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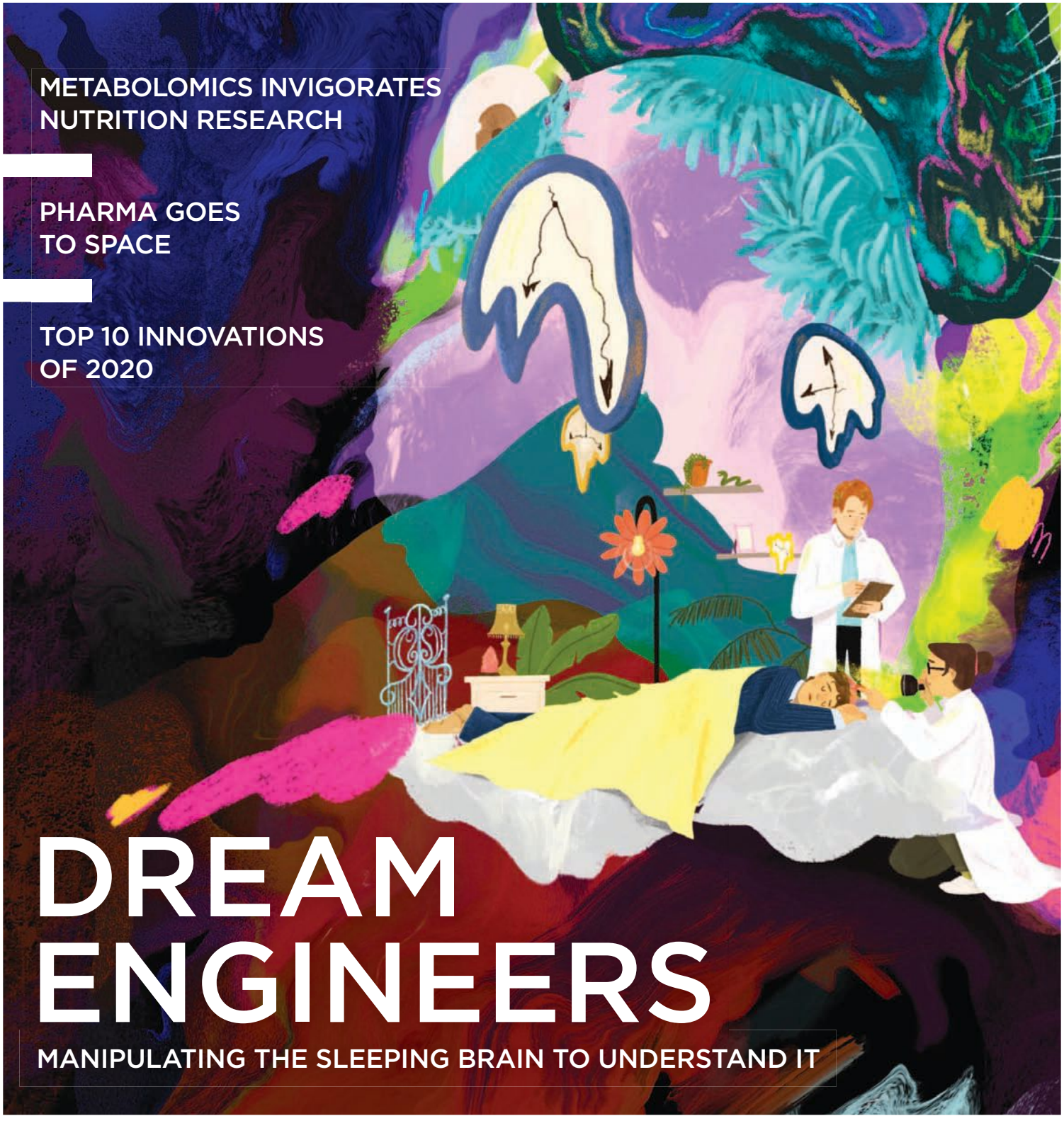
EXPLORING LIFE, INSPIRING INNOVATION



METABOLOMICS INVIGORATES NUTRITION RESEARCH

PHARMA GOES TO SPACE

TOP 10 INNOVATIONS OF 2020

DREAM ENGINEERS

MANIPULATING THE SLEEPING BRAIN TO UNDERSTAND IT

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NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB #E7645)				
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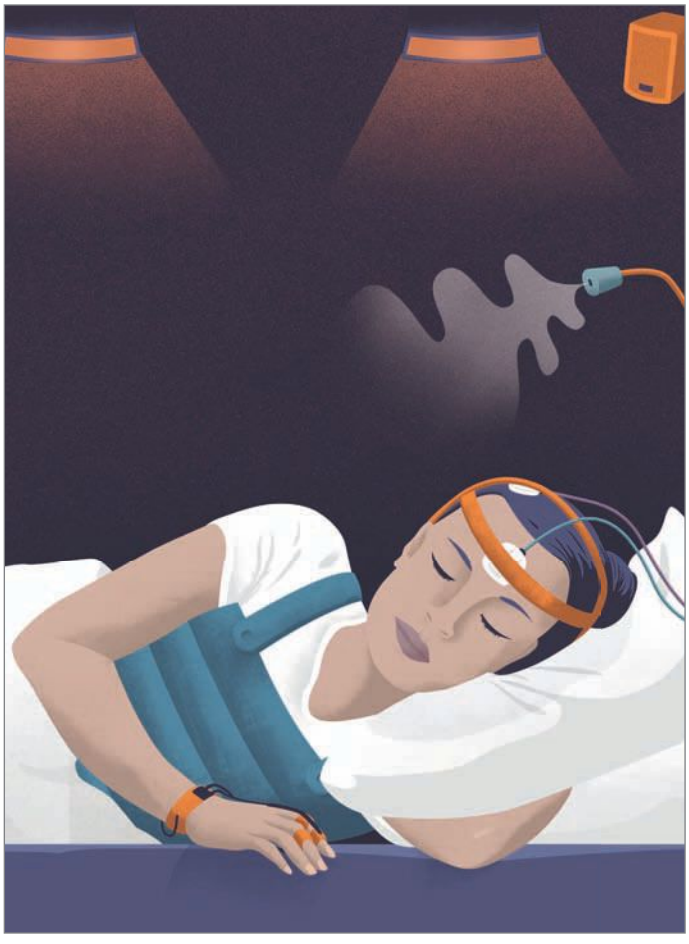

Features

ON THE COVER: © TIFFANY DANG

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

PODCASTS

BY SCIENTISTS FOR SCIENTISTS

The Scientist Speaks is a podcast produced by *The Scientist's* Creative Services Team. Once a month, we bring you the stories behind news-worthy molecular biology research.

SHARING RESEARCH, INSPIRING SCIENTISTS

LabTalk is a special edition podcast series produced by *The Scientist's* Creative Services Team, where we explore topics at the leading edge of innovative research.

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PUZZLE ON PAGE 11

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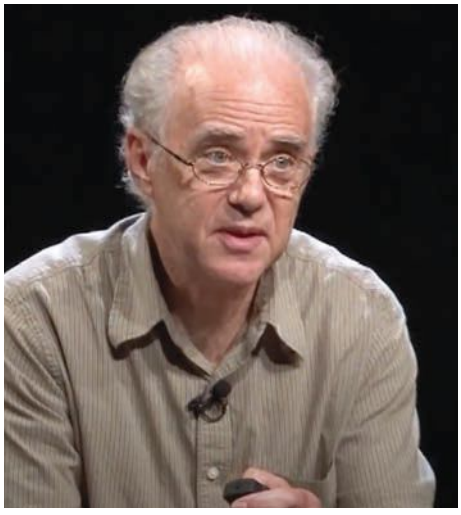



THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Discovery Against All Odds
Watch Nobel Laureate Rita Levi-Montalcini tell the story of how she continued her transformative cell biology research as World War II raged.

VIDEO
What's in a Dream?
See Reading Frames coauthor Robert Stickgold discuss the relationship between sleeping, dreaming, and memory.

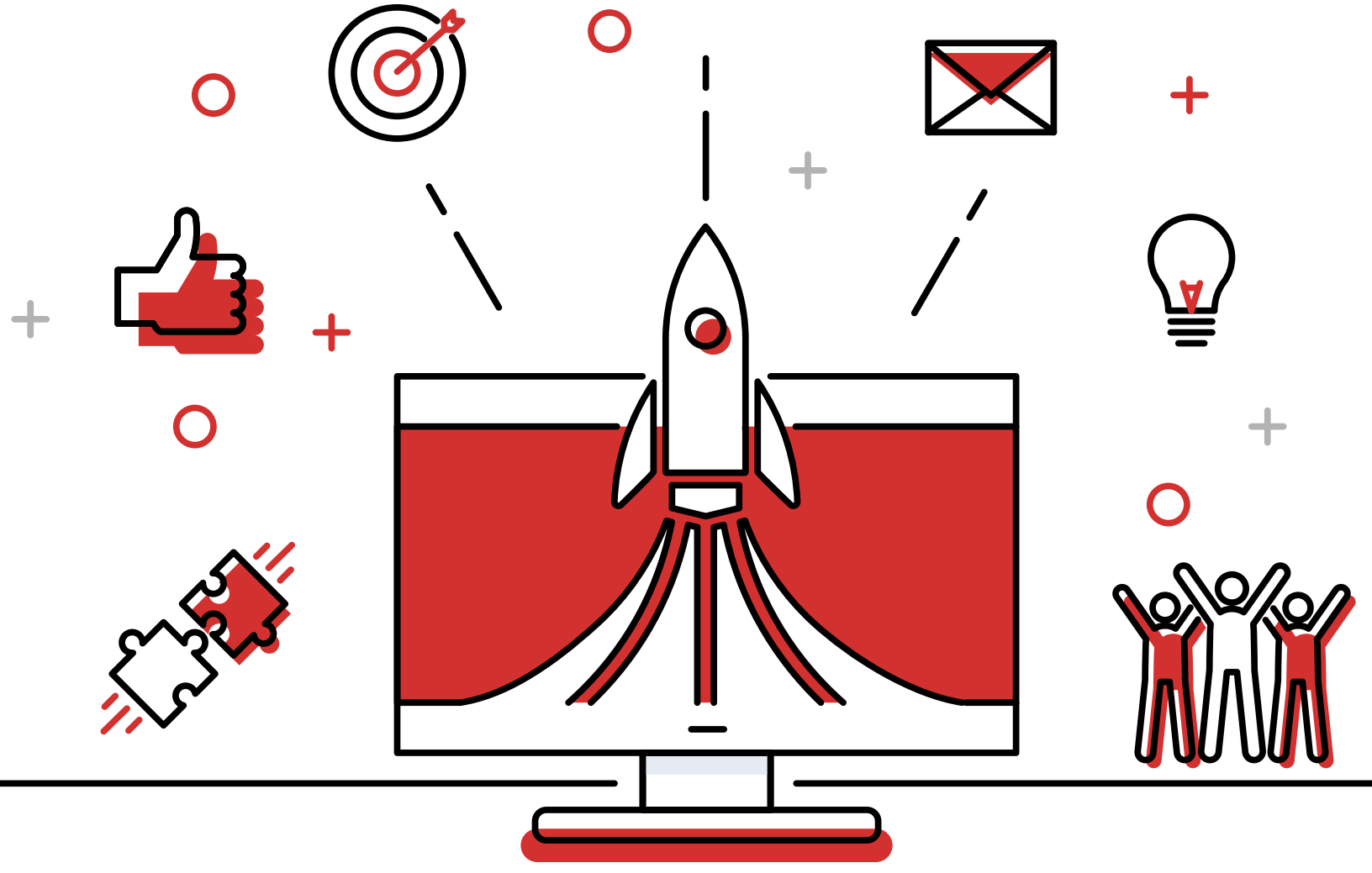
VIDEO
Rocket Pharma
Take a peek inside one Israeli company's push to send pharmaceutical research into space.

AS ALWAYS, FIND BREAKING NEWS EVERY DAY ON OUR WEBSITE.

Coming next month

- Scientists are learning that SARS-CoV-2 can jump from mom to unborn baby, but it's rare.
- Researchers use optical illusions to probe the visual perception of dogs.
- In an effort to decolonize science, indigenous researchers take the lead on collaborations with scientists from the global North.
- Photoactivated caspases give researchers control over apoptosis in human cells.

AND MUCH MORE



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AZBEES
ASBPE Awards of Excellence
2020
WEBSITE OF THE YEAR

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Contributors

As a medical student at the University of Groningen in the Netherlands, **Sibrandes Poppema** worked in a pathology lab, and he enjoyed the research so much that he decided to also pursue a doctorate in pathology. Poppema says he immediately set his sights on studying Hodgkin's disease, a type of lymphoma. "It's a cancer," he says, "but it's a cancer where less than one percent of it is tumor cells—ninety-nine percent are reactive cells," immune cells that are reacting to something in their environment. After receiving his medical degree in 1974 and his pathology doctorate in 1979, he completed two postdocs, one at the Christian-Albrecht University of Kiel in Germany and one at Harvard Medical School in Boston, before returning to the University of Groningen as an immunopathology professor. In 1987, he moved to Canada to join the leadership of the Cross Cancer Institute at the University of Alberta, but after eight years again returned to the University of Groningen, where he served as the pathology department chair, then dean of medical faculty, and finally, president of the university. A couple years ago, he moved to Malaysia to become a professor at Sunway University, where he is lobbying the Malaysian government to open a new medical school.

Over the course of his career, Poppema recalls looking at the CVs of some prospective graduate students and feeling impressed by a high number of citations on their publications. But, after further investigation, Poppema was dismayed to learn that, in some cases, many of those citations were all a bunch of "gobbledygook"—a few obscure journals made up the bulk of the citations. On page 14, he writes about this practice, which he calls "citation padding." The practice, he says, is "simply not fair."

When **Antonio Zadra** was a teenager, he had a lucid dream that changed the course of his career. After that dream, he began keeping a dream diary and devoured every book in the library on sleep and dreams. He graduated from McGill University with a bachelor's degree in psychology and stayed there to pursue graduate studies focused on dreaming. He eventually earned a master's in experimental psychology and a doctorate in clinical psychology. Zadra's own dreams continue to influence his research interests today at the Université de Montréal, where he is a psychology professor. He says he is fascinated by recurring characters in his dreams, the neurobiology of parasomnias such as sleepwalking, and the origins and content of nightmares.

Robert Stickgold didn't research dreams until much later in his career. He graduated from Harvard University with a bachelor's degree in biochemistry in 1966 and received a doctorate in biochemistry from the University of Wisconsin–Madison in 1972. Stickgold first became interested in dreaming when he read an article by psychiatrist Allan Hobson that argued that the content of dreams was essentially random—an assertion that Stickgold rejected. Despite their differences in opinion, he went on to work in Hobson's lab before becoming a psychiatry professor at Beth Israel Deaconess Medical Center and Harvard Medical School, where he studies the function of sleep and dreams on memory consolidation and how defects in this process contribute to psychiatric disorders.

At a sleep conference a few years ago, Zadra asked Stickgold if he wanted to coauthor a journal article on pervasive myths about dreams, but their list grew so long that the two decided to write an entire book—*When Brains Dream*, which publishes in January 2021. On page 57, read an essay from the pair about the possible functions of dreaming.

Max Kozlov's interest in science started early. His family had immigrated from Ukraine to the Boston area a few years before Kozlov was born, and as a young child, he couldn't speak English. His grandparents would often take him to the city's Museum of Science, "and they had a lot of interactive exhibit components that didn't require you to know English," he says. "I think that made me curious about the world around me." He went on to work at the museum while in high school, giving him an early taste of science communication. Around the same time, he also began volunteering to be a subject in research studies; he estimates he's participated in around 40 so far. They pay well, he explains, and "I'm so curious what researchers are working on and what it's like to be in a research study."

Kozlov studied cognitive neuroscience at Brown University, and, after graduating in May of this year, went on to do a AAAS Mass Media Fellowship at the *St. Louis Post-Dispatch*. "I found myself, and I still find myself thinking, 'Man, I have the coolest job, because I just get to call up people and listen to them talk about . . . what motivates them, and hear about some of the coolest research that's going on, and every single day I learn something completely new,'" he says. Currently an intern with *The Scientist*, Kozlov has already written multiple stories for the website, and his first pieces for the print magazine appear on pages 49 and 51.

NOR HIDAYAH HASSAN, SUNWAY CORPORATION; VINCENT ZADRA; BRUCE WAHL, BIDMC; LAUREN SHIN

Science Is My Copilot

As the world around us seems increasingly volatile, protecting and respecting the integrity of research and evidence becomes more important than ever.

BY BOB GRANT

Sitting down to write this editorial, I thought I would look back at the one I wrote for our first issue of 2020 to get a sense, at the end of an extremely unpredictable and disconcerting year, of how I was feeling going into it. "In those halcyon days of boyhood, one date stuck in my mind as 'the future'—2020," I wrote in *The Scientist*'s January/February 2020 issue. "That year, difficult to imagine but endlessly entertaining to dream about, was when everything would be different. World peace would be a reality. Technology would solve humanity's and the planet's ailments. And yes, cars would fly."

I knew that my childish fantasies had failed to materialize well in advance of last January, but early in the year I had no idea how wrong I would be about 2020. This year has shown us all that, despite humanity's decades of scientific, technological, and social progress, nature (human and otherwise) still harbors the power to bring us to our knees. We also learned that the time required to go from futuristic dream to dystopian nightmare is the veritable blink of an eye.

I, like the rest of us, have been trying my best to cope with the harsh new realities of the worst infectious disease pandemic in a century. One of the things that buoyed my spirit in the darkest hours was the sense that the life science community was rising to the challenge of COVID-19, with several labs pivoting to study the disease and the virus that causes it; drug companies and independent scientists speeding the development of tests, treatments, and vaccines; and researchers anticipating the need to study the societal and mental health effects of the pandemic. Other times, despondency took over, as news of publication misconduct surrounding COVID-19 broke and it became apparent that, in some countries, policy and science regarding the appropriate path toward coronavirus mitigation were seriously out of step.

Still, as I reflect on 2020, I see the heartbeat of research pulsing through the turmoil, even when science was sidelined, ignored, or contravened. Evidence of discovery in the face of numerous challenges can be seen in the pages of this issue, where we highlight the winners of our annual Top 10 Innovations competition (see page 38). Several of the winning submissions—including a rapid and portable SARS-CoV-2 test and antibody kits to help characterize the plasma of patients who have recovered from COVID-19, to name but a couple—address the pandemic head-on. Other winning products represent advanced development of tried-and-true laboratory technologies, such as microfluidics and single-cell analyses, that could potentially help battle the scourge of COVID-19.

