dy

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ADVANCING PRO

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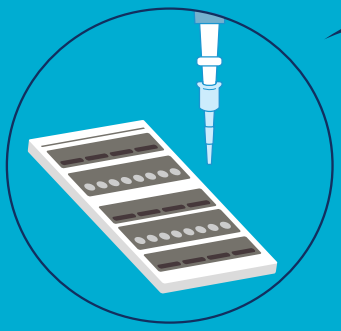
STEP 1: LOAD

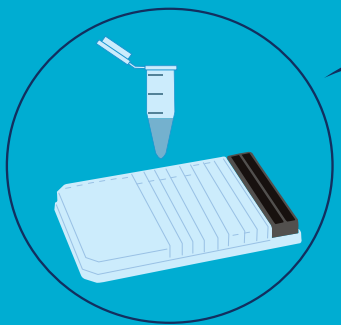


Load your sample
onto the cartridge

AUTOMATED W

relative protein size and

STEP 1: LOAD

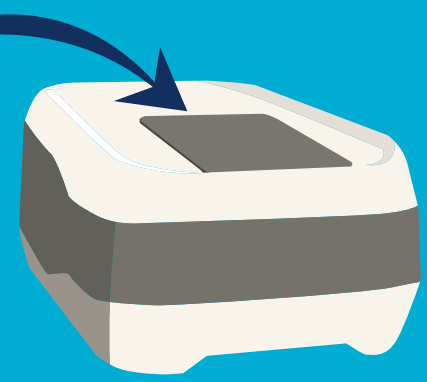


Sample and reagents
are loaded onto a plate

OTEIN RESEARCH WITH MICROFLUIDICS

ELISAs utilize microfluidic cartridges to quantitate protein levels in solution
ng minimal hands-on setup, low sample volume, and no manual washes

STEP 2: RUN



Come back in an hour
to analyzed data

BENEFITS:

Enjoy a rapid automated process, with no manual washes, incubations or reagent additions

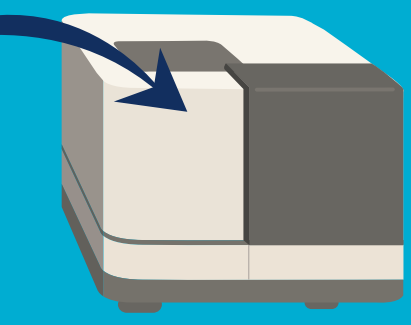
Reproducibly quantitate levels of single or multiple analytes in solution

Rely on accuracy of built-in standard curves and triplicates

AN AUTOMATED ELISA CAN ANALYZE UP TO 72 SAMPLES IN 1 HOUR

WESTERN BLOTS utilize microfluidic or capillary cartridges to analyze
abundance, minimizing setup, sample volume, time to results, and reagent waste

STEP 2: RUN



Come back in an hour
to analyzed data

BENEFITS:

Enjoy faster Westerns, with no gels or transfers

Experience less hands-on time

Rely on higher reproducibility

AUTOMATED WESTERN BLOTS CAN YIELD 24 DATA POINTS IN ~3 HOURS

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Mol Neurobiol, 27:687-94, 2007). The resulting structure better reflected the blood-brain barrier's electrical resistance properties and the expression of various tight junction proteins, small-molecule transporters, and efflux pumps seen in the blood-brain barrier in vivo, says Deli. The permeability of this "triple-culture" model, when tested with 19 drugs, was similar to that in vivo in a mouse.

Deli's lab uses rat cells for most triple-culture studies. Rat- or mouse-based models are suited to complex triple culture models, as all three cell types can be readily obtained and data correlated with in vivo experiments. These rodent-cell models are also relevant to research involving knockouts or transgenic cell lines, or in drug screening where these animals are used in preclinical studies. Other researchers have played mix-and-match, growing bovine endothelial cells with rodent astrocytes, for example. Different brain sizes can necessitate this; larger bovine or porcine brains yield more cells, and can be obtained at slaughterhouses, says Brodin.

Despite the benefits of animal-derived cultures, however, researchers acknowledge that human cells are the most relevant to understanding human physiology. "The lack of a good source of human endothelial cells until a few years ago has been recognized as one of the main barriers in the field," says Peter Searson of Johns Hopkins University.

In 2012, researchers successfully derived brain endothelial cells from human stem cells, a method that's now gaining traction, particularly in microfluidic and 3-D models that aim to recapitulate the morphology and biology of the blood-brain barrier (*Nat Biotechnol,* 30:783-91, 2012). Having access to these cells is a huge boon, though the differentiation protocol is "tedious, time-consuming, and expensive," Searson says, requiring about 10 days to complete. "But the relative benefits are enough that I think there will be more implementation as time goes on," he adds.

Deciding which species and how many cell types to use is a matter of practicality as well as of the research question at hand. Each cell type plays a distinct role. Astrocytes, which are now used in some way in most models, induce endothelial growth by releasing as-yet poorly understood signals. Pericytes boost barrier properties such as electrical resistance and the expression of efflux transporters in rat and human stem cell–derived models, but their functions are less evident in bovine and porcine models.