And in mid-November, as I write this piece, news broke about the Pfizer/BioNTech and Moderna vaccines being reportedly more than 90 percent effective in Phase 3 trials. These results are preliminary, but the scientific community and the world are responding with all the hope and positivity that one might expect.

It is my sincere hope that 2021 will be a better year than 2020. If this is to happen, research must continue to forge ahead, unimpeded by politicization and supported by the public, governments, and private parties alike. Never before has it been clearer that evidence, logic, and science are the keys to delivering us from the suffering of the past several months and the months to come.

We at *The Scientist* look forward to a new year and to following and reporting on the innovation and discovery that rise from the ashes of 2020. There will be much to keep tabs on, from continued vaccine development and testing to the long-term biological impacts of COVID-19. As case numbers rise around the world, and especially in the US, we know that there are more challenges and heartbreak ahead. But we will keep a close eye on the possible paths out of this pandemic, with science as our touchstone and navigator. ■

Editor-in-Chief
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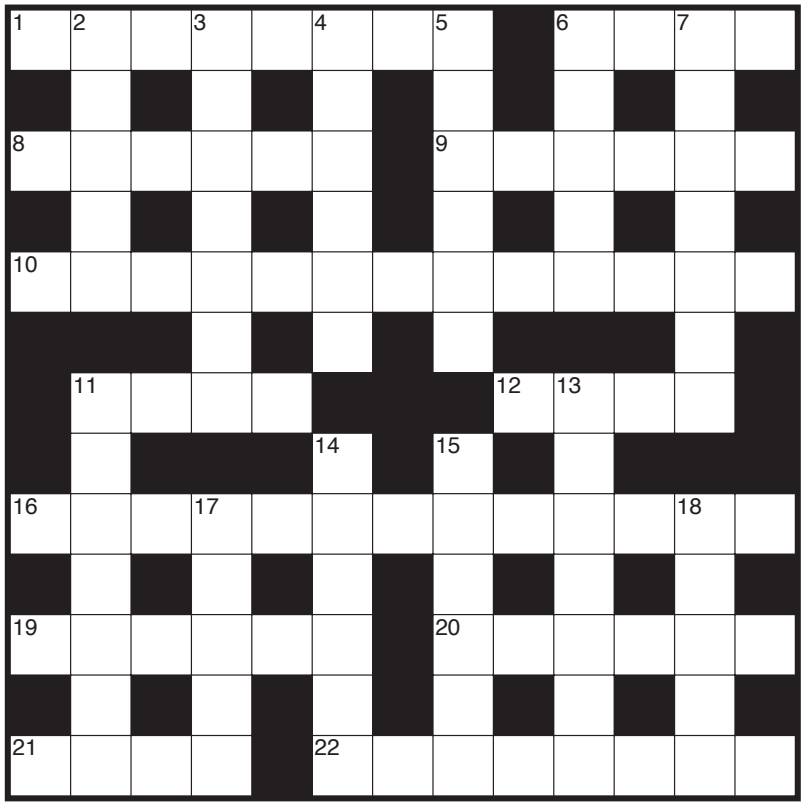
ANDRZEJ KRAUZE

Speaking of Science



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

BY EMILY COX AND HENRY RATHVON

ACROSS

1. Like muscle tissue with sarcomeres
6. Sir Humphry who isolated sodium
8. Unit of temperature named for William Thomson
9. Proteins that form microfilaments
10. Virus that infects prokaryotes
11. Slender projection of a neuron
12. Match up, as chromosomes during meiosis
16. Neurons in the cerebellum with many dendrites (2 wds.)
19. Vessel typically carrying oxygenated blood
20. Prove Darwin right?
21. Newtonian trio
22. Outermost germ layer of an embryo

DOWN

2. Sheath enclosing the spinal cord
3. Outside the body, as a test-tube experiment (2 wds.)
4. Arctic plain affected by global warming
5. Specimen of microscopic algae
6. Like Leeuwenhoek and Huygens
7. Acetic liquid
11. Where schools are held behind glass
13. Pharyngeal tonsil
14. Pepsin or trypsin
15. Flaw that might cause a mutation
17. Citric acid cycle's name
18. Organ with a tissue of hepatocytes

Answer key on page 5

It is a great day for science. It is a great day for humanity. When you realize your vaccine has a 90 percent effectiveness, that's overwhelming.

—**Albert Bourla**, chairman and chief executive of pharmaceutical company Pfizer, speaking to CNBC after the firm announced preliminary results from a Phase 3 trial of its COVID-19 vaccine, developed in collaboration with BioNTech, that suggest it is 90 percent effective in preventing the disease (November 9)

This is really a spectacular number. I wasn't expecting it to be this high. I was preparing myself for something like 55 percent.

—**Akiko Iwasaki**, an immunologist at Yale University, commenting on the Pfizer/BioNTech announcement (*The New York Times*, November 9)

Harnessing the Most Powerful Cells Crucial to Accelerating Curative Medicines

IsoPlexis has emerged as the gold standard for single-cell functional proteomics through its unique applications of functional immune landscaping, intracellular signaling omics, and high-plex automated immunoassays. IsoPlexis' platform prioritizes function over form to fill the gap missing with traditional technologies. Historically, researchers have characterized the immune system using protein expression markers, but these expression profiles do not always translate to cellular function. When developing immunotherapies, a high priority for scientists is to identify polyfunctional cells (single cells that secrete multiple cytokines) but these cells cannot be characterized when measuring protein expression markers. IsoPlexis' platform overcomes this challenge while addressing many of the pain points associated with scaling these types of assays, streamlining the proteomics workflow into one automated hub instrument to advance precision, curative medicines.

The Gold Standard for Functional Single-Cell Proteomics to Advance Curative Medicines

Basic, translational, and clinical scientists already use IsoPlexis' proteomics technology extensively. It has helped scientists better understand the mechanisms guiding immune responses to pathogens, inflammation, and therapeutic agents or approaches.

Precision Biomarkers for Accelerating & Improving Immune Therapies

Characterizing immune cell function is essential for understanding the relationship between immune cells and cancer cells and improving the therapeutic efficacy of immune-oncology approaches. IsoPlexis has a proven track record[1-4] of helping scientists discover links between immune cell function and therapeutic outcomes. In a 2018 study published in the journal *Blood*, Rossi et al. used IsoPlexis' single-cell secretomic analysis to establish a link between polyfunctional chimeric antigen receptor (CAR) T cells in pre-infusion CD19 CAR products and patient responses to treatment.[1]

The same platform used in the *Blood* study was used by researchers Parisi et al. to predict the anti-tumor response of a novel kinetically engineered IL-2 agonist, NKTR-214, with adoptive cell transfer (ACT) compared to the conventional IL-2 combination therapy. IsoPlexis' platform found that ACT with NKTR-214 resulted in increased proliferation, homing, and persistence of anti-tumor T cells in a murine melanoma model. This resulted in superior anti-tumor activity, and the use of NKTR-214 led to an increase of polyfunctional T cells in murine spleens and tumors. Enhanced polyfunctionality of T and NK cells in the peripheral blood of human patients suggested that NKTR-214 has the potential to improve the anti-tumor effects of ACT in humans. These results highlight critical insights of functional immune profiling for uncovering biomarkers that help predict treatment response.[5]

Identifying Key Prognostic Biomarkers of Inflammation in COVID-19

IsoPlexis' functional immune landscaping has been used to investigate the mechanism of COVID-19 inflammation within a variety of cell types. In a study recently published in *Cell*, researchers Su et al. conducted deep functional immune profiling of COVID-19 patients ranging in disease severity compared to healthy samples. They found that with increasing disease severity as measured by the WHO Ordinal Scale (WOS), CD-4$^+$ T cell, CD-8$^+$ T cell, and NK cell percentages dropped while the proportion of monocytes increased. Su et al. noted a surprising similarity between healthy subjects and mild COVID-19 cases, as well as between moderate and severe cases. IsoPlexis' functional immune landscaping was able to shed light on the functional mechanisms behind the differences in disease severity.

The severity of COVID-19 correlated with an increase of polyfunctionality in CD8$^+$ T cells, followed by a significant drop in function at severe stages of the disease, but "unlike the case of CD8$^+$ and CD4$^+$ T cells, the [polyfunctional strength index (PSI) of monocytes] monotonically increases with disease severity, suggesting that monocytes contribute to the pro-inflammatory condition of moderate or severe COVID-19.[6]" Researchers Su et al. used IsoPlexis' uniquely correlative metrics to assess the relationship between highly functional cell subsets and COVID-19 disease progression. Monocytes showed a sharp increase in function between the mild and moderate disease stages, while the monocyte population and PSI continued to increase between moderate and severe cases. These unique findings, enabled by IsoPlexis' single-cell functional proteomics suggest that monocytes may contribute to the pro-inflammatory environment that is characteristic of moderate and severe COVID-19 cases.

A New Layer of Multiplexed Proteomic Biology: Intracellular Signaling Omics for Hyper-Powered Targeted Therapies

With IsoPlexis' intracellular signaling omics, researchers can identify functional pathways driving therapeutic resistance and develop combination therapies to combat resistant cell states and resolve tumor heterogeneity. IsoPlexis' intracellular proteome solutions identify polyfunctional cell subsets to provide a comprehensive picture of altered signal transduction networks in tumors, which allow researchers to identify whether therapies targeting protein signaling networks are effective. In another study published in *Nature Communications*, Su et al. utilized IsoPlexis' technology to characterize resistance pathways in mutant melanoma cells. IsoPlexis' solutions identified two distinct subpopulations of cells which took different paths to drug

resistance. IsoPlexis' intracellular signaling omics provided the insights for researchers to identify combination therapies to combat this resistance that were both effective and low in cytotoxicity.[7]

Highly Multiplexed Automated Proteomics for Identifying Druggable Targets to Treat Cancer Metastasis

In contrast to traditional technologies, IsoPlexis' high-plex walk-away immunoassays provide researchers the ability to highly multiplex (e.g. 30+ cytokines) and fully automate proteomics for accelerated insights. The CodePlex family of solutions uses ultra-small sample volumes (11 µL per sample), enabling critical applications across research disciplines from cancer immunology to infectious diseases. In a study led by Denis Wirtz at Johns Hopkins University, researchers investigated the role of cytokines in promoting or preventing metastatic cancer cell phenotypes. These researchers used the CodePlex technology to simultaneously measure the concentration of 24 soluble molecules. Both cytokines IL-6 and IL-8 were secreted at high concentrations in a specific ratio, while proteins typically associated with promoting tumor metastasis and progression were not elevated, suggesting that both of these cytokines are responsible for driving the density-dependent cell migration within 3D matrices. Wirtz's team found a synergistic IL-6 and IL-8 mediated paracrine signaling pathway which may provide a new therapeutic target against metastatic cancer cells.[8]

Sparking the Next Big Breakthrough

The IsoLight system was the first solution to solve numerous instrumentation issues of a typical proteomics workflow by consolidating a multi-faceted and laborious process into one automated, walk-away proteomics system. This system is most often used by pharmaceutical companies and core facilities at leading institutions worldwide due to its high throughput. Now the gold standard of single-cell functional proteomics IsoPlexis is known for is available in three different formats: IsoSpark, IsoLight, and IsoSpark Duo.

The introduction of the IsoSpark, a personalized proteomics system for any laboratory, makes unique functional proteomics accessible to every lab, providing an integrated and flexible solution for accelerating curative medicines. At only 30% of the size of the IsoLight, the IsoSpark's smaller footprint and lower throughput is perfect for researchers wanting to apply functional immune landscaping, intracellular signaling omics, and high-plex automated immunoassays to their research.

The IsoSpark runs up to four chips simultaneously, and both the IsoLight and the IsoSpark Duo run 8 chips for higher throughput. The IsoSpark Duo is ideal for complete functional immune landscaping with the ability to analyze multiple cell types simultaneously in one run.

A New Era for Discovery Biology

IsoPlexis' functional proteomics is changing how the world thinks about immune cell characterization, showing how harnessing the most powerful cells is changing immune medicine.

The IsoSpark, the IsoLight, and the IsoSpark Duo, along with functional immune landscaping, intracellular signaling omics, and high-plex automated immunoassays are the latest innovations from IsoPlexis. The IsoSpark, introduced on November 11th, is currently available for pre-order in North America and Europe with shipping planned for January 2021.

Experience the innovation that has unlocked the next generation of personalized medicines with unique functional proteomics. Bring IsoPlexis' gold standard single-cell functional proteomics technology to your laboratory to accelerate the development of curative medicines.

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The Problem with Citation Cartels

Gaming citations is marring the integrity of institutions, researchers, and journals by manipulating the scientific literature.

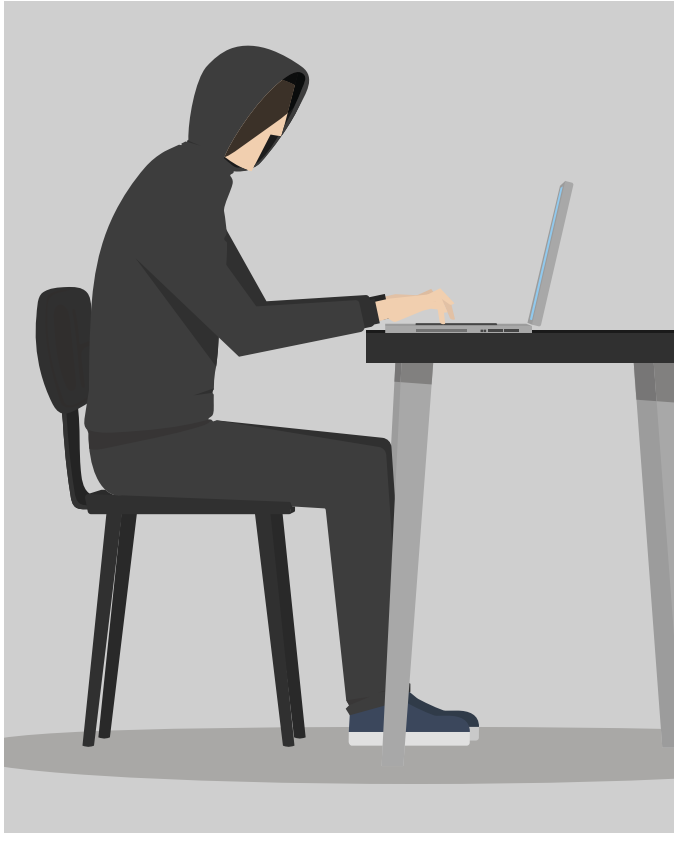
BY SIBRANDES POPPEMA

Scientific papers are the recordkeepers of progress in research. Each year researchers publish millions of papers in more than 30,000 journals. The scientific community measures the quality of those papers in a number of ways, including the perceived quality of the journal (as reflected by the title's impact factor) and the number of citations a specific paper accumulates. The careers of scientists and the reputation of their institutions depend on the number and prestige of the papers they produce, but even more so on the citations attracted by these papers.

In recent years, there have been several episodes of scientific fraud, including completely made-up data, massaged or doctored figures, multiple publications of the same data, theft of complete articles, plagiarism of text, and self-plagiarism. And some scientists have come up with another way to artificially boost the number of citations to their work.

Citation cartels, where journals, authors, and institutions conspire to inflate citation numbers, have existed for a long time. In 2016, researchers developed an algorithm to recognize suspicious citation patterns, including groups of authors that disproportionately cite one another and groups of journals that cite each other frequently to increase the impact factors of their publications. Recently, I came across yet another expression of this predatory behavior: so-called support service consultancies that provide language and other editorial support to individual authors and to journals sometimes advise contributors to add a number of citations to their articles and the articles of colleagues. Some of these consultancies are also active in organizing conferences and can advise that citations be added to conference proceedings. In this manner, a single editor can drive hundreds of citations in the direction of his own articles or those of colleagues that may be in his circle.

The advent of electronic publishing and authors' need to find outlets for their papers resulted in thousands of new journals, frequently with the imprimatur of "international" and promises of open access and wide circulation. The birth of predatory journals wasn't far behind. Recently a group of authors published a consensus definition of such publications: "Predatory journals and publishers are entities that prioritize self-interest at the expense of scholarship and are characterized by false or misleading information, deviation from best editorial and publication practices, a lack of transparency, and/or the use of aggressive and indiscriminate solicitation practices."



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These journals can act as milk cows where every single article in an issue may cite a specific paper or a series of papers. Sometimes the citations are more or less on topic, but in other instances, there is absolutely no relationship between the content of the article and the citations. The peculiar part is that the journal that the editor is supposedly working for is not profiting at all—it is just providing citations to other journals. It's easy enough to spot if someone at the journal would pay attention. Such practices can lead an article to accrue more than 150 citations in the same year that it was published.

How insidious is this type of citation manipulation? In one example, an individual—acting as author, editor, and consultant—was able to use at least 15 journals as citation providers to articles published by five scientists at three universities. The problem is rampant in Scopus, which includes a high number of the new "international" journals. In fact, a

listing in Scopus seems to be a criterion to be targeted in this type of citation manipulation.

Why is this important? First, these individuals who are not only authors, but also editors and consultants, and their colleagues obtain hundreds of citations and outshine their colleagues who play by the rules. Guess who's more likely to get tenure or that plum promotion? Second, the numbers are staggering. For one university, I found that of the nearly 700 Scopus-listed papers its researchers published in 2019, the citation numbers of at least 20 appear to have been boosted in this way. Almost 60 percent of the citations to published studies from this university came from 15 manipulated journals, and this significantly padded the citation numbers of the 20 articles. These suspect citations drove the citations per paper (C/P) average for this university up to 2.50 for the year, whereas without them, the C/P would have been 1.08. Because citations per paper and/or citations per faculty are criteria in the Quacquarelli Symonds ranking and the Times Higher Education World University Rankings, this also artificially inflates the quantitative standing of the university.

What can be done about citation cartels? First, editors and editorial boards of legitimate journals should be paying attention and correcting their colleagues. They carry a responsibility. When every single article in a journal is citing a specific article or group of articles, something is likely amiss. When the subjects of the cited and citing articles are unrelated, it is a dead giveaway. Before publication of a journal issue, this could be analyzed. In fact, checking for appropriateness of citations is a major task for reviewers and editors. Journals should also reconsider the practice of using outside support service consultants as editors of articles or special issues that result from conferences.

Citation cartels, where journals, authors, and institutions conspire to inflate citation numbers, have existed for a long time.