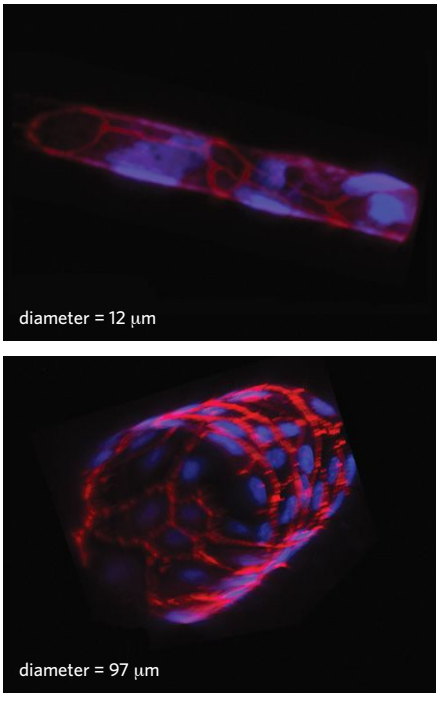
When investigating basic biology such as how pathogens enter the brain, or how disease affects the blood-brain barrier, including all three cell types is likely to be important. And while triple cultures can be time-consuming and expensive, they also require more expertise. "If you're studying the role of one receptor on endothelial cells and it's sufficiently expressed in a single cell [type] culture model, then there may not be a need to attempt triple cultures," Deli says. The same goes for testing drug molecules, where the most important barrier property is a high electrical resistance, she adds. "The question of which cell type induces the blood-brain barrier properties is not as important as the quality of the endothelial layer."

Choosing a barrier shape

Microvessels and capillaries in vivo are tubular, and increasing evidence suggests this architecture is critical to blood-brain barrier functions. "Our ultimate goal is to recapitulate the 3-D geometry of the vasculature in the brain with endothelial cells, astrocytes, and pericytes that mimic at least some of the aspects of the functionality," says Searson.

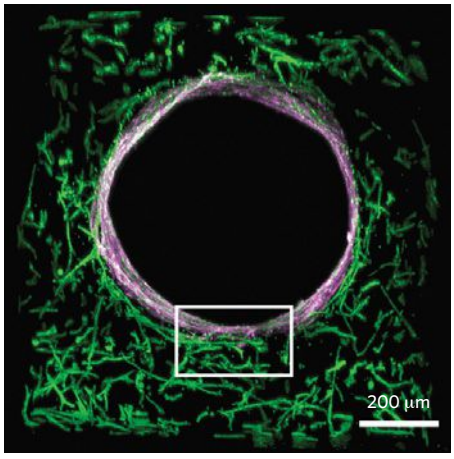
In a 2013 study, he and his colleagues compared how endothelial cells grew when cultured on flat surfaces versus on rods of dimensions similar to blood vessels. Unlike endothelial cells from other tissues, which grow along the length of the rods, human stem cell–derived brain vascular endothelial cells prefer wrapping around them (*Front Neuroeng,* doi:10.3389/fneng.2013.00007, 2013). "They're programmed to like the curvature, which we think promotes their being able to form those tight junctions with themselves," he says. Similarly, astrocytes take on different shapes depending on culture conditions. In 2015, Searson and his colleagues described growing astrocytes along a cylindrical cell culture such that the cells mimicked their star-shape in vivo morphology, with long extensions radiating from the cell body to form contact points known as end feet along a blood vessel (*Biomaterials* 42:134-43, 2015).

Fluid flow within microfluidic and 3-D cultures also mimics the shear stress that endothelial cells experience when blood flows through vessels, which triggers the cells to produce higher levels of barrier-specific proteins, similar to levels observed in vivo. To study how culture conditions affect results, Anna Herland, a research associate at Harvard University, and her colleagues constructed a triple-culture microfluidic model and exposed cells to tumor necrosis factor α, a



ENCIRCLING CELLS: Confocal micrographs show stem-cell derived human brain endothelial cells grown around glass rounds to form microvessels of different diameters connected by cell-cell junctions (red).

PLOS ONE, DOI:10.1371/JOURNAL.PONE.0152105, 2016



MICROFLUIDIC MODEL: Confocal micrographs depicting top-down and cross-sectional views of a chip made from endothelial cells (pink) surrounded by a gel containing astrocytes (green)

common inflammatory protein. They found marked differences in the roles of astrocytes and pericytes in responding to inflammation, a distinction that hasn't been seen in static co-culture models (*PLOS ONE,* doi:10.1371/journal.pone.0150360, 2016). "The [3-D microfluidic] device really affected the inflammatory response," Herland says. "Pericytes contributed very little in other systems, but in close contact with endothelial cells their expression of cytokines was very different."

Three-dimensional and microfluidic models are especially useful for imaging studies to elucidate the workings of the blood-brain barrier and the roles of different cell types. Their barrier properties are typically gauged using dyes, cell morphology, or the expression of transporters and tight junction proteins. The electrical resistance across a tight junction is tough to determine in many 3-D cultures, however, making static 2-D cultures the better choice for studying whether a small molecule can cross the barrier.

The small scale and high cost of 3-D and microfluidic devices, combined with the expertise needed to assemble them in the lab, pose additional constraints. For drug screening and other high-throughput applications—the most common use of in vitro models—static cultures will probably continue to be the gold standard, Searson says. "The objective there is not to mimic the original tissue as closely as possible, but to replicate one aspect of function that can be used for high-throughput measurements."

Picking the best model for your work

Even among standard static culture models, researchers rely on many different configurations of cells. Monocultures of endothelial cells, co-cultures with astrocytes and endothelial cells, and triple cultures that also include pericytes are all widely used. Choosing the right one depends on how the drug molecule being studied interacts with different cell types, Brodin says.

Two-dimensional models typically rely on a permeable membrane surface that forms a small well inside a larger well. Models using only endothelial cells grow them on this membrane, and track how molecules travel across the cells into the larger well. In some co-cultures, astrocytes are added to the bottom-facing side of the membrane, so that they are in contact with endothelial cells. In noncontact models, astrocytes are grown at the base of the large well, and endothelial cells at the base of the small well.

To study a small molecule that's not easily metabolized, a model that places astrocytes and endothelial cells in close contact—on either side of the membrane—may be ideal to understand how the substance is transported across the barrier. But if the molecule is prone to being metabolized—typically by astrocytes—Brodin recommends a noncontact model, which permits astrocytes and endothelial cells to be more easily separated during the course of the experiment by removing the membranous insert that contains endothelial cells from the larger, astrocyte-containing well. For preclinical studies in animal cells, a rat model with all three cell types—endothelial cells and pericytes on the membrane, astrocytes at the bottom of the large well—may be best, he says.