Scopus itself has all the data necessary to detect this malpractice. Red flags include a large number of citations to an article within the first year. And for authors who wish to steer clear of citation cartel activities: when an editor, a reviewer, or a support service asks you to add inappropriate references, do not oblige and do report the request to the journal. ■

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Notebook

ALYONA KOSHKINA

I Spy

In 1960, in a milestone that would remain classified for more than three decades, the US Air Force, CIA, and private industry partners launched the world's first photo-snapping satellite into orbit. Known as Corona, the satellite and its successors would be sent on periodic missions for the following 12 years, chiefly targeting sites of strategic interest such as military airfields and missile silos within the territories of the Soviet Union and its allies. Once the satellite had shot its strip of film, it would release the photos in a parachute-equipped capsule over the Pacific, to be retrieved by a military plane before it could hit the water.

"I think it's [a] really great example of human ingenuity that they were able to take such astounding photos already back then," says Volker Radeloff, an ecologist at the University of Wisconsin–Madison. After the program and its 800,000 images were declassified in 1995, Radeloff recognized that repository as a potential treasure trove of ecological information, a chance to trace changes in landscapes over time. He and his colleagues have since mined the images to reveal changes in forest cover along the Latvian-Russian border and a surge in tree-felling that occurred in Romania in the 1960s.

Radeloff's former student Catalina Munteanu, who coauthored the Romania study, was speaking a couple years ago with some ecologists who were involved in a project to tease out the interplay between agriculture and biodiversity on the Kazakh steppe, when the topic of the bobak marmot (*Marmota bobak*) came up—specifically, the difficulty of getting historical data on the rodents' populations. These marmots are important from an ecological point of view, Munteanu notes, because they turn over soil and fertilize it with their feces, and their abandoned burrows provide homes for other animals such as foxes and burrowing owls. "They sustain a lot of biodiver-

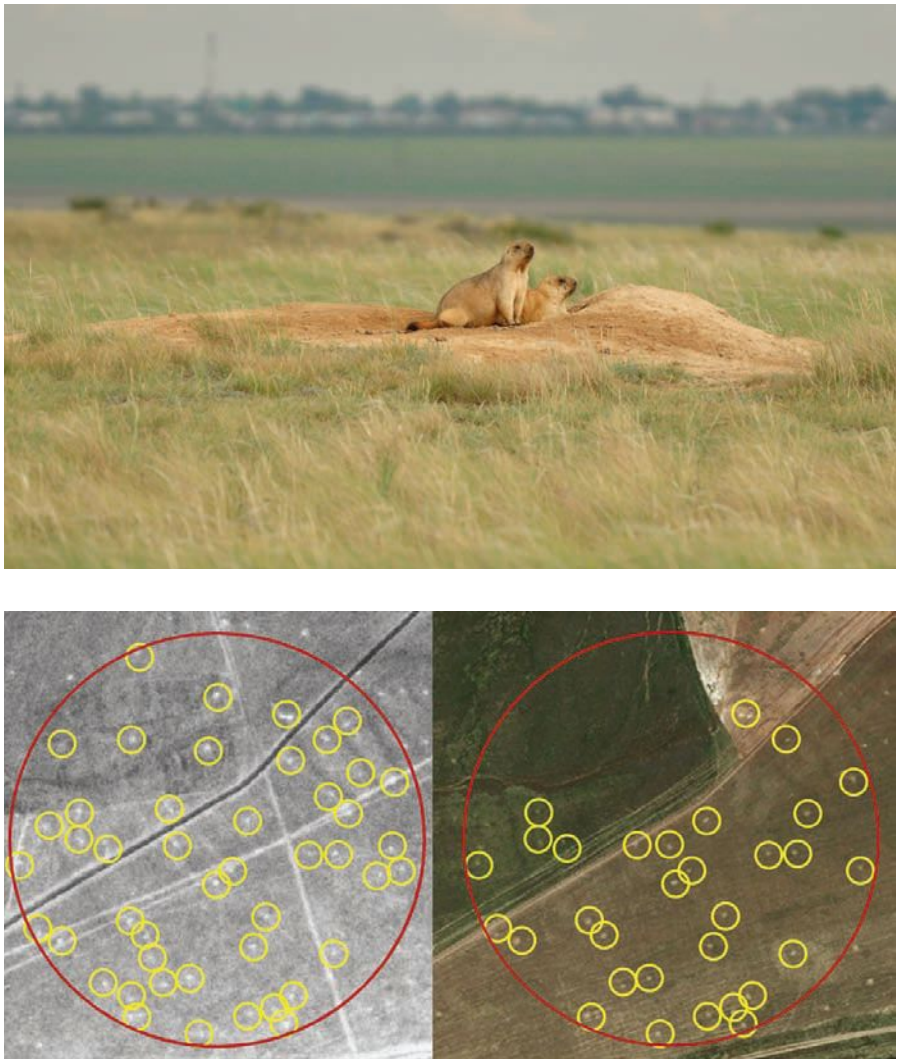
PEERING THROUGH TIME: Researchers used images taken by the Corona satellite system decades ago to track declines in marmot populations in Kazakhstan.

sity in the steppe, and they are a very good indicator for the overall health of the ecosystem," says Munteanu, who at the time was a postdoc at the Leibniz Institute of Agricultural Development in Transition Economies in Germany. The ecologists wanted to know how the marmots had fared given dramatic land-use changes in Kazakhstan during the Soviet era and after the USSR's collapse.

Munteanu suggested to the researchers that they might use Corona photos to track Cold War–era marmot burrows. It was meant half-jokingly, she says. "But then we pulled out some images, and we saw that we could actually see the marmot burrows." The researchers could find them thanks to mounds of soil that pile up as the marmots tunnel into the ground—on the satellite images these appeared as little spots, a few meters across, that were lighter-colored than their surroundings.

The team compared Corona images taken in 1968 and '69, soon after a Soviet campaign to ramp up food production motivated the conversion of many grasslands in northern Kazakhstan to cropland, with modern satellite imagery taken of the same areas between 1999 and 2017. Munteanu and her colleagues saw that, initially, the animals seemed to do fine in the newly-cultivated fields—in fact, the overall density of burrows was higher in the fields than in grasslands across both the 1960s images and their more contemporary counterparts.

But although the marmots are philopatric—meaning that they normally return to the same burrows year after year, generation after generation—about 60 percent of the historical burrows on land cultivated since the 1960s had disappeared, as indicated by a lack of halos of freshly turned soil in the more recent images. Across the studied area, the number of burrows declined by 14 percent over the period encompassed by the study, with long-cultivated lands showing the steepest declines. Meanwhile, the number of burrows in grasslands that remained uncultivated rose by 17 percent (*Proc R Soc B*, 287:20192897, 2020). "It wasn't the single event of converting the marmots' habitat, but it was the persistent agriculture that led to the decline of the species" in the area, Munteanu posits.

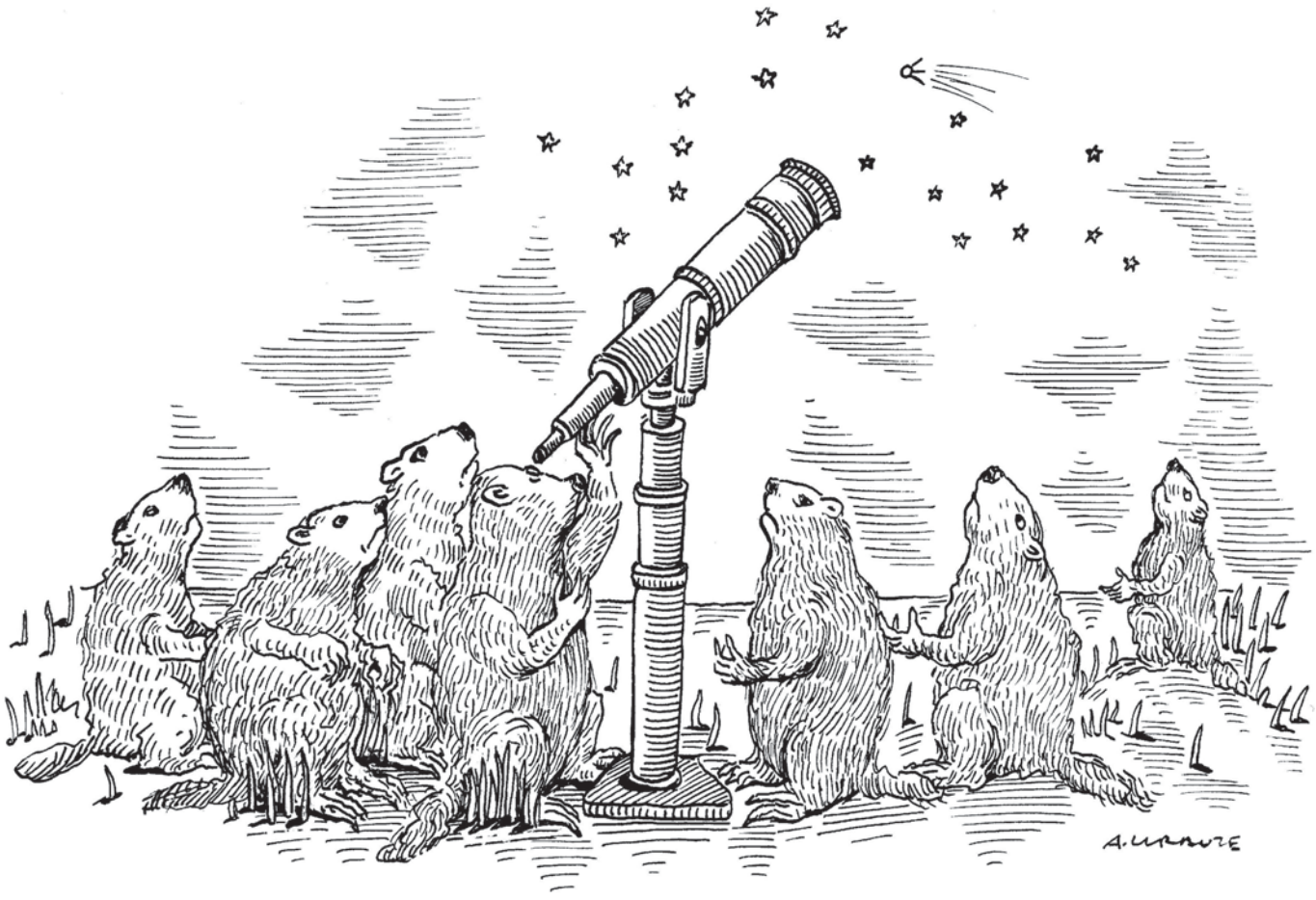


MARMOT MAPS: Marmot burrows are visible on satellite images thanks to the mounds of soil that the rodents make as they tunnel into the ground (above). Many of the burrows researchers observed in images taken in the 1960s (below left) had been abandoned by the 2000s (below right).

Munteanu, now a postdoc at Humboldt University, suspects that what's happening with the marmots is that "when one shock occurs—their homes are destroyed by cropping—they tend to come back [and rebuild], and they do this again and again," so there aren't obvious population declines in the short term. "But when we look at these long time spans . . . we realize that this coming back and rebuilding means investing a lot of energy in maintaining the housing," leaving less energy for tasks such as gathering food and caring for young, and eventually causing a decline in the population. A key takeaway for humans making planning decisions that affect the environment, she says, is to consider such potential long-term ecological effects when making changes to the landscape.

Radeloff, who was not involved in the research but has collaborated with several

ALYONA KOSHKINA; *PROC R SOC B*, 287:20192897, 2020.



of its authors, agrees. "It highlights that we need historical information to understand how species are affected by agriculture and other human activities," he says.

The study is an interesting and rare example of an ecological application for Corona data, says Melanie Vanderhoof, a geographer with the US Geological Survey who was not involved in the project but uses satellite images in her own research. "One of the major limitations in relying on satellite imagery for lots of applications is the date range over which the satellite imagery is available," she notes. "It can be hugely advantageous" to make use of earlier satellite imagery when possible. That said, the black-and-white Corona images aren't as information-rich as those from newer satellites, which can capture reflected light in various color and infrared bands, she notes, making the Corona images harder to analyze accurately using artificial intelligence techniques. For that reason, she says, "it seems like [Corona] imagery would work best for localized applications that don't require automating the detection or image classification process."

The new research convincingly ties changes in land use to a decline in marmot populations, says Ken Armitage, an emeritus professor at the University of Kansas who studied the animals for decades. For him, though, a drawback of the study is its "inability to distinguish between grazed and ungrazed grassland" in the satellite images. That's important, he says, because other studies have found that marmots do well in grasslands grazed by larger animals, but not in areas filled with tough, tall grasses. Despite the downsides of cultivated fields from marmots' point of view, then, "they could well be doing much better on the cropland then they did on the ungrazed grassland," he says.

Munteanu acknowledges that her team's approach was unable to distinguish between these two grassland states, but says this doesn't affect the study's finding that persistent agriculture was associated with declining marmot populations. She and her colleagues are now working on using spy satellite data to examine the effects of livestock grazing on the Kazakh steppe. "We hope that we can find new ways to evaluate biodiversity based on these historical data sources,"

ANDRZEJ KRAUZE

she says. “I think there are endless possibilities for this [satellite] data . . . and many of them relate to areas that maybe are otherwise out of reach for scientists.”

—Shawna Williams

Diversifying Neuroscience

Search among the millions of volunteers in the world’s brain-based genomic studies, and you will be hard-pressed to find people of African ancestry. The largest meta-analysis of genome-wide association studies (GWAS) of Parkinson’s disease to date, for example, didn’t include any individuals of primarily African descent among its more than 1.4 million participants, nor did a 2019 meta-analysis of GWASs examining depression. Only 4 percent of all neurological disorder research contained in the GWAS database of the National Human Genome Research Institute includes minority participants.

Databases such as these are critical to research on brain disorders and to genetics-based precision medicine. Yet the lack of data from non-Europeans means that researchers know very little about the genetic variants associated with disease risk in people of African descent, or about genetic biomarkers for disease severity, drug response, or side effects.

This lack of representation so stunned Daniel Weinberger, the director of the Johns Hopkins University–affiliated Lieber Institute for Brain Development, that he published a paper in *Neuron* this summer addressing the lack of African representation in neuroscience (107:407–11, 2020). It was troubling, he says, to acknowledge his own institution’s culpability. “We felt that we, like many people in biomedical research, had underexplored this ancestry, and we became conscious of the fact that most of what we were publishing . . . were studies on people of European ancestry,” Weinberger tells *The Scientist*. “This was no longer an acceptable thing for our institute to be doing.”

While several other organizations have launched their own efforts to improve diversity in genomics research, what sets the AANRI apart is its community buy-in.

By then, Weinberger had also helped establish an initiative rooted in the community surrounding the Lieber Institute’s laboratory in Baltimore. He and his collaborators set up the Afri-

GROUP EFFORT: A partnership between the Lieber Institute for Brain Development, the African-American Clergy Medical Research Initiative, and Morgan State University aims to boost representation of people of African descent in brain-based genomic research. Here, the Lieber Institute's Rahul Bharadwaj examines brain tissue.

LIEBER INSTITUTE FOR BRAIN DEVELOPMENT

can Ancestry Neuroscience Research Initiative (AANRI) in 2019 as a partnership between the institute, the African-American Clergy Medical Research Initiative, and Morgan State University, a historically Black college or university (HBCU), with the intention of increasing diversity among research participants and creating a more diverse community of neuroscientists. The Lieber Institute has been collecting brain donations since 2010, including roughly 700 brains donated by the relatives of people of African descent. According to Weinberger, it's the largest and most well-curated collection of African ancestry brain tissue in the world.

The initiative's work to systematically analyze these samples is divided into three phases. During the first phase, which began in early 2020, researchers are extensively sequencing 200 brains, including DNA, RNA, bisulfite (methylation detection), and peptide sequencing. COVID-19 put the work on pause earlier this year, but the team plans to usher another 300 brains through phase one before moving into phase two, when a subset of those 500 brains will undergo single-cell sequencing and multi-omics to study gene expression. Phase three will look back over the previous two phases and add samples to fill any remaining information gaps. The data will be shared publicly throughout the process, according to Weinberger.

While several other organizations, including the Broad Institute of MIT and Harvard University and the National Institutes of Health, have launched their own efforts to improve diversity in genomics research, what sets the AANRI apart is its community buy-in, says community leader Reverend Alvin Hathaway. Having met with descendants of the infamous Tuskegee Experiment—during which Black men were deceived about treatment for syphilis by researchers studying the disease—Hathaway says, "I know suspicion. I understand distrust." He facilitated the partnership with Morgan State, which will include the AANRI in its curriculum, host an annual symposium, and send faculty and students to the Lieber Institute for collaborations. Hathaway has also leveraged connections with state officials, financiers, doctors, and educators to bring in funding totaling more than $3 million.

Partnerships with HBCUs will put data directly in the hands of scientists of color, says Mima Akinsanya, a neuro-

immunology fellow at the NIH who is not involved in the AANRI. "You can't say that you are interested in diversity or recruitment of African-Americans and not have any connection with an HBCU." Akinsanya adds that having a member of the clergy involved in the initiative will help reassure the public about research integrity.

One of the most immediate priorities of the AANRI is to better understand how genetic and racial diversity manifests in disease. People of African ancestry fall into some of the world's most genetically diverse lineages, and genome-wide "polygenic risk scores" used to predict illness based on data from people of European ancestry are significantly less useful in assessing risk within African descendants. Including more individuals in ongoing research—and therefore capturing more of the diversity inherent in the human genome—will make these scores more predictive for a broader sweep of people, Weinberger says.

With a more complete understanding of the scope of human diversity, researchers could also design better animal models to study mental health disorders in humans, says Bianca Jones Marlin, a neuroscientist at Columbia University who studies the epigenetic impacts of trauma and was not involved in the AANRI. Human GWAS help Marlin know which genes to target when designing her animal experiments, she explains. "As researchers, we should dive at the opportunity to diversify our pool because it's going to lead to better data," she says. "We're hungry for that as scientists."

We became conscious of the fact that most of what we were publishing . . . were studies on people of European ancestry.

—Daniel Weinberger
Lieber Institute for Brain Development

Understanding more about how genetic diversity influences an individual person's susceptibility to disease could lead to advances in precision medicine that benefit all, agrees Kafui Dzirasa, a neuroengineer at Duke University Medical Center and one of Weinberger's coauthors on the *Neuron* paper. "Locked within these genetic differences, there might be medications that could be created" to better take each individual's genetic background into account, he says. "There's an important case to be made around equity, but there's also an important scientific case around biological discovery that advances health for everyone."

—Amanda Heidt

THE DREAM ENGINEERS

Researchers have long wanted to manipulate the sleeping brain's wanderings. Technologies new and old are now helping them to do so.