Getting started with a single cell type in a static culture doesn't require any special expertise beyond cell culture techniques. Microfluidic models are trickier, however. For now, only one microfluidic model is commercially available, manufactured by Alabama-based SynVivo. (Cost runs from $990–$2460 for a pack of 10, not including cells, media, and matrix. The company also sells single chips for $99–$129, but that doesn't include tubing, needles, etc., and one chip may not suffice for an experiment.) But most researchers build their own lab-made devices, and concur that if an experiment requires more-complex barrier models, such as triple co-cultures or microfluidic devices, it's best to reach out to laboratories that have the experience doing this. "We're seeing an explosion of diverse new devices, designed depending on individual lab teams and their needs," Deli says. ■

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CRISPR'ing Human Stem Cells

Two popular techniques—inducing pluripotent stem cells and gene editing—require special care when combined.

BY KELLY RAE CHI

The past decade has seen the birth of two incredibly useful biological tools, and now scientists are beginning to marry them. The first is human induced pluripotent stem cells (iPSCs). Nobel Prize–winning advances, beginning with mice in 2006 and subsequently in humans, showed that it was possible to revert adult skin cells to pluripotent stem cells, which can in turn be coaxed to become nearly any cell type. These cells are the cell-scale embodiment of a person's genome, and provide researchers with the ability to create cell types that would be otherwise impossible to cull from the living body. iPSCs offer powerful new ways to model monogenetic and complex human diseases and to tailor cell-based therapies.

The second tool is the CRISPR-Cas9 system, which allows easy and precise editing of any region of the genome. When it comes to traditional immortalized cell lines, such as HeLa or HEK293, cutting with CRISPR is something a relative newcomer can learn in week. A second wave of CRISPR-based methods that work by boosting or dampening gene-expression levels, rather than cutting genes, has made the tool even more useful.

Together, these techniques are more than the sum of their parts. CRISPR'ing human iPSCs allows researchers to manipulate genes to study their functions in the context of specific diseases, or to correct genetic defects in patient cells. One challenge that seamless gene editing helps address is the genetic variability across different iPSC lines that has prevented researchers from directly comparing them; CRISPR-Cas9 allows researchers to make new control cells for a particular individual that differ by only a single nucleotide.

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Although both CRISPR-Cas9 and human iPSCs have become commonplace in the lab, it's not necessarily easy to unite the two. Researchers who want to study gene function are finding that human iPSCs can be difficult to maintain in culture and to manipulate with CRISPR, says Chad Cowan of the Harvard Stem Cell Institute in Cambridge, Massachusetts.

The Scientist talked to experts who use both tools, and put together this basic guide on what to expect when you're CRISPR'ing human stem cells, advice that applies to both human embryonic stem cells (ESCs) and iPSCs.

The basic workflow

Editing genes or controlling gene expression in human iPSCs begins with two parallel work streams: 1) growing a reliable supply of pluripotent cells and 2) designing guide RNA, a 20-nucleotide sequence that targets the gene you wish to alter.

To transfect the cells with the guide RNA and the Cas9 (or related/derivative) nuclease, electroporation is the dominant delivery method, because it allows researchers to get large amounts of DNA and protein into the cells' nuclei while keeping them alive. There are automated benchtop systems such as Lonza's Nucleofector device and kits or Thermo-

SAMPLE PROTOCOLS

Establishment of Genome-edited Human Pluripotent Stem Cell Lines: From Targeting to Isolation
J Vis Exp, 108:e53583, 2016.
This 10-minute video takes users through the process of preparing human pluripotent stem cells for editing using CRISPR-Cas9, and explains how the details differ from older editing platforms (zinc finger nucleases and transcription activator-like effector nucleases or TALENs).

Genome Editing in Human Pluripotent Stem Cells
Cold Spring Harb Protoc, doi:10.1101/pdb.top086819, 2016.
This step-by-step protocol includes guide RNA strategies and approaches for disrupting, adding, or replacing DNA sequences in human pluripotent stem cells—with some troubleshooting tips.

Genome Editing in Human Pluripotent Stem Cells: Approaches, Pitfalls, and Solutions.
Cell Stem Cell, 18:53-65, 2016.
This article takes users through the workflow of editing human pluripotent stem cells and through applications, including a table with recommendations specific for CRISPR-Cas9 and TALENs.

Multi-Kilobase Homozygous Targeted Gene Replacement in Human Induced Pluripotent Stem Cells
Nucleic Acids Res, 43:e21, 2015.
Researchers explain how they were able to knock in a 2.7-kilobase replacement gene into up to 11 percent of human iPSCs (without selection steps), including how they designed the targeting vectors.

Genome Editing in Human Stem Cells
Methods Enzymol, 546:119–38, 2014.
This protocol presents a method for using CRISPR-Cas9 editing in human iPSCs with simple transfection, selection, and characterization of edited cells. The article also includes alternative methods such as viral transfection.

Fisher Scientific's line of Neon Transfection system products available. Try to avoid lentiviral delivery at all costs—in part because human pluripotent stem cells can naturally silence genes delivered this way—except for high-throughput cell screens, says Mohammad Mandegar, a postdoctoral researcher in Bruce Conklin's lab at the Gladstone Institute of Cardiovascular Disease.

Antibiotic resistance is one way to select colonies that harbor your modification. Researchers use resistance in combination with a fluorescent reporter and fluorescence-activated cell sorting (FACS) to enrich for transfected cells. These cells are then expanded on single- or multi-well plates.

Investigators routinely assess pluripotency by tracking cell morphology, pluripotency markers, and the cells' capacity to differentiate. Karyotyping—typically done every 10–20 passages or after any manipulation including gene editing—ensures that the cells have the normal number of chromosomes. iPSC lines are widely available for purchase, and many core facilities and companies derive iPSCs as a service. Protocols are plentiful, too. Bottom line: find the methods that work for you, and make sure you have a good mastery of the cells before you begin editing, says cell biologist Dirk Hockemeyer of the University of California, Berkeley.

Human pluripotent stem cells versus human tumor cell lines

Try CRISPR-Cas9 tools in a human cancer cell line first. When you're ready to move into human pluripotent stem cells, keep in mind that human iPSCs are much more labor-intensive, finicky, and expensive to maintain. (See *The Scientist*, April 2013, "Pluripotent Until Needed.")

An experienced researcher can take about a month to learn the basics of human iPSC culture. Even then, because new protocols and reagents surface regularly, working with these cells is a constant learning process, says Susan Byrne, a postdoctoral researcher in the Harvard Medical School laboratory of George Church. Adds Cowan, "I tell undergraduates that

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within six months, you'll be an expert once you begin working with these and have the right mentorship. But people tend to [initially] underestimate that."

Keep in mind that each stem cell line is unique, a quality that affects your ability to culture the cells and target them with guide RNAs. "The variation we see depends on the individual donor and reprogramming method," Byrne says. "Sometimes we have to optimize the protocol for a particular stem cell line."

In general, even with a healthy cell line (i.e., one that clones and freeze-thaws well) and with your maintenance protocol perfected, you should expect 10-fold lower efficiency incorporating edits into human iPSCs and ESCs compared with cancer cell lines, says Linzhao Cheng of the Johns Hopkins School of Medicine.

Getting to know your gene

Study your gene locus to understand those sequences that are likely to be transcribed and to strategize the best possible alteration. For example, it's possible that even a disrupted gene still shows some biological activity after editing. In that case, you might consider a double cut in order to excise a larger region, Byrne says.

Choose several guide RNAs and test them empirically. A spate of software is available for selecting guide RNAs. But keep in mind that even a specific guide RNA might not work well, or at all. One simple, overlooked reason stems from the

fact that you may not have a reference genome on which you can base your guide design. In addition, you'll need to consider optimizing the guide's activity, says bioengineer Prashant Mali of the University of California, San Diego. A paper published by his group last year in *Nature Methods* presents general design rules for selecting active guide RNAs (*Nat Methods*, 12:823-26, 2015).

Knocking out versus precision editing

When you use CRISPR-Cas9 to cut a gene, two major molecular pathways step in to perform DNA repair and ultimately determine the fidelity of the subsequent edit. Both repair pathways occur at the same time. The pathway used for knocking out genes, called nonhomologous end joining (NHEJ), creates small insertions or deletions in the DNA that disrupt the gene's function. The other, homology-directed repair (HDR), creates precise nucleotide changes based on a stretch of donor DNA.

Researchers using conditions that favor HDR over NHEJ report that the edit fares worse in human pluripotent stem cells than in traditional immortalized cell lines, introducing the correct alteration in only 1 out of 100 transfected iPSCs in a given experiment. It's a major challenge in the field, but Hockemeyer says that his group can manage. "If you have a very good tissue-culture workflow, that only means in order to get 2 clones, you have to pick 200. A student can do that within 3 hours," he says.

Hockemeyer and others are working on new strategies to tip the scales toward HDR, for instance, by applying specific chemicals or using Cas9s from different species. "I think that it's completely reasonable that we—meaning our lab plus the field—will have solved this issue in the next few years," he says.

Conklin's group has developed a rapid digital PCR–based test to quantify the rates of HDR and NHEJ repair, finding in several cell types—including human iPSCs—that the two rates did not correlate with one another (*Sci Rep*, 6:23549, 2016). That suggests that it would be wise to measure both rates rather than one, especially when aiming for HDR. (HDR efficiency rates in iPSCs were less than 0.2 percent in this study.)

Characterizing your cut

Before or after differentiation, depending on your question, measuring or observing a specific cell phenotype—such as its shape or the presence of particular markers—is the most basic way to verify you've made the cut in the right place. Supplying your edited cells with DNA complementary to your edited gene should rescue that phenotype. If your cell phenotype is too subtle to measure a change, you could try making a second edit in the gene that would restore it to wild type, Hockemeyer says.

Researchers also spot-check the genome for their edit, for example, using PCR and Sanger sequencing of those regions. A free algorithm called TIDE (tide.nki.nl/; for Tracking of Indels by DEcomposition) reconstructs the identity of intended mutations and how often they occur, based on PCR and Sanger sequencing data in cell populations.

Sequencing all of the protein-coding genes, or the exome, may well become the method of choice for verifying edits. "[Sequencing is] probably the most complete analysis of your system. It's an investment you're going to want to make if that's going to be the cell line you'll use for the next 10 years. You need to make sure it's perfect," Mali says.

On the other hand, keep in mind that there will be genetic differences that accumulate over time, no matter the cell line. "We cannot argue those away," Hockemeyer says.

Off-target effects

One major way to prevent off-target edits is to use at least two or more sufficiently different guides designed to target the same spot, which makes it nearly impossible that both would give you the same off-target result.

In general, off-target effects should not be a major concern, at least for most basic researchers, Cowan says. It turns out that off-target effects occur much less often in human pluripotent stem cells than in cancer cell lines, Cheng says. But if you are really concerned, then whole-exome sequencing may be your best bet, he adds.