BY CATHERINE OFFORD

Adam Haar Horowitz is the first to admit that whispering to strangers as they fall asleep "seems a little creepy." He'd been mulling over the idea with fellow MIT master's student Ishaan Grover a few years ago while thinking about ways to influence the dreamlike visions people see at sleep onset, a state known as hypnagogia. The pair wondered if quietly saying words or phrases to people in hypnagogia might influence the content of their thoughts and visions, thereby serving both as a tool to investigate human cognition and, ultimately, as a means to help people wield control over their dreaming brains.

Haar Horowitz didn't end up whispering into strangers' ears, but he, Grover, and other collaborators did find a way to execute the basic concept, using a more practical solution: a device that fits into a person's hand to monitor changes in heart rate, muscle tone, and skin conductance—all of which help researchers determine the moment at which someone dozes off—paired with a computer or smartphone app that automatically plays audio prompts and records people's spoken responses. The app "would speak to people when it guessed that they were at the end of hypnagogia before they go into something deeper," Haar Horowitz says. After reporting what they were thinking about right at that moment, people would be allowed to nod off again, and the whole process could be repeated.

In experiments detailed in Haar Horowitz's master's thesis and a scientific paper published earlier this year, people interacted easily with the setup, known as Dormio. They chatted with it, albeit somewhat nonsensically, about what they could see and feel as they slipped in and out of wakefulness. One volunteer,

prompted by Dormio to think about a fork, described dreaming about a family that was "happy to see the fork. And they're putting it in a pumpkin," according to Haar Horowitz's thesis. Another participant, told to think about a tree, described "a tree from my childhood, from my backyard. It never asked for anything."

Dream researchers who spoke with *The Scientist* say Dormio marks an exciting step for a field traditionally limited by scientists' inability to interact with their study participants. Many experts define dreams broadly as any subjective experiences people have while asleep, although most projects rely on dream reports collected specifically from people woken up from rapid-eye movement (REM) sleep, the stage of sleep at which people are most likely to experience emotional, narrative dreams (though not the only sleep stage in which dreams can occur; see "The Stages of Dreaming" on page 25). This time-consuming approach has been something of an obstacle to researchers interested in manipulating dreams—an important aspect of dream research, says Haar Horowitz, now a research assistant in the Fluid Interfaces research group at MIT's Media Lab. After all, he says, "you can't do controlled experimentation on dreams without an ability to control dreams."

Haar Horowitz is one of a small but growing group of researchers who call themselves dream engineers and are exploring various methods to influence people's thoughts at sleep onset and during sleep itself. Some tools, like Dormio, use aural stimuli, while others harness sights or smells, or employ more-complex technologies such as noninvasive brain stimulation. With a recent wave of studies demonstrating the promise of such approaches, neuroscientists and psychologists may be able to learn more not only about how and why dreams are generated, but about possible health and cognitive applications of dream control.

Such techniques could help give dream researchers the control they're after, says Harvard Medical School dream researcher Robert Stickgold, Haar Horowitz's mentor and collaborator. With these approaches, "we can use the scientific method."

Sweet dreams

Attempts to influence dreaming are by no means new. People dating as far back as the ancient Egyptians have been known to fast to induce vivid dreams, while scientists, philosophers, and artists have been experimenting for centuries with hashish, opium, and other drugs to conjure dreamy visions in and out of sleep. Many cultures continue to hold beliefs about the dream-altering effects of certain foods—folklore in many Western societies holds that cheese can induce vivid dreams, although there's little scientific research on the topic. And demand for lucid dreaming classes designed to help people take control of their in-dream environments has taken off in the last few years, helped along by Christopher Nolan's dream-twisting 2010 blockbuster, *Inception*.

Dream engineers are interested in finding reliable, researcher-controlled ways to induce lucidity—where a dreamer becomes aware of being in a dream and may be able to exert control over their actions and their environment, as well as other sensations such as flying, to investigate how those sensations are generated and whether they're associated with any benefits for the person experiencing them. One method that's received significant interest as a way to manipulate dream sensations is noninvasive brain stimulation, which uses a magnetic coil or scalp electrodes to influence electrical activity in the dreamer's brain.

In 2013, a small study applied 10 minutes of transcranial direct current stimulation (tDCS) to people in REM sleep, and concluded, based on reports people made after being woken up from REM sleep, that the procedure increased lucidity in dreams when compared to a sham procedure.[2] A similar study the following year[3] that applied either a sham procedure or bursts of transcranial alternating current stimulation (tACS)—which is thought to be better than tDCS at influencing brain oscillations—concluded that 40 Hz currents during REM could also promote dreamers to become more self-aware. However, in both studies, the effect was weak, and in the 2013 study it was only observed among people who said they already frequently experienced lucid dreams. That group is unlikely to be representative of the general population, for which researchers estimate that up to 50 percent may never have experienced dream lucidity.

> We treat studying dreams as another way to understand what the mind and brain are doing during sleep.
>
> —Erin Wamsley, Furman University

The University of Montreal's Tore Nielsen, who directs the Dream and Nightmare Laboratory at the Center for Advanced Research in Sleep Medicine, is unconvinced that noninvasive brain stimulation works as a lucidity inducer. Like many dream researchers, Nielsen says he has experienced his fair share of lucid and otherwise extraordinary dreams. He and his colleagues recently carried out their own study of tACS using stringent study conditions: for researchers to confirm a participant's report of lucid dreaming, that person had to give a signal on becoming lucid—flicking their eyes from left to right under their closed eyelids three times—and that had to happen during REM sleep, as determined by electroencephalography (EEG) analyses of their brain activity. "Much to our chagrin, we failed to replicate" the earlier findings, Nielsen says. Although some participants did the eye-flick signal during REM sleep and subsequently described vivid dreams, people were no more likely to have lucid dreams after receiving tACS than they were if they'd had the sham procedure.[4]

Noninvasive brain stimulation may have other uses in dream manipulation, particularly for studying the relative roles of different brain regions in generating common dream experiences. Queen Mary University of London's Valdas Noreika and colleagues, for example, recently used 10-minute sessions of tDCS to disrupt activity in the sensorimotor cortex of 10 volunteers while they were in REM sleep. The researchers woke people from REM sleep shortly after each session, and asked them to fill out questionnaires on what they'd been dreaming about—and specifically, whether they'd been engaged in movements such as lifting objects or walking. The results showed that people who had received tDCS reported experiencing less movement in their dreams than people receiving a sham procedure, suggesting that normal sensorimotor cortex activity is required for those dream sensations, Noreika says.[5] Specifically, "we found that this sensorimotor cortex is responsible for repetitive actions of the dream self . . . such as walking, running, swimming."

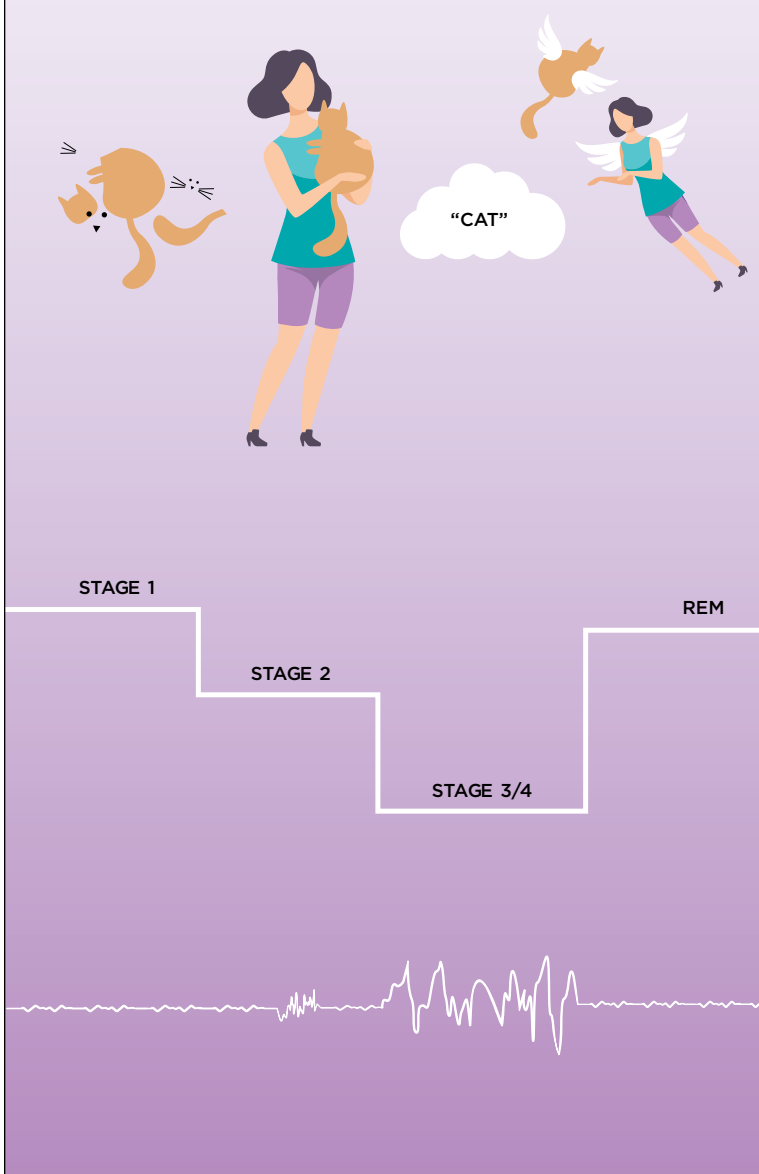
Simpler technologies likely also have their place in the manipulation of dream experiences. Michelle Carr, who did her PhD in Nielsen's lab and is now a postdoc at the University of Rochester, has been experimenting for the last couple of years with techniques to induce self-awareness in dreamers in a lab setting. She's found that using behavioral training to get people to associate sensory stimuli such as lights or sounds with a sense of heightened awareness seems to be an effective way to trigger lucid dreaming.

In a recent study, for example, Carr and her colleagues trained awake volunteers to try to become particularly aware of their surroundings whenever researchers presented them with alternating cues of flashing red LEDs and a beeping noise. Participants subsequently were allowed 90 minutes to doze off for a nap in the lab, while researchers monitored their sleep stages using several techniques including EEG and measurements of electrical activity in the muscles. When a participant entered REM sleep, experimenters triggered the LED and the audio cues in the same alternating pattern they'd played during training. By monitoring eye movements for the agreed-upon eye-flicking signal, collecting dream reports from people woken up by researchers for brief periods mid-nap, and administering questionnaires after the 90 minutes was up, the team found that around 50 percent of the treatment group experienced lucid dreams, compared with just 17 percent in a control group of participants who'd completed the training but hadn't had the lights and sounds played to them during their naps.[6] "It was really cool—some people did [the cue-signal response] up to eight times," Carr says. "Some people who had never before had one had their first lucid dream in the lab."

While the research is still in early stages, Carr says she hopes the findings will encourage further studies intended to trigger certain sensations in dreams, with an eye toward the possible benefits. She and her colleagues recently analyzed dream and mood diaries kept by 20 people over the course of a week and found that higher

THE STAGES OF DREAMING

Neuroscientists used to think that dreaming took place almost exclusively during rapid-eye movement (REM) sleep, a stage of slumber that is often accompanied by complex emotional, narrative-heavy dreams that can involve sensations such as flying or other movements. But in the last few decades, research has shown that people can also have subjective dream-like experiences in non-REM sleep, albeit less frequently and of a different nature. For example, a person thinking about a cat as they doze off into the first stage of sleep—a hallucinatory state known as hypnagogia—may see strange cat visions and experience sensations such as falling. Dreams experienced later in non-REM sleep tend to be more mundane and may involve people or objects that are familiar to the dreamer. Once in very deep sleep, people are more likely to have conceptual thoughts than to experience emotional narratives, if they have any memorable dreams at all.



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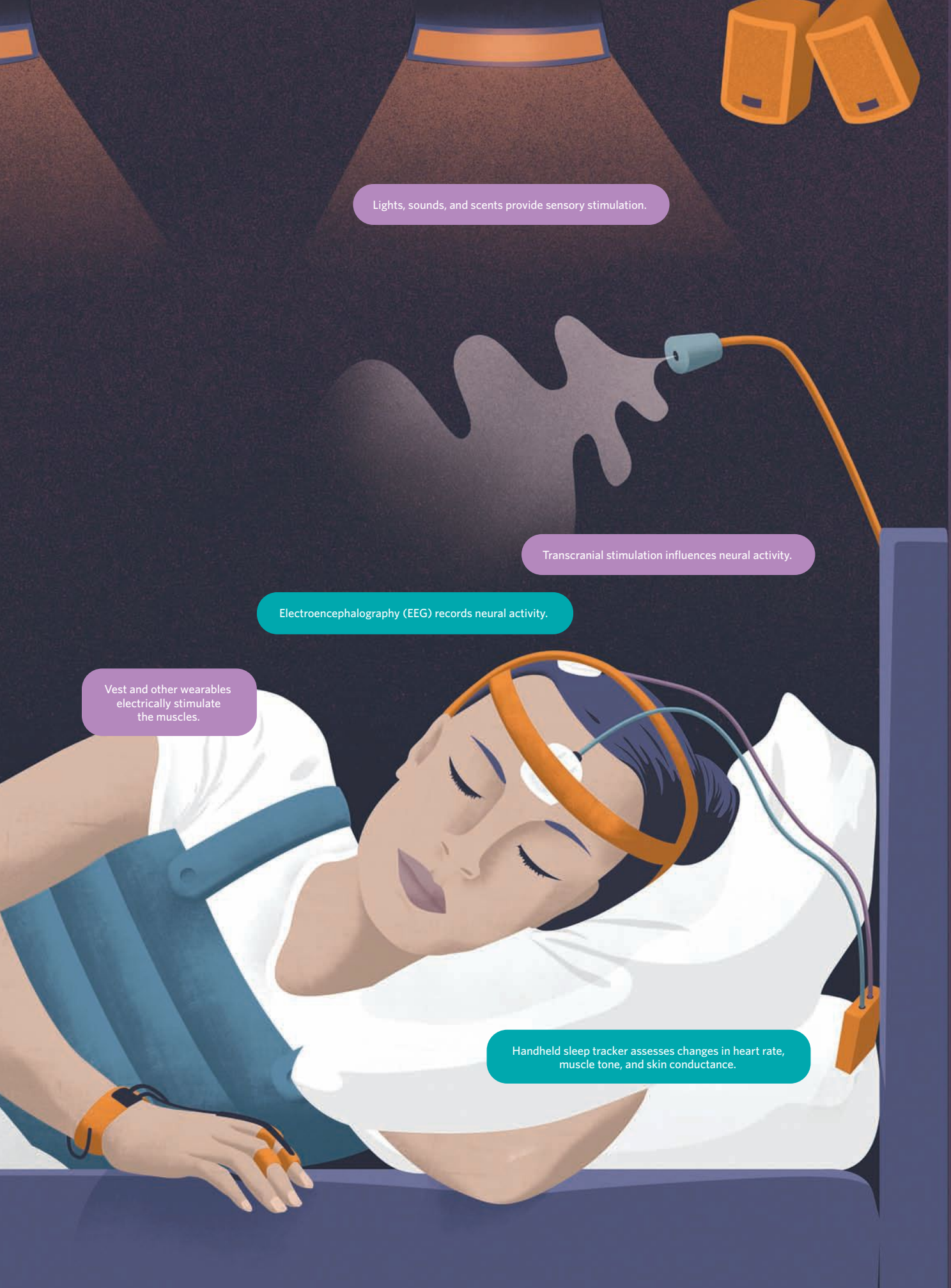
THE DREAM ENGINEER'S TOOLBOX

Researchers use a variety of technologies to monitor (teal) and attempt to modulate (purple) people's dream experiences. While many protocols include pre-sleep training—to encourage people to become more aware of their dreaming selves, for example, or to incubate specific ideas using virtual reality or computer games—a number of dream-influencing approaches can be applied during sleep. Scientists also monitor participants during sleep and collect dream reports as soon as they awake.


Lights, sounds, and scents provide sensory stimulation.
Transcranial stimulation influences neural activity.
Electroencephalography (EEG) records neural activity.
Vest and other wearables electrically stimulate the muscles.
Handheld sleep tracker assesses changes in heart rate, muscle tone, and skin conductance.

lucidity correlated with elevated waking mood the following day.[7] The researchers plan to use their lucidity-inducing techniques to investigate whether the relationship is to some degree causal, Carr says, and whether inducing lucidity has other applications, such as helping people suffering from recurrent nightmares—a common symptom of many mental health conditions including anxiety disorder and post-traumatic stress disorder.

"If we can get [these dreams] to be induced reliably," Carr says, "then we can use them for beneficial purposes."

Incubating ideas

For some dream researchers, it's not just the overall dream experience that's worth manipulating, but also a dream's specific content. Many ancient human civilizations experimented with this idea too, and documented attempts to promote in-dream encounters with various deities, for example. But for Harvard's Stickgold, it was a family trip to Vermont in the 1990s that made him start thinking about the idea.

Falling asleep one evening after a hike up Camel's Hump in the Green Mountains, Stickgold was surprised to feel as though he were scrambling up the side of the mountain, just as he had earlier that day, with the distinct sensation of rocky ground under his hands. Waking up and then dozing off again, he found that he was able to regain this sensation several times before falling into a deeper sleep. Intrigued by the experience, Stickgold says, he wondered about how to try to capture it in an experiment.

There was a stumbling block, however: he'd be unlikely to obtain ethical and administrative approval to lead a gaggle of undergrads on a rock-climbing expedition just to see if they'd go on to dream about the experience. It was only a few years later that an alternative presented itself. "I was in a meeting with a bunch of students one day, pissing and moaning about what a great experiment this would be, but how I would never be able to do it," Stickgold says. "One of the students sitting there just said, 'What about Tetris?' They proceeded to tell me that this happens when you start playing Tetris: you see [the pieces] floating down before your eyes."

That conversation was the seed for what would become a famous study in dream research. Stickgold and colleagues recruited 27 people—10 Tetris experts, 12 Tetris novices, and five patients with memory loss from brain damage—to play seven hours of the computer game over the course of three days. For an hour at the beginning of each night, participants were prompted by an experimenter or by a digitized voice recording to say what they were thinking about into a microcassette recorder or to an experimenter as they fell in and out of sleep. Almost two-thirds of the participants reported dream-like visions of Tetris during sleep onset, and three of the five amnesiacs also reported seeing Tetris-inspired imagery, despite having no conscious memory of the game.[8] One described "thinking about little squares coming down on a screen and trying to put them in place," while another said they'd seen "images that are turned on their side. I don't know what they are from, I wish I could remember, but they are like blocks." Several Tetris experts reported thinking not only of the Tetris they'd been playing during the experiment, but also of older versions of the game they'd played previously.

Nudging the brain to incorporate specific content—a trick known as dream incubation—has proven to be surprisingly practical using computer and virtual reality games. Erin Wamsley, previously a postdoc with Stickgold's group who now runs a lab at Furman University in South Carolina, says that many researchers previously assumed dreams would be most influenced by more-intense experiences. "You can show someone horrible graphic images or very disturbing films with very high emotional content that participants would agree is disturbing or emotional," says Wamsley. "But [that's] not something that triggers people to dream directly about that experience, necessarily. On the other hand, we've had a lot of success causing participants to incorporate new learning experiences into their dreams." Wamsley's now looking into what determines whether a particular experience will be incorporated into a dream.

In 2010, Stickgold, Wamsley, and colleagues got 43 volunteers to play an arcade skiing game called Alpine Racer.[9] Around a third of the dream reports collected from subjects woken up from non-REM sleep over the following nights were related to the game. The nature of the dream content changed as people fell into deeper sleep, however, going from typical comments such as "I get like flashes of that . . . game in my head, virtual reality skiing game," to oblique skiing references such as, "I was picturing stacking wood this time. . . . I felt like I was doing it at . . . a ski resort that I had been to before, like five years ago maybe."

In the last couple of years, the same researchers have also used a simple maze navigation task that participants carry out on a computer to explore how content is incorporated into dreams that occur during different sleep stages.[10] A dream report from someone just falling asleep contained thoughts of swimming above the maze, for example, while one participant woken from REM sleep reported dreaming about walking through it. A typical report from later stages of non-REM sleep involved the dreamer just standing in the middle of a maze waiting for a friend to find them.

This and many other studies have also reported an association between the incorporation of task content into dreams and task performance post-sleep—a finding that adds weight to the prevalent view among sleep researchers that sleep, and perhaps dreaming specifically, plays an important role in memory consolidation. On the basis of current evidence, it's not clear whether dreaming helps drive that consolidation, or is perhaps instead a reflection or byproduct of the process. Stickgold, who explores theories of dreaming with coauthor Antonio Zadra in a new book, *When Brains Dream*, slated for publication in January, hypothesizes that REM sleep plays an active role in consolidating emotional memories and extracting patterns from recent experiences, and that perhaps the dreamlike visions of hypnagogia are the brain's way of tagging relevant content for processing later on in the sleep cycle. (See "Dreaming of Possibilities" on page 57.) Other researchers posit

that dreams serve different functions—Noreika is one of several scientists who think they offer simulation of potential threats and social interactions that the dreamer might encounter in waking life—or perhaps no function at all.

Exploring potential functions of dreams and dream content is a key purpose of dream-influencing technologies such as Dormio, notes Haar Horowitz, who says that the device's ability to interact with dreamers in real time offers the possibility of collecting data more easily compared to traditional dream research, even if hypnagogia and REM sleep aren't exactly equivalent. He's recently launched a number of collaborations, not only with sleep scientists curious about how changing dream content could alter memory or learning, but also with artists and philosophers interested in how dream incubation might boost their creativity.

> You can't do controlled experimentation on dreams without an ability to control dreams.
>
> —Adam Haar Horowitz, MIT

Changing sleep science

Carr, Haar Horowitz, and others organized a workshop at MIT last year for engineers and dream researchers to discuss technologies available to the field, and the group put together a special issue of scientific papers on dream engineering for the journal *Consciousness and Cognition* this summer. "A lot of collaborations developed from that workshop," says Carr, who was managing guest editor for the issue. "I think it's the start of something new."

With this momentum, dream researchers are hopeful that their field will overcome a lingering image problem in sleep science. Even now, "a lot of people view dreaming as a fringe topic, kind of like studying ESP [extrasensory perception] or out-of-body experiences," says Wamsley. "Of course, in my opinion, it's nothing like that at all. In our research on dreaming, we treat studying dreams as another way to understand what the mind and brain are doing during sleep."

Nevertheless, the subjectivity of self-reported dreams remains an issue, she acknowledges. While neuroscientists' attempts to objectively predict what people are dreaming about on the basis of brain imaging techniques such as functional MRI have made strides in the last few years, they're a long way from matching the detail in dreamers' own descriptions, she says. Aware of this obstacle, several groups working on dream engineering seek to demonstrate the value and feasibility of collecting dream reports as part of regular sleep studies.

In a recent study from Björn Rasch's lab at the University of Fribourg in Switzerland, for example, researchers trained people on a word-picture association task, and then subsequently woke them up for dream reports during the night. The team found that people's memory of the task the following morning didn't seem to be affected by the awakenings themselves. The researchers also reported that there was a positive relationship between dreaming of the task during non-REM sleep and memory performance the following morning, but they found no such association when it came to dreams of the task during REM sleep—a clue about sleep's role in memory that would have been overlooked had dream reports not been gathered.[11]

Dream researchers are also looking toward some of the extraordinary implications of manipulating the minds of sleeping people. With the prospect of devices such as Dormio allowing people to interface with their own or other people's dreams, ethical considerations "are paramount here," notes computer scientist Pattie Maes, the head of the Fluid Interfaces group at MIT's Media Lab and a coauthor of a review of the field in *Consciousness and Cognition*.

Stickgold agrees, noting that even after having done it for decades, there's something unique, and even unsettling, about interacting with the minds of people in the not-quite-conscious, not-quite-unconscious world of dreams. "It has an edge of scariness," he says. "We're tapping into an aspect of people's minds that we don't have much control over and they don't have much control over when they're sleeping. We're almost voyeurs, watching their minds do what they decide to do." ■

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YOU ARE WHAT YOU EAT

The study of diet, long plagued by inaccuracies in self-reports, is entering a new age of precision with the methods of metabolomics.

BY AMBER DANCE

Between 2013 and 2014, 19 people were voluntarily locked in a clinic for days at a time—not once, but on four separate occasions. They were fed a different, strict diet on each of their three-day-long visits, and were forbidden to exercise. Computer access and visitation were allowed, so long as guests didn't smuggle in snacks. Subjects turned over all their urine, from morning, afternoon, and night, to researchers.

These participants temporarily sacrificed their freedom to help dietician Gary Frost and colleagues at Imperial College London understand how eating habits influence the relative concentrations of metabolites excreted in urine, and thus how urine could serve as an indicator of a person's diet, which in the team's experiment ranged from healthy to gluttonous.[1] Frost's team anticipated that such metabolomics analyses would provide more-reliable data for nutritionists than the traditional tactic of asking free-roaming subjects what they've been noshing—an approach that is notorious for its huge error rates.

"Most people have a really bad memory of what they're eating. . . . People will deny eating a dessert or forget that they ate a chocolate," says David Wishart, a biochemist who works on metabolomics and nutrition at the University of Alberta but was not involved in Frost's study. On the other hand, he adds, "blood and urine don't lie."

Indeed, Frost's team was able to use the volunteers' data to turn profiles of metabolites in urine into a single score, which they can now use to make inferences about the diets of people whose meals they didn't control.[2] Other researchers seeking objectivity in nutrition research are identifying metabolites that reveal if a person has consumed a specific food.

The approach is not yet perfected or widespread, and researchers are still working to define the metabolites linked to certain dietary qualities or foods. But with careful analyses, scientists are beginning to uncover nuanced information about people's diets, such as how much milk and cheese they consume, or what kind of brew coffee drinkers drink. As the techniques improve and data on diet-metabolite correlations amass, researchers expect to standardize dietary epidemiology, which seeks to elucidate links between specific eating habits and disease risk. Some even see an opportunity to develop personalized nutrition recommendations to help people boost or maintain physical and mental health. Wishart, for example, is the chief informatics officer at a Vancouver-based company called Molecular You that uses metabolomics and other information to advise customers on eating habits to improve their health.

"There is a high expectation that [metabolomics] will play a leading role in deciphering the interactions between diet and health," says Cristina Andrés-Lacueva, a nutrition researcher at the University of Barcelona.

What's in your food?

Before scientists can begin to link metabolites with health and disease, they must detail the relevant biomarkers for consumption of diverse foods. In 2019, Wishart and collaborators from Europe and New Zealand wrapped up a project that aimed to identify biomarkers for various foods and drinks, from Coca-Cola to chicken breast to Gruyère cheese. Potential biomarkers include compounds directly derived from those foods or fluctuations in the concentrations of human metabolites or metabolites produced by the gut microbiome. Called the Food Biomarker Alliance (FoodBAll),[3] the study turned up several promising candidates.

For example, the team investigating blood biomarkers for dairy proposed the sugar alcohol galactitol as an indicator for consumption of cow's milk, and the aromatic compound 3-phenyllactic acid as a signal for cheese ingestion.[4] The FoodBAll collaborators also developed protocols for validating these novel biomarkers and created several online databases to fuel food metabolomics research. (See table on page 36.)

Even beyond the FoodBAll group, once researchers started looking, they

found metabolites that reveal striking details about dietary habits. For example, biochemist Augustin Scalbert, of the International Agency for Research on Cancer, is interested in coffee, which comes in various types. It's also been linked to a range of health effects, so scientists want to know the best biomarkers for what kind, and how much, subjects consume. Scalbert and colleagues compared blood metabolites from 451 individuals from four countries: France, where people often drink espresso; Germany, where drip-filtered coffee is the norm; Greece, where boiled coffee is preferred; and Italy, where an espresso-like shot brewed by a percolator called a moka pot is popular. In France and Germany, the alkaloid trigonelline was the best marker for coffee consumption. But in Greece the best way to estimate someone's coffee intake was quinic acid, Scalbert says, and in Italy, it was the amino acid derivative cyclo(isoleucyl-prolyl).[5] The results suggest the ideal biomarkers might depend on the population under study.

Metabolites and disease risk

Diet has long been linked to cancer risk. But most studies have simply asked people what they eat, and then tracked later cancer diagnoses.

Like many nutrition researchers, epidemiologists at the American Cancer Society are now seeking specific markers for individual foods to obtain more-reliable data on diet-cancer links. A couple of years ago, Marji McCullough and Ying Wang analyzed 1,186 serum metabolites from 91 food groups and individual items, based on a study of 1,369 women who had filled out food-frequency questionnaires as part of the Cancer Prevention Study II. Correlations abounded: the scientists were able to connect 42 of those foods and food groups to 199 different metabolites, including some novel, not-yet-named biomarkers for coffee and for dark fish, a category that includes sardines and salmon.[6] McCullough and her colleagues hope to validate and apply this profiling method to understand cancer risk in future studies. "The field is still young," she says.

Scalbert agrees. "We are learning, little by little, to exploit this information and make the best use of it to understand the link between food intake and different foods . . . and different diets and the risk of cancer."

He was interested in coffee because it is associated with a lower risk for liver cancer and its precursor, chronic liver disease. Liver cancer affects about 33,000 Americans each year, according to the CDC, but a regular coffee habit slashes cancer risk by up to 50 percent.[7] Armed with their knowledge of coffee biomarkers, Scalbert and collaborators investigated banked blood samples collected from male smokers during a trial of nutritional supplements for the prevention of lung cancer in Finland in the 1980s.[8] In this dataset, coffee-drinking was associated with higher blood concentrations of several compounds—including the neurotransmitter serotonin, glycerophospholipids that make up cell membranes, and trigonelline from the coffee beans themselves—and lower concentrations of tyrosine and bile acids.

Then, the researchers compared the data from trial participants who'd later been diagnosed with liver cancer or who died of liver disease before the end of 2012 with the data from volunteers in the same trial who had healthy livers.[9] Those healthy controls tended to have higher levels of the coffee compounds and associated molecules, while tyrosine and bile acids were higher in those who went on to develop liver disease or cancer.

"This is a very nicely done and well-designed study," says Wishart, who was not involved in that particular piece of research but has collaborated with Scalbert on other projects. He says the results fit with other studies suggesting that consuming coffee can diminish inflammation in the gut while promoting the growth of a beneficial microbiome—factors that then limit the damage caused by compounds such as bile acids and tyrosine.

Interpreting diet

Taking a more generalized view of eating habits, Frost and others are attempting to quantify the overall healthfulness of a diet.[10] In the lock-in study, held at the UK's National Institute for Health Research/Wellcome Trust Imperial Clinical Research Facility in London, he and his team fed their subjects four different meal plans: Diet 1 included foods considered healthy by the World Health Organization, including whole-wheat cereal, steamed salmon, and grapes. Diet 4 represented the opposite end of the spectrum, with sugar-coated cereal, fried pork sausages, and milk chocolate. Diets 2 and 3 fell in between those extremes.

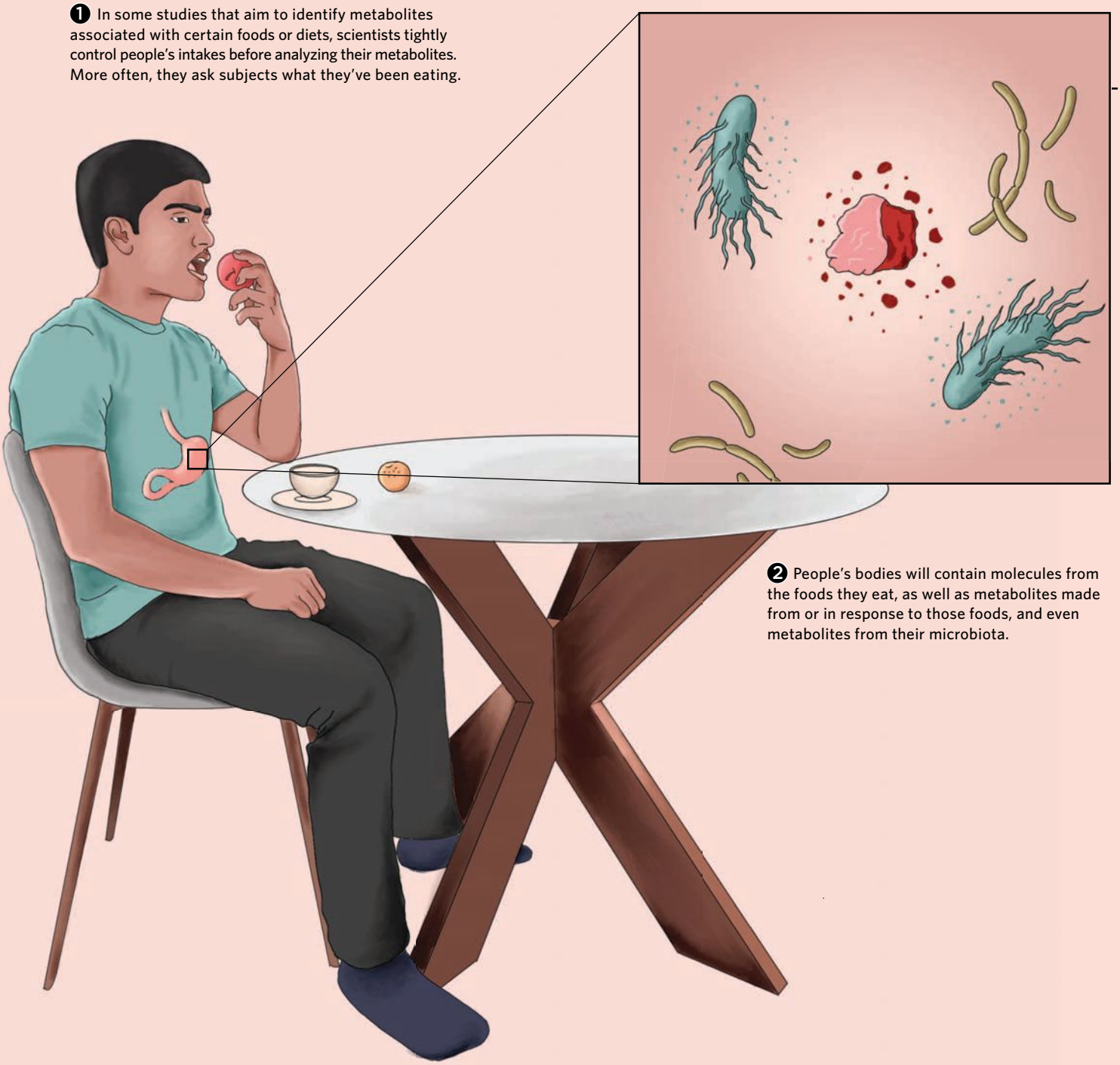
> BLOOD AND URINE DON'T LIE.
>
> —David Wishart, University of Alberta

Within just a few days, the researchers could detect the effects of these menus in the subjects' metabolomic profiles. For people eating the nourishing Diet 1 meals, 19 metabolites appeared at higher concentrations than they did in the urine of people consuming junk food–heavy Diet 4. One of those metabolites, for example, was hippurate, an indicator of fruit and vegetable consumption. Conversely, nine metabolites were higher in people feasting on unhealthy Diet 4 than those on Diet 1. Carnitine, a biomarker for red meat, was one.

To incorporate these individual signals into a broader assessment, data scientist Joram Posma applied machine learning. He used the biomarker levels from participants on Diet 1 or Diet 4 to train a computer algorithm to predict a person's diet quality by profiling their urine. Then, the team tested its model on the data from the intermediate diets. Sure enough, the model correctly iden-

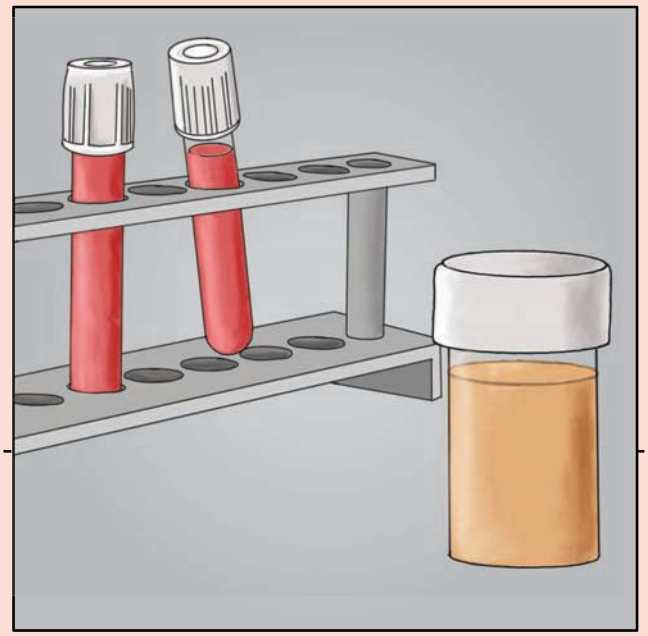
FROM DINNER PLATE TO DATASET

To achieve greater objectivity in nutrition research, which has historically relied on self-reports of what subjects eat, scientists are turning to biomarkers in bodily fluids that reveal details about a person's diet. Much of the work to this point has involved screens to identify novel markers for specific food items (or even for how those foods are prepared). In some cases, researchers have begun to use markers identified in these screens to correlate diet with health risks.