Controlling genes instead of editing them

If you had hoped to knock out a gene that is essential for pluripotency in your pluripotent stem cells, then obviously you're out of luck. You won't be able to get much done with dead cells. In this scenario, you're better off turning gene expression down (or up), and an expanding CRISPR-Cas9 tool set is available for this purpose. These make use of a "dead" version of Cas9, which binds to a predetermined place in the genome but does not cut DNA. Instead, different variations of dead

Cas9 can either inhibit transcription—these are called CRISPR interference or CRISPRi—or they activate it (CRISPRa). (See *The Scientist*, March 2016, "Dial It Up, Dial It Down.")

A recent study found that for loss-of-function screens in iPSCs, CRISPRi was more efficient than traditional CRISPR—silencing the gene of interest in 95 percent of the cells, compared with CRISPR-Cas9, which shut off the gene in only 60 percent to 70 percent of cells (*Cell Stem Cell*, 18:541-53, 2016). That's because CRISPR-Cas9 does not always cause a frameshift mutation (i.e., these mutations remain in-frame), which can leave the protein fully or partially functional. That said, success in silencing genes varies from one locus to another and one cell type to another, says Mandegar, the study's first author.

The major difference between gene-expression control and gene editing is that, with control, you're not permanently

altering the genome. You can perturb the cells, for example, silencing the gene and then reversing that suppression. Also, off-target effects are even less of a concern with CRISPRi and CRISPRa than with CRISPR-Cas9 because of the narrower window in which guide RNAs operate in the former.

One challenge in regulating gene expression is that it is more difficult to select/design a solid set of guide RNAs, Mali says. Ideally, they would avoid the regions of the genome that are coiled tightly around nucleosomes, the protein spools that keep genes silent, but in some cases those are the very regions you'd like to target.

It is also more complicated to deliver CRISPRa and CRISPRi efficiently into human pluripotent stem cells. "It's still really difficult to get it into 70–80 percent of cells unless you're using a viral delivery method. You're typically talking about 30–40 percent of cells," says Cowen, who uses CRISPRa to activate and validate reporter genes. That's helpful because in some cases it is difficult or impossible to differentiate an iPSC into a particular cell type—in his case, renal endothelial cells—to know that his reporter has worked. ■

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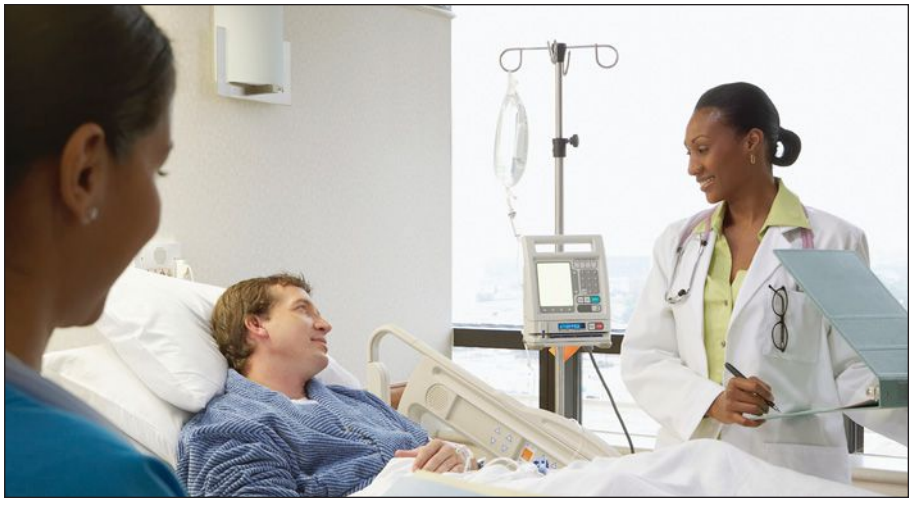
Achieving Rare Success

In the resource-poor world of rare-disease research, basic scientists must rely on clinicians, patient advocates, and their own keen eye for biological connections.

BY JYOTI MADHUSOODANAN



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Becoming a mother changed Heather Etchevers's life in more ways than she expected. After her daughter was born in 1999 with a rare skin condition known as giant congenital melanocytic nevus (CMN), the developmental biologist engaged with patient groups to understand the condition's risks, which include myriad neurological disorders, malignancies, and cancer-like growths. But as the dearth of information about her daughter's condition grew more apparent, she began to see a wealth of research potential. "I realized that things should be getting done that weren't, and I had some special approaches that others weren't doing or implementing at the time," she says.

So Etchevers, who was using functional genomics to study malformations involving embryonic neural crest cells, decided to expand the focus of her research at the French National Institutes of Health (INSERM). But it would be another decade before any projects on CMN got off the ground. Because a rare disorder afflicts, by definition, fewer than 1 in 200,000 people in the U.S., patients are difficult to recruit without the help of a clinician, and clinical trials must be kept small so as to have any hope of filling them (giant CMN affects just 1 in 500,000 individuals). Funds are often scarce for research on conditions with such a small market, and the lack of existing literature and investigators working on the same disease can pose added professional barriers.

Due to these and other obstacles, Etchevers wasn't able to fully launch her own CMN research until her daughter was 10 years old, when the head of the patient advocacy group Naevus Global offered her start-up funds and help getting samples. Even with that support, "publishable results have been much more difficult to achieve, especially while also financially bootstrapping and establishing my credibility as a newcomer in a barely existing field," Etchevers says.

The challenges can seem daunting enough to turn early-career researchers away from studies of rare disorders. "It's often not easy to get young researchers or doctors interested in rare diseases," says Petra Kaufmann, director of the Office of Rare Diseases Research at the National Institutes of Health (NIH). "They're naturally exposed in their education and training to common conditions."

But collectively, rare conditions are not so rare, affecting a total of nearly 25 million Americans. Studies in the NIH Undiagnosed Diseases Program have found that insights from investigations of rare disease can also help improve understanding of more-common conditions. Successes from this program have led to its expansion into an Undiagnosed Diseases Network linking academic medical centers around the U.S., and to rare-disease research playing a key part in the NIH strategic research plan for 2016–2020.