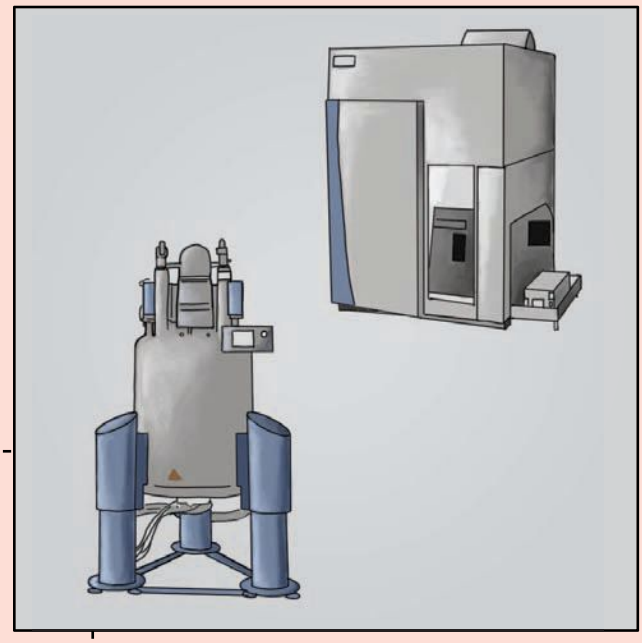


❶ In some studies that aim to identify metabolites associated with certain foods or diets, scientists tightly control people's intakes before analyzing their metabolites. More often, they ask subjects what they've been eating.

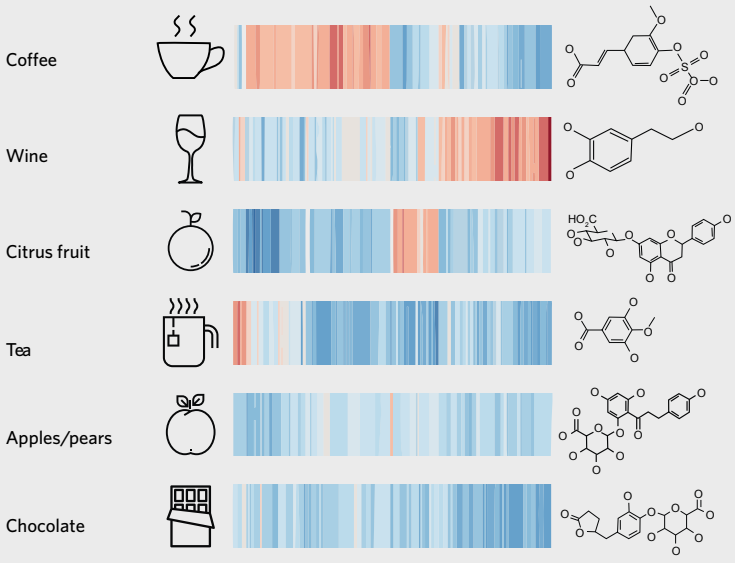
❷ People's bodies will contain molecules from the foods they eat, as well as metabolites made from or in response to those foods, and even metabolites from their microbiota.



❸ Most studies sample blood or urine, but stool, hair, or fingernails might also yield dietary clues.

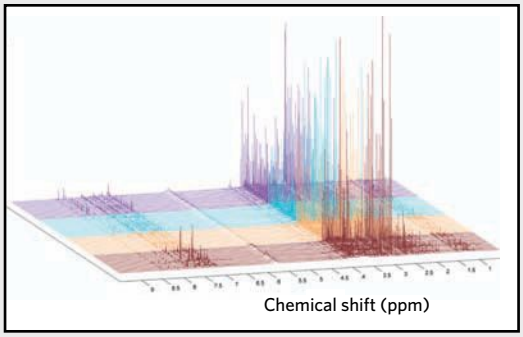


❹ Mass spectrometry allows for highly sensitive analyses of these metabolites in any sample type, even picking up those found at low concentrations. Nuclear magnetic resonance (NMR) provides more reproducible results, but may miss rare molecules.



Data display adapted from *Am J Clin Nutr*, 102:905–13, 2015

Diet 1 (purple) included foods considered healthy by the World Health Organization, including whole-wheat cereal, steamed salmon, and grapes. Diet 4 (brown) represented the opposite end of the spectrum, with sugar-coated cereal, fried pork sausages, and milk chocolate. Diets 2 and 3 fell in between those extremes.



Data image courtesy of Isabel Garcia-Perez, Joram Posma, and Gary Frost

❺ Results can reveal metabolites that are positively (red; example shown) or negatively (blue) associated with specific foods or correlate with the overall healthfulness of a diet.

WHAT'S IN YOUR FOOD?

Researchers involved in the FoodBAll project built several databases to facilitate research on food biomarkers.

Database	Description	Details
FooDB	FooDB lists information on food components including biochemical makeup, health effects, and color, taste and smell.	70,926 compounds in 797 different foods
Phenol-Explorer	Polyphenols are plant compounds with documented health benefits. Phenol-Explorer lists chemical details, which foods contain the compounds, and whether they appear in blood or urine upon consumption.	500 polyphenols found in more than 400 foods
PhytoHub	PhytoHub lists polyphenols and other plant compounds, along with their metabolites produced by humans or other animals.	1,200 compounds in more than 350 foods
Food Compound Exchange	FoodComEx is a virtual library of food-derived compounds that different labs possess, so scientists can easily share them for study.	1,203 compounds

tified Diet 2 eaters as having relatively healthy metabolomes, and Diet 3 diners as skewing closer to the unhealthy patterns.[1] The team also validated the model in other cohorts from Denmark and the UK. Those with healthier reported diets had metabolomes more similar to those of locked-in subjects who ate Diet 1.

"This paper is interesting as it combines an intervention study with observational studies in two different cohorts," says Scalbert, who was not involved in the research. "This has often been done for specific foods, but more rarely for whole diets."

Based on a metabolite profile, the team can now calculate a single Dietary Metabotype Score (DMS) to represent how healthy a person's eating habits are.[2] "We can say where you are on the spectrum," says Frost.

Crucially, the scores are objective, with no interference from faulty recollections by eaters, adds Isabel Garcia-Perez, a chemist on the project who is now putting the algorithm into practice. In a trial of clients seeing dieticians collaborating on the project, Garcia-Perez will use dietary scores to give providers an idea of a person's eating habits before they meet, and to determine how closely clients follow, or don't follow, their prescribed eating plans. She predicts that clients will be more motivated to keep up with the recommended diets if their metabolome provides frequent, reliable feedback on their adherence to the instructions.

McCullough and colleagues are seeking easy markers for broad dietary patterns in blood. They specifically looked for metabolites that would likely indicate scores on four different diet measures: the alternate Mediterranean diet score, the alternate Healthy Eating Index, the Dietary Approaches to Stop Hypertension, and the Healthy Eating Index. Top predictors for high scores on these indices, reflecting a healthy diet, included markers for fish consumption such as the omega-3 fatty acid DHA, and the vitamin carotene from fruits and vegetables.[11]

Ultimately, says McCullough, this kind of research may lead to affordable blood tests that indicate a person's true dietary patterns—an objective measure that clinicians could use to assess disease risk and advise patients. "That's far in the future," she says, "but there's a lot of potential." ■

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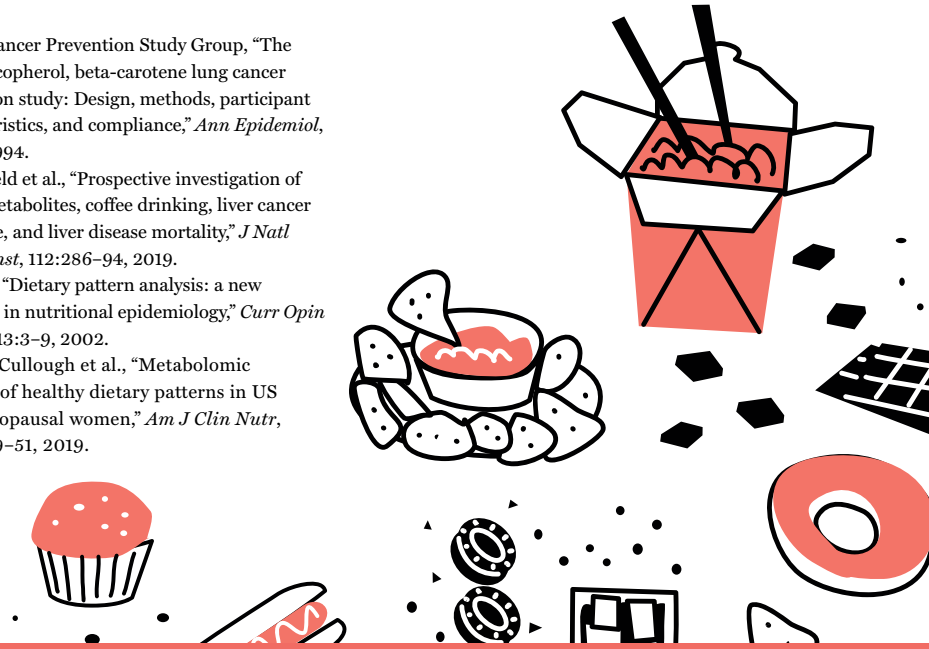

PERSONALIZED NUTRITION

While nutritionists agree on the basic ingredients of a healthy meal plan—lots of fruits and veggies, for example—the general guidelines don't speak to differences between individual diners.

"People respond to foods differently," points out biochemist David Wishart of the University of Alberta. "Particularly with vitamins, there's a fair bit of variability." Vitamin processing may vary with age, genetic makeup, and physiology—for example, if someone is obese, fat-soluble vitamins might be stored in fat tissue instead of circulated through the body, he says. Thus, a glass of vitamin C–rich orange juice might in fact provide different vitamin C effects for different drinkers.

That's why he and others are developing personalized nutrition plans that recommend specific foods, supplements, or even exercises to improve health for individual clients. Wishart is the chief informatics officer for Molecular You, a company in Canada aiming to boost people's health with detailed prescriptions based on metabolomics and other information. But at this point, some experts caution, it's unclear how personalizing diets can improve the health of individuals, aside from populations with specific nutritional needs or allergies.

In 2016, researchers from the Weizmann Institute of Science in Israel launched DayTwo, a company focused on blood-sugar control for people with type 2 diabetes and prediabetes. In diabetics, blood glucose spikes after eating can boost their risk for cardiovascular disease.

DayTwo's approach is based on a study published by its scientific cofounders in 2015. The scientists had monitored glucose levels continually for a week in 800 people as they collectively consumed nearly 47,000 meals (*Cell*, 163:1079–94, 2015). Using machine learning to incorporate glucose patterns, dietary habits, and other factors, the team developed an algorithm to predict glucose levels after consuming specific meals.

The team then offered 12 new subjects the opportunity to receive dietary recommendations informed by its computer model. Participants worked with dieticians to devise meals that matched their preferences, then underwent glucose-level monitoring for a week while eating those meals to provide input data for the algorithm. The team then used the algorithm to predict "good" diets that avoided spikes, or "bad" diets that created them, for each individual. The subjects followed each of those diets for a week. Sure enough, blood-sugar spikes were higher in 10 people when they were on the "bad" diet. The algorithm performed as well as human experts in identifying whether meals would be "good" or "bad" for an individual, but outpaced the experts in that it could do the same for new meals, without any data on that person's previous glucose responses to those foods.

Molecular You launched its own metabolomics-based nutrition services to doctors in 2018, and to consumers last year. Company scientists delineated "safe" blood or urine concentrations for a slew of metabolites, based on the scientific literature and other sources. (The US Food and Drug Administration offers guidelines on safe levels of various compounds in foods, but not in bodily fluids.) Molecular You nutritionists combine company algorithms with their own experience to help customers reach those ideal ranges.

A common, though not universal, recommendation is to eat less red meat, says company CEO Robert Fraser. Other tips are more individualized. For example, if people don't have enough vitamin B, the company might recommend supplements. "There's a lot of variety in what people need," says Fraser. "There's usually something very personal for each person." In an as-yet unpublished study, the company analyzed about 150 customers who followed their Molecular You plan for 100 days. Those who stuck to the recommendation nudged all of their metabolite values into that "safe range," Fraser says.

Not everyone is ready to buy in. "I'm not a strong believer of personalized nutrition," says Augustin Scalbert, a biochemist at the international Agency for Research on Cancer in France. Diet is just so complex, he explains, it will be difficult to prove any interventions are working.

From a rapid molecular test for COVID-19 to tools that can characterize the antibodies produced in the plasma of patients recovering from the disease, this year's winners reflect the research community's shared focus in a challenging year.

BY *THE SCIENTIST* STAFF

We know the old saw: necessity is the mother of invention. Well, 2020 has shown us that a global pandemic is one serious mother. Typically, our Top 10 Innovations competition focuses on laboratory technologies, tools designed to plumb the mysteries of basic biology. But as biologists turned their sights to understanding SARS-CoV-2, the innovation landscape changed accordingly, with new tools developed and existing technologies bent to address the pandemic. So this year at *The Scientist*, our annual contest incorporates inventions aimed at understanding and ultimately solving the COVID-19 problem.

Among our independent judges' picks for 2020's Top 10 Innovations were core laboratory technologies—such as a single-cell proteome analyzer and a desktop gene synthesizer—alongside pandemic-focused products, including a rapid COVID-19 test, a tool that can capture antibody profiles from the blood plasma of convalescing coronavirus patients, and a platform for characterizing glycans in the spike protein that studs the surface of SARS-CoV-2. The competition among stellar submissions was so steep that this year's Top 10 actually contains 12 products, thanks to a couple of ties.

As challenging as 2020 has been for all of us, this tumultuous year has given birth to promising products and approaches for elucidating the complex world of biology. And even more than that, 2020 has shown that the scientific community, when faced with a shared problem, can rise to the challenge and come together to refocus, research, and innovate. Here, *The Scientist* presents the tools and technologies that make up this year's Top 10 Innovations.

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AbCellera
Celium™

In late March, biotech firm AbCellera hosted a call with 40 researchers to review the data they'd collected on potential antibodies against SARS-CoV-2. Using AbCellera's high-throughput microfluidics and single-cell analysis tools to probe samples of COVID-19 patients, the company's team had deciphered the genetic sequences encoding hundreds of antibodies that might treat the disease. Sifting through all of that data by hand was tedious, though, so the team fed it into Celium, a data visualization tool that intersects hundreds of high-quality data points for those antibodies to reveal which ones might work best in patients as a potential therapy. In real time, on the call, the researchers used Celium to probe those relationships and home in on the LY-CoV555 antibody that, months later, entered clinical trials as a possible COVID-19 treatment, says Maia Smith, a bioinformatics engineer at AbCellera and designer of Celium. "I think that kind of says it all."

Before Celium came on the market in 2017, scientists sending their samples to AbCellera for analysis would get back complex spreadsheets of data that were difficult to navigate, and it was hard to know where to start, Smith says. Using Celium, data are presented in a visual format and the tool "helps you identify the right molecule for your needs," Fernando Corrêa, a protein engineer at Kodiak Sciences in Palo Alto, California tells *The Scientist*. He's partnered with AbCellera to identify antibodies to treat retinal diseases, and says the company's package of microfluidics, single-cell analysis, and data visualization tool "streamlines the process of antibody discovery in a user-friendly manner."

KAMDAR: *"AbCellera's response to the pandemic underscores the real power of the Celium platform at the intersection of biology and AI to make new antibody discoveries at a blazing speed."*

ABCELLERA

THE JUDGES

PAUL BLAINEY
Associate professor of biological engineering at MIT and a core member of the Broad Institute of MIT and Harvard University. The Blainey lab integrates new microfluidic, optical, molecular, and computational tools for application in biology and medicine. The group emphasizes quantitative single-cell and single-molecule approaches, aiming to enable studies that generate data with the power to reveal the workings of natural and engineered biological systems across a range of scales.

CHARMION CRUICKSHANK-QUINN
Application scientist at Agilent Technologies. Previously, she was a postdoctoral fellow at the University of Colorado Denver - Anschutz Medical Campus, a research fellow at National Jewish Health in Denver, and a graduate student at the State University of New York at Buffalo, where she worked in the instrument center.

KIM KAMDAR
Managing partner at Domain Associates, a healthcare-focused venture fund creating and investing in biopharma, device, and diagnostic companies. She began her career as a scientist and pursued drug-discovery research at Novartis/Syngenta for nine years.

ROBERT MEAGHER
Principal member of Technical Staff at Sandia National Laboratories. His main research interest is the development of novel techniques and devices for nucleic acid analysis, particularly applied to problems in infectious disease, biodefense, and microbial communities. Most recently this has led to approaches for simplified molecular diagnostics for emerging viral pathogens which are suitable for use at the point-of-need or in the developing world. Meagher's comments represent his professional opinion but do not necessarily represent the views of the US Department of Energy or the United States government.

Editor's Note: The judges considered dozens of entries submitted for a variety of life science products by companies and users. The judging panel evaluated submissions with only basic instructions from The Scientist*, and its members were invited to participate based on their familiarity with life science tools and technologies. They have no financial ties to the products or companies involved in the competition. In this issue of* The Scientist*, any advertisements placed by winners named in this article were purchased after our independent judges selected the winning products and had no bearing on the outcome of the competition.*

2 Abbott ID NOW COVID-19 Test

Since 2014, Abbott's ID NOW system has helped physicians detect influenzas A and B, strep A, respiratory syncytial virus (RSV), and most recently SARS-CoV-2, in less than 15 minutes. The toaster-size device works by heating nasal samples in an acidic solution that cracks open the envelope of the viruses, exposing their RNA, which ID NOW amplifies at a constant temperature instead of the heating and cooling cycles that PCR machines use. Gaining emergency authorization from the US Food and Drug Administration in late March, the COVID-19 ID NOW test was one of the first tests accessible to the US public.

Norman Moore, Abbott's director of scientific affairs for infectious diseases, says the test's short turnaround time is critical to stopping viral spread. "You're the most infectious early on—and if we don't have that result in that timely fashion, what does it help if a molecular test comes back two weeks later?" he tells *The Scientist*.