And for researchers who venture into studies of such disorders, the experience can be uniquely rewarding. The work is almost always novel—some researchers find themselves essentially starting new fields—and projects typically involve collaborations with clinicians, patient advocates, and the patients whom discoveries may potentially benefit.

"In the next few years, there will be hundreds if not thousands of rare diseases that will be identified based on genomic data and exome sequencing," says Hudson Freeze, director of the human genetics program at the Sanford Burnham Prebys Medical Discovery Institute in San Diego. "Each one of those is a potential project for a basic scientist to stitch together disease mechanisms." (See "Exome Exercises," *The Scientist*, July 2016.)

Making contact

Freeze spent his early career focused on understanding how sugars are added to and removed from proteins in the slime mold model *Dictyostelium discoideum*. At the time, his work focused on how cer-

tain lysosomal enzymes malfunctioned when the glycoproteins lacked a sugar called mannose-6-phosphate. But after a colleague sent him patients' cells that showed similarly aberrant glycoproteins, and Freeze later met the patients whose cells he had studied, his career took a turn.

Inspired by their stories, Freeze and his colleagues began to search for other patients who suffered from this set of conditions, known as congenital disorders of glycosylation. In the late 1990s, the researchers stumbled across a listserv run by patients' families—all trying to understand their children's disorders—who were more than happy to work with Freeze and his team. For more than 20 years, Freeze has continued to collaborate with those families and others as he studies the underlying mechanisms of a range of rare glycosylation disorders in search of effective treatments. "It was not part of my big plan to do this," he says. "It was just a series of opportunities that presented themselves."

Developmental biologist Hamed Jafar-Nejad of Baylor College of Medicine in Texas had a similar segue into rare-disease research when he discovered that the gene he was manipulating in mice was the same gene that was mutated in Alagille syndrome, which causes liver damage and affects many other organs. Now, as he studies how specific mutations affect metabolic pathways in animal models, he keeps an eye out for clinical links. "Although at heart I'm still a developmental biologist, I see my projects differently," he says. "If exome sequencing identifies [the genes we're working on] as being mutated in human patients, I can't say, 'I'm going to ignore this and wait and see how these genes work in my genetic screen.'"

Freeze suspects that experiences like his and Jafar-Nejad's will become more common among basic researchers as sequencing efforts continue to unveil the mutations underlying rare diseases. And those basic scientists will be key to advancing the study of rare disease. "It's going to be especially important [to establish clinical connections] when someone has been working on a particular set of genes in a model system, and then you find a disorder that's affected by those genes," he says.

Making those connections isn't easy, however. For now, such discoveries are either serendipitous or stem from the efforts of a motivated person—researcher, patient, family member, or advocate—keeping abreast of the literature; there's no centralized database that matches studies in model organisms to human rare diseases. One database, maintained by the NIH and the National Center for Advancing Translational Sciences (NCATS), the Global Rare Diseases Patient Registry Data Repository (GRDR), stores de-identified patient information, including symptoms, medications, or genetic test results, but does not yet link these data to preclinical studies. To keep a lookout for disease links, scientists should search genes and mutations of interest in databases such as GRDR, and also in PubMed and OMIM (Online Mendelian Inheritance in Man), which include case reports of individual patients, Etchevers says.

Once you find a rare disorder that you'd like to learn more about, try broadening your search beyond your disease of interest, suggests Devaveena Dey, a research fellow at Harvard University. "Be prepared to gather information from unrelated fields." Dey transitioned from studying cardiac stem cells to working on the signaling pathways involved in fibrodysplasia ossificans progressiva, a rare condition in which muscles, ligaments, and tendons are gradually replaced by bone. "If you have a question but don't know how to address it or get the protocols, start looking at other known diseases, or think about where the same problem or pathway might exist in other organs and tissues."

Collaborating with the clinic

A crucial component of success with rare-disease research is establishing strong connections with physicians and the patient community. Unlike better-known conditions such as diabetes or some cancers, for which the disease's natural history is well established and research can continue independent of the clinic, most rare-disease studies must rely heavily on input from those affected and the clinicians who care for them.

When starting her work on CMN, Etchevers already knew families and patients affected by the condition, but needed physicians to help collect patient samples and monitor study participants. In part to establish her credentials, she successfully crowdfunded a proposal to establish a biobank of such materials. "It was hard for me as a non-doctor to get physicians to contribute," Etchevers says. "By putting the biobank together and getting the necessary ethical approvals and things, it made me look more serious to people who want to collaborate."

In the next few years, there will be hundreds if not thousands of rare diseases that will be identified based on genomic data and exome sequencing.

—Hudson Freeze
Sanford Burnham Prebys
Medical Discovery Institute

To help scientists pick up skills such as working with physicians, consortia that are part of the Rare Diseases Clinical Research Network (RDCRN) offer mentoring and hands-on training. Universities, hospitals, and local groups of clinical researchers also occasionally provide such learning opportunities. In 2014, an initiative of RDCRN, which is part of NCATS, established a national training program that teaches postdocs and early-career faculty to design studies that have sufficient statistical power despite small numbers of patients; to find funding opportunities; and to establish and work with biobanks and registries. Trainees attend webinars twice a month and meet at the beginning and end of the program to discuss their individual projects. "We're taking it beyond the regional level to bring common standards and homogeneity to the training," says Kaufmann.

Patients and patient advocacy groups are also key. In addition to aiding patient recruitment for clinical studies, the

patient community can help secure funding. For example, Jafar-Nejad's work on disorders caused by a defective *NGLY1* gene began when a foundation established by a patient's family offered funding support. Funding from patient advocacy groups also commonly supports many academic projects; even if they're not providing the funds themselves, representatives from these groups help keep track of new research on a particular rare disease and of drugs being developed for more-common disorders that may be relevant. "If these groups weren't being really proactive and strong advocates, much of the work wouldn't get done," says Eleanor Perfetto, professor of pharmaceutical health services research at the University of Maryland, who last year received a grant from the National Organization for Rare Disorders (NORD) to train patient advocates to effectively collaborate with researchers.