With more than 23,000 ID NOW devices in use in the US, mainly in urgent care clinics and pharmacies, Moore says his team is developing tests compatible with the platform for other infectious diseases, such as sexually transmitted infections.

J.D. Zipkin, chief medical officer of GoHealth Urgent Care, which partnered with San Francisco International Airport to administer the ID NOW COVID-19 test to travelers, calls the test a game changer. "[Abbott] took a platform that's already really good at detecting very specific disease states and applied it to the biggest pandemic need that we have in this country," he says.

The ID NOW platform costs $4,500 and each COVID-19 test costs $40.

CRUICKSHANK-QUINN: "*The ability to receive COVID-19 test results from a throat or nasal swab in under 15 minutes can provide hospitals, schools, or any other institution with the ability to quickly test persons to determine those who would need to self-isolate at home. Since it is light-weight and portable it can be used in the field and at mobile sites like drive-thru testing locations.*"

ABBOTT

BioLegend TotalSeq™-C Human Universal Cocktail v1.0

In 2017, researchers from the New York Genome Center published a new approach called CITE-seq that allows scientists to assess proteins in individual cells at the same time they are doing single-cell transcriptomics. CITE-seq works by linking antibodies with oligonucleotides that can eventually be sequenced to reveal whether target proteins were present and joined to their corresponding antibodies. Life science company BioLegend licensed CITE-seq and developed the TotalSeq™-C Human Universal Cocktail v1.0, a collection of 130 oligo-linked antibodies for massive screening of the cell-surface proteins of individual cells, for use on a single-cell sequencing platform from 10X Genomics.

In contrast to proteomics approaches based on visual assessment of tagged proteins, "there's no theoretical limit anymore as to how many proteins you can [screen for]," says BioLegend's Head of Proteogenomics Kristopher "Kit" Nazor, adding that the company is already working to expand the number of antibodies included in the cocktail. "That increases the opportunity for unbiased discovery massively."

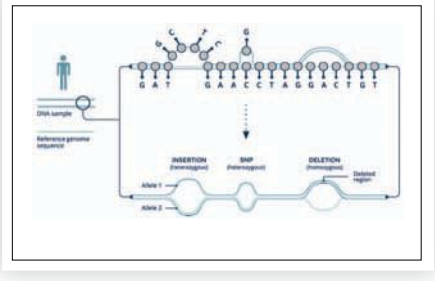
"It's groundbreaking in many ways," says immunologist and genomicist Alexandra-Chloé Villani of Massachusetts General Hospital, Harvard Medical School, and the Broad Institute of MIT and Harvard University. Like many researchers, Villani, who is one of the coordinators of the immune cell segment of the Human Cell Atlas, pivoted this year to studying COVID-19. She has already used BioLegend's cocktail, launched in early August at a price of $5,350 for five single-use vials, to analyze blood samples from nearly 300 patients who tested positive for SARS-CoV-2.

"When you have surface protein and RNA in the same cell, it really helps us to derive a more granular definition of the immune cells involved" in response to infection, says Villani. "I actually know a lot of colleagues across the United States and Europe that have used this same panel to analyze their COVID cohorts . . . which means we'll be able to combine all of our data and compare. And that's incredible."

MEAGHER: *"This is a really nice merging of next-gen sequencing as a digital readout for sequence barcodes and single-cell barcoding technology to enable single-cell quantitative proteomics."*

Seven Bridges GRAF™

The release of the human reference genome in 2013 was a tremendous leap forward for biology, but as far as actually representing humanity, it fell quite short. Our genomes are rife with variants not present in the reference genome, which was built from a small sampling of individuals, primarily of European descent. To account for human genetic diversity, bioinformatics firm Seven Bridges has developed a genomic analysis platform called GRAF that attempts to include all possible iterations of genetic sequences at any given locus. The resulting GRAF/Pan Genome Reference is a graph of the known variants at particular points in the genome, rather than a linear reference sequence. When genomes are aligned to the GRAF reference, any deletions, insertions, single nucleotide polymorphisms, or other variations are therefore not missed as they might be when aligned to the linear reference genome.



With the goal of boosting the presence of underrepresented groups in genomic research, Seven Bridges announced in June that access to its GRAF Germline Variant Detection Workflow and GRAF/Pan Genome Reference would be free to academic researchers. "This is the first production-grade workflow that incorporates ancestry information and diversity of the human genome to provide improved variant calls and alignment," says the company's chief scientific officer, Brandi Davis-Dusenbery.

"The hope is that, by accounting for that complexity in the analysis, you will see things you were missing," says Bruce Gelb, the director of the Mindich Child Health and Development Institute at the Icahn School of Medicine at Mt. Sinai. "That's been an idea floating around for a few years, but nobody prior to what Seven Bridges is doing implemented a graph-based approach that is practical. They're the first to do that."

Gelb has been using the GRAF platform to search for variants related to congenital heart defects and comparing those variants to what turns up when he uses traditional sequence analyses. So far, he says, it appears that GRAF is identifying some variants that would otherwise have been overlooked.

CRUICKSHANK-QUINN: *"The fact that Seven Bridges GRAF is being made freely available to academic institutions will certainly pave the way towards precision medicine by allowing research advancement in under-represented populations without the struggle of cost to academic researchers."*

BIOLEGEND; SEVEN BRIDGES

4 OXGENE TESSA

A central challenge to delivering gene therapies to patients' cells is the cost of making adeno-associated virus (AAV), a common vector for genes of interest, says Ryan Cawood, CEO of UK-based biotech company OXGENE. "The first AAV gene therapy product that was approved in the EU cost a million pounds per dose," he says. "If you wanted to treat a disease [with a therapy targeting a large organ] that you could apply to thousands of people, you just simply couldn't make enough of it at a cost that would make it viable."

Currently, Cawood says, batches of cultured human cells are transfected with multiple plasmids to induce them to make the AAV vectors containing a selected gene. But the plasmids are expensive to make, and the transfection process isn't very efficient. By contrast, infection with adenoviruses naturally induces cells to activate replication of AAVs. The problem is, the adenoviruses also replicate themselves and contaminate the resulting AAV product. To get around this issue, OXGENE devised a genetic switch that shuts down an adenovirus's activity halfway through its life cycle within a cell, so that it programs the cell to churn out AAV particles but not to make adenovirus. "When the virus goes in, you only get AAV coming out; you don't get any more of the adenovirus coming back out," Cawood says. The company began selling its research-grade viral vector, which it calls TESSA, in September, and plans to begin offering clinical-grade material next year, he adds. The cost for the research-grade vector starts at £5,000, and depends on the size of the batch of cells to be infected.

BLAINEY: *"Supports translation of gene therapies. Demonstrates the biotechnical value of biological engineering."*

5 Codex DNA BioXp™ 3250 System

Biotech firm Codex DNA released the BioXp™ 3250 system in August 2020 as a follow-up to BioXp™ 3200, released in 2014. The automated platform for on-demand DNA assembly and amplification allows researchers to synthesize genes and genomes faster than ever, with the potential to accelerate the development of vaccines, diagnostics, and treatments, says Peter Duncan, director of product management at Codex DNA. The equipment can be used on cancer cells or a variety of infectious agents, including SARS-CoV-2.

Without BioXp™ 3250 or its predecessor, labs that want to synthesize DNA fragments, clones, or whole genomes have to send samples out to be processed by a third party. In addition to having to deal with transit, such processing could take weeks or months. With the BioXp™ 3250, priced at $100,000, DNA sequences up to 7,000 base pairs in length can be assembled in a matter of days, with the push of a button.

Rather than having to code genetic script on a computer for specific experiments, customers can order a module that comes in about two days, ready to go. The module has a barcode containing all the necessary information; when scanned by the device, instructions for synthesizing the desired DNA are uploaded. A lab technician merely needs to insert the module into the device and press start, Duncan says.

"The BioXp has enabled us to perform simple subcloning steps hands-free," Mark Tornetta, VP of Biologics Discovery at Tavotek Biotherapeutics, tells *The Scientist* in an email, describing how the lab uses the device to generate NGS libraries. "All of these methods [that are run] on the BioXP save us time and cost to perform."

BLAINEY: *"Democratizing gene synthesis by placing capability in individual labs for faster turnaround and lower costs at high throughput."*

OXGENE; CODEX DNA

6

IsoPlexis
Single-Cell Intracellular Proteome

The Single-Cell Intracellular Proteome solution from IsoPlexis grew out of several labs at Caltech, all seeking better ways to monitor protein-protein interactions in cancer cells with the goal of developing targeted treatments. With traditional methods such as Western blot, mass spectrometry, and flow cytometry, only a couple of protein types can be tracked at a given time. With Isoplexis's system, launched in July, researchers can monitor 30 or more protein pathways, with results available on the same day.

With previous technology, phosphorylation was used to identify the function of the individual proteins, with no insight as to how they work together. The Single-Cell Intracellular Proteome reveals the function the same way, but is also able to provide the context of entire protein signaling pathways, uncovering how the network operates as a whole.

Understanding the entire network of cellular pathways allows researchers to better understand the downstream effects of aberrant cells, says Sean Mackay, CEO and cofounder of IsoPlexis. In cancers, he adds, this approach helps evaluate the efficacy of targeted treatments such as antibody therapies or small-molecule drugs.

"Such pathways basically define how cells are activated, [which] is particularly important for cancer, where activated phosphoprotein signaling is not only a hallmark of cancer," says James Heath, who ran the Caltech lab that created the technology eight years ago, "but is a major focus of targeted inhibitors."

MEAGHER: *"The Single-Cell Intracellular Proteome solution uses innovative microfluidics to scale down what looks like well-established ELISA chemistry down to the level of single cells."*

7

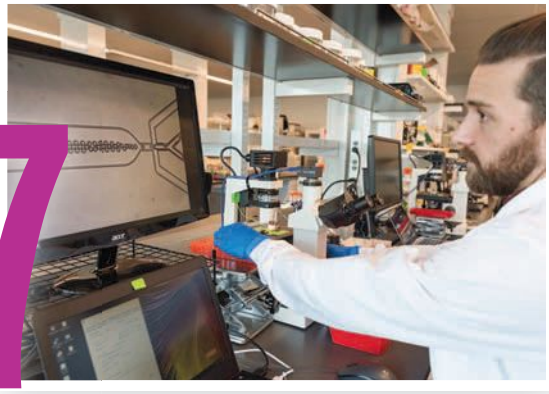
GigaGen
Surge

Scientists have used intravenous immunoglobulin (IVIG) to treat immunodeficient or immunosuppressed patients and convalescent plasma to treat infectious diseases for more than a century. And plasma is one of many treatments now being tried for COVID-19. But biological samples drawn from donors are not the most standardized therapeutics. Enter GigaGen's Surge platform, which uses single-cell sequencing to "capture and recreate" libraries of antibodies from plasma donors. To create these libraries, the company runs donors' blood samples through the Surge platform to isolate individual antibody-producing B cells into microdroplets and extract the RNA that encodes the antibodies. From these genetic sequences they can create a "blueprint of that person's immune system," says GigaGen CEO David Johnson.

Company researchers then select some of those antibodies to engineer in mammalian cells to create a recombinant antibody treatment, which they say is much more potent than convalescent plasma or IVIG, based on in vitro experiments and tests in animal models. GigaGen does not currently plan to sell Surge, but rather has been using the platform to develop treatments for cancers, immunodeficiency disorders, and, most recently, COVID-19. GigaGen hopes to start clinical trials on their COVID-19 treatment, which uses more than 12,500 antibodies from 16 donors, in early 2021. The goal of Surge is to "tease apart the complexity of the immune system," says Johnson, and then tailor antibody treatments that elicit the strongest response.

Fred and Vicki Modell, who founded the Jeffrey Modell Foundation after their son Jeffrey died at 15 due to complications from primary immunodeficiency, say they have been searching for an alternative to IVIG, which is sometimes in short supply and can lead to side effects in many patients. "[GigaGen] is giving the greatest gift of all—they're giving hope to [immunodeficient] patients," Fred Modell says.

CRUICKSHANK-QUINN: *"By combining single-cell emulsion droplet microfluidics technology, genomics, and protein library engineering, this antibody drug therapy, if successful, could revolutionize COVID-19 treatment as well as treatments for many different diseases."*



ISOPLEXIS; GIGAGEN

10X Genomics Chromium Single Cell Multiome ATAC + Gene Expression

A few years ago, 10X Genomics launched an assay, ATAC-seq, to identify regions of open chromatin in single cells; the product won a spot in *The Scientist*'s 2019 Top 10 Innovations. According to product marketing manager Laura DeMare, it wasn't long before customers were clamoring for more, with feedback to the effect of, "'This is great, but we'd really love to get the gene expression information and the ATAC-seq information in the same cell.'" In September, 10X rolled out Chromium Single Cell ATAC + Gene Expression, which harvests both epigenetic and gene expression data from individual nuclei.

The platform tags mRNA and open chromatin fragments from each nucleus with DNA barcodes, DeMare explains, and the nucleic acids are then amplified and analyzed. "You can begin to actually link which regulatory elements in the genome are turning on or off genes," she says. It costs approximately $2,400 per reaction for the reagents and a microfluidic chip.

Ansu Satpathy, an immunologist at Stanford University School of Medicine and a former postdoc of ATAC-seq codeveloper Howard Chang, tells *The Scientist* that he's using the new assay to investigate the effects of epigenetic changes associated with T cell exhaustion in tumor samples biopsied from cancer patients. When exhausted, T cells become less effective at battling cancer, and "what we're doing now with the RNA and ATAC method combined is asking, How do each of those molecular switches regulate genes that lead to this dysfunctional outcome in the cell?" Satpathy says.

KAMDAR: *"This approach allows, for the first time, the simultaneous profiling of the epigenome and transcriptome from the same single cell, enabling a better understanding of cell functionality."*

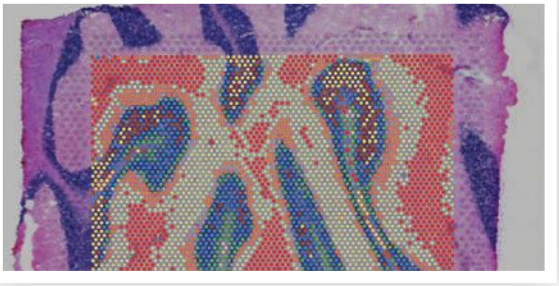
10X Genomics Visium Spatial Gene Expression Solution

Over the last several years, single-cell transcriptomics has provided a wealth of gene expression information for individual cells and cell types. Now, 10X Genomics advances the newer technology of spatial transcriptomics, which provides whole transcriptome data for just one or a few cells, and reveals exactly where in a tissue sample that gene expression is taking place. The Visium Spatial Gene Expression Solution, launched in October 2019, exposes 55-micrometer areas at 5,000 locations within a tissue sample to mRNA-binding oligonucleotides, and overlays the resulting gene expression data with histological images.

The technology was developed and originally marketed by Swedish company Spatial Transcriptomics, which 10X Genomics acquired in 2018. Then 10X developed the product further before launching Visium last year. The Visium Spatial Gene Expression Solution, which sells for $1,000 per sample, has smaller and more densely packed spots—and five times more of them—than it did when the company inherited it, says Nikhil Rao, 10X Genomics's director of strategic marketing for the spatial platform. This increases resolution, he explains. "We also improved the sensitivity of the assay dramatically, being able to pick up tens of thousands of unique molecular identifiers per spot."

Rao says that many of Visium's users focus on neuroscience, studying neurodegenerative diseases, for example. But the product is also being used in developmental biology, oncology, and immunology. Johns Hopkins University computational biologist Elana Fertig has used Visium to understand how a cancer can resist treatment. "By virtue of having the spatial information of these cells, you can really figure out the molecular mechanisms where they interact directly, because you can see if the cells are interacting physically," she explains.

MEAGHER: *"This is another frontier in biology: not just single-cell or few-cell gene expression, but now collecting gene expression data with spatial resolution at the level of a few cells."*



10X GENOMICS

Inscripta, Inc.
Onyx™ Digital Genome Engineering Platform

While CRISPR-based genome editing has become a widely used technique in labs all over the globe, there are research questions that require a scale of nucleotide tinkering that can be cumbersome, if not prohibitive, for some labs. Inscripta Inc.'s Onyx™ Digital Genome Engineering Platform offers a solution—fully automated genome-engineered libraries with hundreds of thousands of single edits in microbial genomes. The benchtop device, which launched in October 2019 and sells for $347,000, allows users to plant desired variants in the DNA of *E. coli* bacteria and *S. cerevisiae* yeast, and the instrument takes care of the rest.

The platform combines everything from the algorithms for optimizing the editing process to the microfluidics for handling cells to the reagents themselves. "Biologists don't have to worry about the technical optimization anymore and can go ahead and focus on any problem in biology now," says Nandini Krishnamurthy, the vice president of applications development at Inscripta.

Shelley Copley, a molecular biologist at the University of Colorado Boulder, is an early tester of Onyx. She's using it to examine the effects of synonymous mutations, those that don't change the resulting protein, on fitness in *E. coli*. "The high-throughput part of it is critical to be able to address this," she says. Rather than attempt to engineer each mutation she wants to examine one by one, Onyx enables Copley to generate all 50,000 variants. Her team can then move straight to the fitness assays. "I don't know of any other technology that can do it."

KAMDAR: *"CRISPR is a powerful tool for editing genomes and allowing functional assessments that can elucidate causality and improve our understanding of genome biology. But those outcomes will not be achieved without overcoming a number of the technical and scalability challenges. This is what the Onyx Digital Genome Engineering Platform enables."*

MOBILion
SLIM

John McLean, a bioanalytical chemist at Vanderbilt University, wants to know exactly what's in a puff of gas, down to a vaporized blood or tissue sample's very last lipid molecule. For years, he has used mass spectrometry to catalog compounds in a sample by weight. Sometimes different molecules can have the same mass and the same atomic composition, making it hard to distinguish them. Ion mobility separation runs gas samples down meter-long tubes to differentiate molecules by shape and structure, getting around the mass issue. But because the technique was designed decades ago, it hasn't achieved the same resolution as mass spectrometry. To achieve a similar resolution, the ion separation instrument would need a 13-meter tube.