Patients, too, are often involved in the research, and it's important to clarify what their role will be—sample contributors, funders, collaborators, or something else—and what costs will be covered. "Both sides need to have a lot of transparency up front," Perfetto says. "Researchers need to be aware of, understand, and embrace the role that patients can play in helping their research and making it not just richer, but more relevant to the patients."

Rare diseases, common insights

The genetic and clinical research methods that rare-disease researchers employ are slowly gaining traction for studies of more-widespread diseases. Many common conditions are not single diseases but have varied origins, and as exome sequencing expands into clinical care, the promise of precision medicine continues to motivate the creation of smaller and smaller disease subgroups. This approach poses many of the same challenges that rare-disease researchers are familiar with: developing close clinical collaborations, recruiting sufficient patients for a trial, and finding statistically significant results in small cohorts. (See "Clinical Matchmaker," *The Scientist*, June 2015.) Rare-disease researchers are proficient in studies of one or a few patients, for example. While such small cohorts were once frowned upon, proponents of personalized medicine consider clinical trials of smaller and smaller patient subgroups the ultimate goal. "Before precision medicine became a catchword, [our methods] weren't always taken seriously," says Etchevers. "I'd hear things like, 'That's not the way to sample a population'—but we're not sampling a population, we're identifying unique individuals. I hope that more-common conditions will take a page from the books of rare disease researchers."

Sometimes there is more-direct overlap between common and rare diseases. In 2008, the NIH established its Undiagnosed Diseases Program, which combines genomic sequencing with clinical data to identify the origins of undiagnosed conditions. Case studies from the program have revealed many links between rare and common diseases. For example, a teenager with bone and neurological abnormalities was found to carry a mutation in one gene, *SMS*, that affects bone development and leads to osteoporosis. His condition, dubbed Snyder-Robinson syndrome, is being used as a model to test preventive options for osteoporosis, which is common in aging individuals even though they lack *SMS* mutations. Similarly, studies of progeria, a rare syndrome that causes premature aging, have revealed cellular workings that are important in many aging-related conditions, such as brittle bones or vascular disease. Based on these and other successes, the NIH program expanded in 2015 to include several clinical sites.

"The more we do rare-disease research, the more we then identify cellular pathways and processes that can apply to more-common disease," says genetic specialist Debra Regier at the Children's National Medical Center in Washington, DC. "We never know what we might be able to extrapolate." ■

Jyoti Madhusoodanan is a freelance writer based in San Jose, California.

Tapping Into the Artful Brain

Similar reductionist approaches inform both art and brain science.

BY ERIC KANDEL

In 1959, C.P. Snow, the molecular physicist who later became a novelist, delivered a lecture in which he declared that Western intellectual life is divided into two cultures: that of the sciences, which are concerned with the physical nature of the universe, and that of the humanities—literature and the arts—which are concerned with the nature of human experience. Too often, humanists constrain themselves to a cultural echo chamber, maintaining little contact with the realm of the sciences. But Snow, having lived in and experienced both cultures, had the insight that this divide came about because neither discipline understood the other's methodologies or goals. He argued that by bridging the chasm between their two cultures, scientists and humanists could not only further the pursuit of human knowledge but also benefit human society.

This divide has always been of interest to me because I began my academic endeavors as a student of history and literature seeking to understand the artist's response to the rise of Hitler and Fascism in Germany before World War II. Later, I would turn to study the human mind, first through the lens of psychoanalysis, and ultimately through biological exploration of the brain itself. Throughout this journey, I have straddled the divide between science and the arts. My scientific research on the brain did not supplant my studies of art and the human experience, but served as a bridge that allowed me to come back to the humanities—a means of understanding the human mind and its creativity in all their mystery, complexity, and beauty.

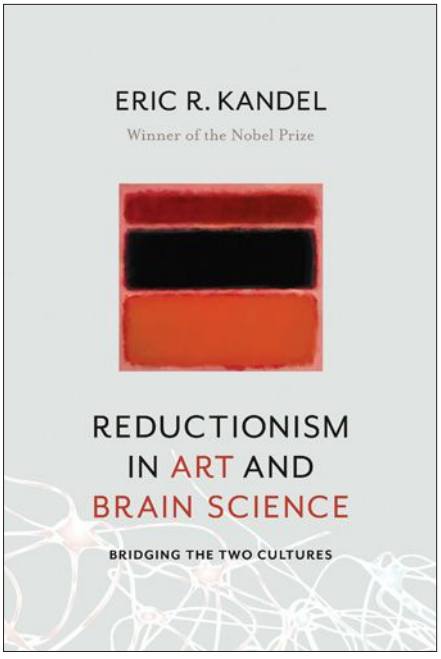
In my latest book, *Reductionism in Art and Brain Science*, I illustrate two ways in which brain science and art can bridge the gap between the two cultures. I show that modern brain science is beginning to directly address questions that are central to humanistic thought. For example, studying the biology of a viewer's response to a work of art can yield humanistic insights not only into the biological makeup of the beholder's mind, but also into the cultural and psychological implications of works of art. Moreover, artists, like scientists, often experiment with different approaches toward achieving their goal. In this way, I argue that visual artists often employ methodologies that are strikingly similar to those used by scientists.

The central challenge of science in the 21st century is to understand the human mind in biological terms. The possibility of meeting that challenge opened up in the late 20th century, when cognitive psychology—the science of mind—merged with neuroscience, the science of the brain. The result was a new biological field of inquiry that has allowed us to address a range of questions about ourselves: How do we perceive, learn, and remember? What is the nature of emotion, empathy, and consciousness?

In this book I ask how the new biological science of mind has begun to engage with visual art. I focus on abstract art because it is highly suitable for scientific exploration, and we now have the beginnings of an intellectually satisfying understanding of how the brain responds to abstract as opposed to figurative art. I discuss how modern science has begun to uncover the rules and mechanisms that underlie vision, and I explain the processes by which our brain re-creates the visual world, and the broader implications of this for our perception of art.

In my life as a scientist, I have often benefitted from taking a reductionist approach. I try to explore a large problem that interests me—such as the problem of memory storage—by initially focusing on its simplest example and exploring it as deeply as possible. In this book, I show that many artists have taken a similar approach to their work, and that reductionism has played a critical role in the evolution of modern art. I trace the transition from figurative to abstract art in the seminal works of Turner, Monet, Kandinsky, Mondrian, as well as Schoenberg. I then examine how the New York School of Pollock, de Kooning, Rothko, Morris Louis, and the heirs of this school, such as Flavin and James Turrell, used a new reductionist approach to arrive at their particular forms of abstract expressionism in the postwar era. Finally, I show how Katz, Warhol, Close, and Sandback built upon these advances to reimagine figurative and minimal art.

Examining modern art through the new biological science of mind provides not only a deeper understanding of what makes us who we are, but also encourages a series of meaningful dialogues between the sciences and the humanities. ■



Columbia University Press, September 2016

Eric Kandel is a Nobel Prize–winning brain scientist and psychiatrist at Columbia University and a senior investigator of the Howard Hughes Medical Institute. Read an excerpt from Reductionism in Art and Brain Science *at the-scientist.com.*

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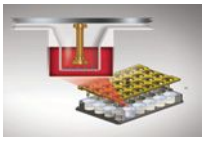

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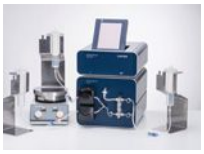

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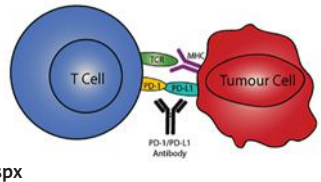

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Pick a Card, 1934

BY CATHERINE OFFORD

In 1926, American medium Mina Crandon held a séance in Boston. Well-known for her claims to channel dead relatives' thoughts and to move objects with her mind, Crandon had drawn a following that included celebrities such as Arthur Conan Doyle and a mathematics professor and former associate editor of *Scientific American*, J. Malcom Bird.

However, at least one audience member at this particular séance—a young botanist named Joseph Banks Rhine—was unimpressed. Crandon, he claimed in a review of the performance, had not made a megaphone levitate, as the audience had believed. Instead, he wrote, she'd simply kicked it into the air. Crandon's supporters were outraged; Doyle reportedly penned a scathing response containing the line, "J. B. Rhine is an ass."

Yet Rhine, who himself had recently attended a lecture by Doyle on the evidence for extra-sensory perception (ESP), was not an unbeliever in psychic powers. Far from it, says Pace University's Terence Hines, a psychologist and author of *Pseudoscience and the Paranormal* (2003). "There was great interest in showing ESP was real," and J.B. Rhine, a scientist, believed he had the tools.

Ditching plant science, Rhine moved to Duke University in 1927 to set about demonstrating ESP through experiments. The most famous of these experiments involved guessing the symbol on a card concealed by an experimenter. Thousands of subjects were tested to see if they could "receive" symbols through ESP, and Rhine soon reported astonishing successes. From a deck containing five different symbols, one of Rhine's assistants, Hubert Pearce, apparently received the correct symbol 40 percent of the time—double what would be expected by chance.

But other researchers, including parapsychologists, found Rhine's experiments concerning. "He wasn't a very good experimentalist," says Hines. "Lab" conditions were loosely controlled: the receiver was allowed to shuffle the cards; some trials were conducted in Rhine's car. The quality of the cards themselves also left something to be desired. "When the cards were printed," says Hines, "sometimes a little bit of the design showed through." Symbols printed on thicker card stock proved to yield less accurate responses, he adds.

An idiosyncratic application of statistics allowed Rhine to detect results where other researchers failed. "He would do a series of studies and some people would score sometimes significantly above chance," says Hines. "He said that was evidence for ESP. Sometimes, people would score significantly below chance, and that was evidence for ESP missing, which, he said, was much more mysterious."

Unfazed by growing criticism, Rhine published *Extra-Sensory Perception* in 1934, declaring ESP "an actual and demonstrable occurrence." Three decades later, he founded the Institute for Parapsychology as part of the Foundation for Research on the Nature of Man, now the Rhine Research Center, in North Carolina. And despite decades of failure to replicate his findings, the appeal of ESP persists. One likely explanation, suspects Hines, is that humans are "terrible" at processing coincidence. "People have subjective experiences that lead them to believe 'something' is happening," he says. "Plus, it's a very attractive idea. It'd be nifty it were true." ■

DIVINING AN ANSWER: J.B. Rhine's early experiments at Duke University employed a set of cards named "the Zener deck" after its inventor, Karl Zener, one of Rhine's collaborators. The deck consists of 25 cards, with five of each symbol: square, circle, cross, squiggly lines, and star. Here, Rhine is shown testing a woman for ESP using the cards in the presence of an assistant (right). "You shuffle the cards and hopefully randomize them," explains Terence Hines of Pace University. "In the simplest version, I'm sitting across the table from you and you're the subject or 'receiver.' I lay a card face down, you don't know what it is, and you guess." Although Zener cards are now considered "old hat" in parapsychology research, Hines notes, there's no shortage of the cards online for contemporary ESP enthusiasts to work with.

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