Making a linear tube that length is impractical due to constraints on lab space. So a few years ago, Richard Smith of Pacific Northwest National Laboratory and colleagues began brainstorming ways to get ions to turn corners. That discussion led to the development of MOBILion's SLIM, or Structures for Lossless Ion Manipulation, an instrument with a 13-meter track cut as switchbacks in two circuit boards that fit in a 3-meter-long box; the device provides data on the size and shape of compounds in samples in minutes. SLIM "reveals the unseen," says Laura Maxon, MOBILion's head of business development and corporate strategy, "without the sacrifice of time." This first iteration of SLIM, which MOBILion began deploying as a Beta version to early adopter collaborators the second quarter of 2020, is built for scientists in a pharmaceutical or clinical research academic environment. The price is competitive with existing technologies, she notes, and the company plans to design the instrument for use in the clinic to identify biomarkers of disease.

"What we're seeing today, from MOBILion on SLIM, is just the tip of the iceberg," McLean says. "There's a lot of untapped potential . . . from an analytical standpoint," so "people should really expect huge advances for these technologies."

BLAINEY: *"Ion-selective chromatography is central to biochemistry. Nice integration of microelectronic technology with biotechnology."*

DANIEL JEFFREY STUDIOS; INSCRIPTA, INC.

LAB TOOLS
THE SCIENTIST WEBINAR SERIES

ONDEMAND | Genetic and Spatial Heterogeneity in Human Papillomavirus-Associated Oropharyngeal Cancer

Head and neck cancers arising from the upper aerodigestive tract are the sixth leading cause of cancer-related mortality, with over 550,000 new cases per year worldwide. Though it continues to be prevalent, the etiology of oropharyngeal cancer (OPC; cancer of the tonsil and base of tongue) has completely changed in the last 30 years. Now, human papillomavirus (HPV) is the leading cause of OPC. While patients with viral OPC tend to be younger and have a superior responses to treatment and better prognoses compared with non-viral-OPC patients, the biological differences between these cancers are not well understood due to the paucity of genomic data in the viral-OPC population. The underlying genetic drivers of diverse cancer cell phenotypes, or "tumoral heterogeneity," affect clinical outcomes but have not been studied in detail.

In this webinar sponsored by 10x Genomics, Joseph Powell discusses how heterogeneous subpopulations of HPV+ head and neck cancer cells drive unique disease states, cell–cell interactions, and microenvironment dynamics, and have implications for cancer behavior, metastasis, and response to treatment.

JOSEPH POWELL, PHD
Associate Professor
Garvan Institute of Medical Research

ORIGINALLY AIRED TUESDAY, SEPTEMBER 8, 2020

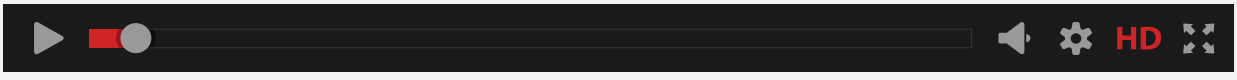
WATCH NOW!
www.the-scientist.com/oropharyngeal-cancer-10x

TOPICS COVERED

- How tumoral heterogeneity in head and neck cancer affects clinical outcomes
- How to study heterogeneous subpopulations of HPV+ cancer cells using single-cell and spatial techniques

WEBINAR SPONSORED BY

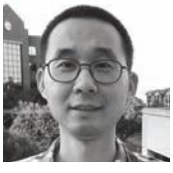
10x GENOMICS



TS WEBINARS

ONDEMAND | How Cancer Evades the Immune System

The body's first line of defense against cancer is the immune system. Yet many tumors evade the immune system and even recruit key immune cells to aid in tumor development. In this webinar, brought to you by *The Scientist* and sponsored by 10x Genomics and Codex DNA, Chuanhui Han discusses how cancer avoids immune system attack after radiation treatment, and Vineet Gupta explores how cancer tricks immune myeloid cells into promoting tumor growth. The speakers also review therapeutic approaches for preventing cancer's manipulation of the immune system.



CHUANHUI HAN, PHD
Postdoctoral Researcher
Laboratory of Yang-Xin Fu, MD, PhD
UT Southwestern Medical Center

VINEET GUPTA, PHD
The Charles Arthur Weaver Chair of Cancer Research
Vice Chair for Innovation, Department of Internal Medicine
Director, Drug Discovery Center
Rush University Medical Center

ORIGINALLY AIRED WEDNESDAY, SEPTEMBER 16, 2020

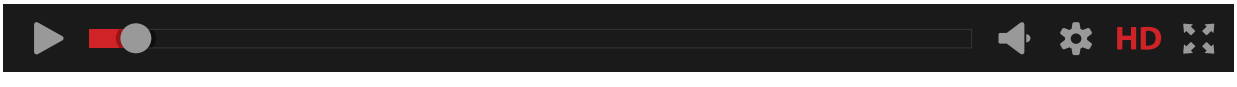
WATCH NOW!
www.the-scientist.com /cancer-evades-the-immune-system

TOPICS COVERED

- Caspases: The mystery of radiation
- Integrin activation as a novel therapeutic strategy against cancer

WEBINAR SPONSORED BY

10x GENOMICS CODEX DNA



ONDEMAND | Unpacking the Genetic Contribution of Glia to Parkinson's Disease

LAB TOOLS THE SCIENTIST WEBINAR SERIES

Parkinson's disease is the second most common neurodegenerative disorder. It is characterized by misfolded alpha-synuclein deposits and dopaminergic neuron death, which lead to progressive motor impairment and disability. Despite extensive efforts, there are no disease-modifying therapies available for Parkinson's disease or related "alpha-synucleinopathies." Glia may represent a source of untapped therapeutic potential.

In this webinar sponsored by BioLegend, Abby Olsen, Associate Neurologist at Brigham and Women's Hospital, discusses how an innovative *Drosophila* model helps explore the genetic contribution of glia to Parkinson's disease pathogenesis. She reviews how forward genetic screens identify novel glial genes and potential therapeutic targets for downstream investigation in mammalian systems and patients.

ABBY OLSEN, MD, PHD
Associate Neurologist
Brigham and Women's Hospital
Instructor in Neurology
Harvard Medical School

ORIGINALLY AIRED THURSDAY, SEPTEMBER 17, 2020

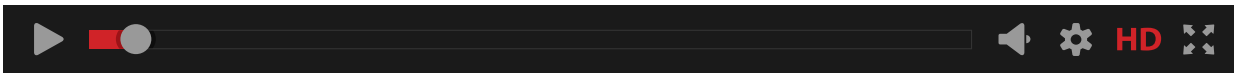
WATCH NOW!
www.the-scientist.com/Glia-in-Parkinsons-Disease

TOPICS COVERED

- A *Drosophila* model of neurodegenerative alpha-synucleinopathies
- The role of alpha-synuclein in glia
- The unique transcriptional signature of alpha-synuclein in glia in Parkinson's disease
- The pathogenic effects and mechanisms of Parkinson's disease candidate genes when expressed in the glia
- Genetic screens to identify novel glial genes and potential therapeutic targets

WEBINAR SPONSORED BY

BioLegend®



ONDEMAND | Optimizing Lab Ultrafiltration Workflows: From Molecule Separation to Diagnostics

LAB TOOLS THE SCIENTIST WEBINAR SERIES

Ultrafiltration techniques are becoming increasingly critical in research and process applications. With the wide range of lab-based research applications and molecules in life science, environmental, clinical, and other industrial sectors, it is important to understand both the shared and specific key principles and methods needed to optimize ultrafiltration procedures. In this webinar sponsored by Sartorius, experts explain how optimization allows researchers to maximize user-specific results and reduce specimen-to-report timelines.

ADAM GREEN
Manager, Lab Filtration Product Management
Sartorius

KLAUS SCHOENE
Lead Application Scientist
Sartorius

ORIGINALLY AIRED MONDAY, SEPTEMBER 21, 2020

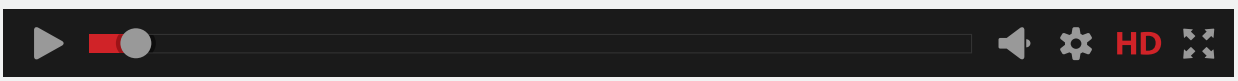
WATCH NOW!
www.the-scientist.com/ultrafiltration-workflows-sartorius

TOPICS COVERED

- The latest guidance on innovative methods, products, tools, and considerations needed to optimize lab ultrafiltration workflows
- How cell culture molecule workflows can be improved

WEBINAR SPONSORED BY

SARTORIUS



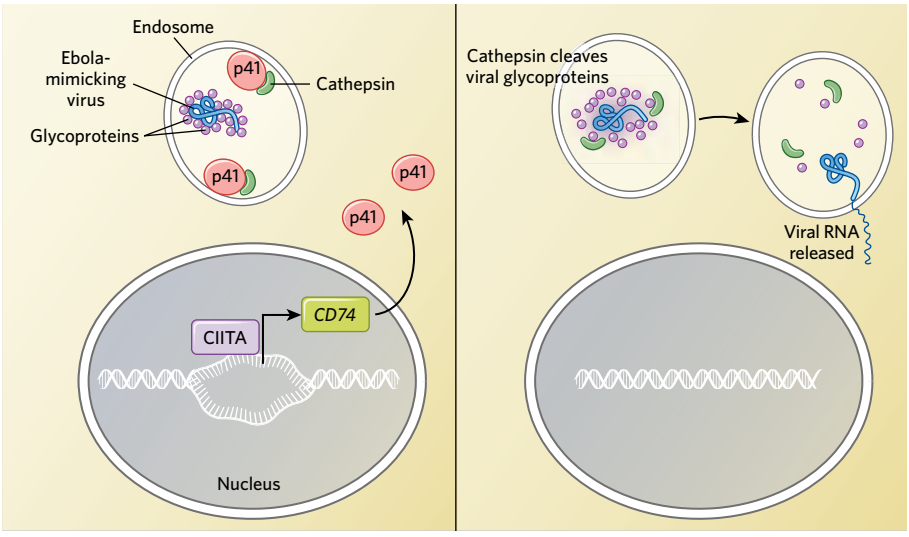
The Literature

IMMUNOLOGY

Viral Defense System

THE PAPER

A. Bruchez et al., "MHC class II transactivator CIITA induces cell resistance to Ebola virus and SARS-like coronaviruses," *Science*, 370:241–47, 2020.



TRAP AND KILL: Overexpressing the transcription factor CIITA in cultured human cells turns on a gene called *CD74*, producing the protein p41, which binds to cathepsin proteases in the endosome (left). When the cells are exposed to an Ebola-mimicking virus, the p41-bound cathepsins are unable to cleave off the virus's glycoprotein, stopping it from fusing with the membrane and thus trapping it inside the endosome. Later, the virus is likely brought to lysosomes and destroyed (not pictured). In a cell where *CD74* is not overexpressed (right), cathepsins cleave the virus's glycoproteins, enabling it to fuse with the side of the endosome and release its genetic material into the cytosol.

Earlier this year, immunologist Adam Lacy-Hulbert of the Benaroya Research Institute in Seattle and his former postdoc Anna Bruchez were writing up their discovery of a previously unknown immune pathway that defends cells against Ebola virus. Then SARS-CoV-2 hit the US. The two suspected that the pathway provided broad antiviral defense, so they decided to test it against the novel coronavirus.

In the Ebola experiments, Lacy-Hulbert, Bruchez, and their colleagues had been using a genetic screen called transposon-mediated gene activation to search for natural antiviral mechanisms within cultured human bone cancer cells. Transposons, mobile genetic elements found throughout the genome, can be added to cells to knock out genes they randomly insert into. The team had integrated a promoter sequence into the transposons so that, in addition to knocking out some genes, they would turn other genes on. After adding these transposons to flasks of human cells, Bruchez introduced viruses engineered to express an Ebola glycoprotein, killing most of the cells. The team genotyped the few cells that remained and discovered two genes that were crucial to the cells' survival: *NPC1* and *CIITA*.

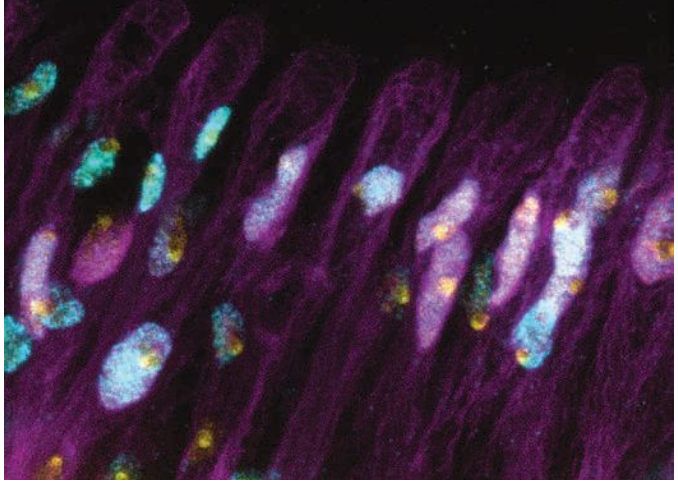
NPC1 encodes the receptor that Ebola virus binds to, but *CIITA* was more mysterious. It encodes a transcription factor that regulates major histocompatibility complex (MHC) genes, which code for the cell surface proteins that present foreign substances to adaptive immune cells. But the team's cultures lacked adaptive immune cells (such as T cells), suggesting a more primitive type of defense was occurring. To find out what it was, the team knocked down each of the genes that *CIITA* regulates, and found one, *CD74* (which encodes part of the MHC), that was key to cell survival. One isoform in particular, *p41*, could keep *CD74* knockout cells alive in the face of the virus with the Ebola glycoprotein. Electron microscopy showed that, in cells expressing *p41*, the glycoprotein remained trapped inside the endosomes that housed the engineered viruses after they were internalized by the cell. The p41 protein binds to proteases called cathepsins, preventing the enzymes from cleaving the Ebola glycoprotein, thus stopping the fusion of the virus with the endosome and the release of the viral genome into the cell, the team demonstrated.

The group found that p41 inhibited entry of SARS-CoV-2 into the cells as well. Lacy-Hulbert suggests that this pathway could trigger broad resistance to viruses and that this "might have been *CIITA* and *CD74*'s original role. Then that activity got co-opted into the adaptive immune system, which evolved later."

Virologist Yong-Hui Zheng of Michigan State University says the study makes a strong case that *CIITA* and *CD74* mitigate Ebola infection in vitro via cathepsins. But he notes that a 2012 study in mice showed that knocking out cathepsins does not prevent Ebola infection, and he questions the importance of the *CIITA/CD74* pathway as a primary antiviral mechanism in animals and humans. In addition, SARS-CoV-2 does not depend solely on cathepsins to infect cells, he notes. But Lacy-Hulbert says it's likely the viruses use different proteases for entry depending on the cell type they're infecting, and it's possible that *CIITA* and *CD74 p41* can block other proteases as well. He adds, "the pathways we have identified are likely to be important in animals and humans, but may need to act in combination with other pathways."

—Rachael Moeller Gorman

STUCK IN AMBER: The record-setting sperm was found in a new ostracod species called *Myanmarcypris hui*.



PACKED FULL: When exposed to oxidative stress, *Drosophila* brain cells (glia shown above) can develop more than two sets of chromosomes—a state that may protect the brain from damage.

PALEONTOLOGY

Oldest Sperm

THE PAPER

H. Wang et al., "Exceptional preservation of reproductive organs and giant sperm in Cretaceous ostracods," *Proc R Soc B,* 287:20201661, 2020.

Something giant lingers in a tiny piece of amber the size of a postage stamp. It's the world's oldest sperm, and it's relatively big—nearly five times longer than the creature from which it came.

The sperm dates back 100 million years and belongs to an ancient ostracod, a relative of millimeter-long crustaceans still alive today. It's almost 50 million years older than the previously catalogued oldest sperm, according to Nanjing University paleontologists He Wang and Bo Wang, who studied it.

Timing was key to the perfect preservation of the sperm, which is rarely fossilized. The researchers found a tangled clump of sperm and four tiny eggs in a female that was petrified in amber along with 38 other females, males, and juveniles, belonging to a genus of ostracods that had not been identified before. Because the sperm was still inside the female, she must have mated "maybe a matter of minutes or hours before" tree resin fell and trapped her, study coauthor David Horne, a paleontologist at Queen Mary University of London, tells *The Scientist*.

Not only was finding the sperm surprising, but so was how much sperm was in the female. Modern ostracods produce the largest volume of spermatozoa in the animal kingdom. As it turns out, ancient ostracods also produced really large sperm a hundred million years ago. Producing voluminous sperm, even though it takes a lot of energy, can physically block other sperm from penetrating the egg. And it appears to have been an evolutionarily advantageous strategy of reproduction for longer than researchers thought, the scientists say.

"There are a couple of categories of stories that gather a lot of attention in paleontology: one is exceptional preservation and the other is weird sex stuff," says Gene Hunt, a paleobiologist at the Smithsonian National Museum of Natural History who was not involved with the study. "This is a perfect intersection of those two kinds of stories."

—Max Kozlov

CELL BIOLOGY

Old Fly Brains

THE PAPER

S. Nandakumar et al., "Polyploidy in the adult *Drosophila* brain," *eLife*, 9:e54385, 2020.

Over a lifetime, mature brain cells face a gauntlet of oxidative stress, DNA damage, and other dangers that can lead to neurodegeneration. In response, *Drosophila*'s brain cells acquire additional sets of chromosomes beyond the normal two. These chromosome-packed cells appear to be more resistant to cell death, suggesting polyploidy plays a protective role in the fly brain, University of Michigan molecular cell biologist Shyama Nandakumar and colleagues found.

Although the researchers knew that cell damage can lead to the accumulation of additional sets of chromosomes in some cases, such as in the human liver and in the brains of patients with Alzheimer's disease, not much was known about the mechanism behind it or what its function could be.

To study polyploidy, Nandakumar and the rest of the team in Laura Buttitta's lab used a brain dissection technique developed by teammate Olga Grushko, along with highly sensitive flow cytometry. When aging fly brains were exposed to oxidative stress, cells in certain areas such as the optic lobes were more likely to obtain extra chromosome sets than cells in other spots. The cells acquired the extra sets after reverting back into the cell cycle. And notably, as the number of polyploid cells in an area grew, the rate of local cell death declined.

The findings came as a surprise, Nandakumar notes. "It took us a while to convince ourselves that what we were observing was actually true."

The study "fits with the emerging theme that polyploidy emerges as a response to tissue stress," Donald Fox, a genomicist at Duke University Medical Center who was not involved with the research, writes in an email to *The Scientist*. "And the fact that these polyploid cells are in the brain raises really interesting questions about the role of polyploidy in aged/stressed neural circuits, and how this might impact behavior."

—Lisa Winter

HE WANG & XIANGDONG ZHAO, NANJING UNIVERSITY; NANDAKUMAR ET AL., 2020

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TheScientist

Gloria Echeverria: Cracking Cancer

Assistant Professor in the Department of Medicine, Baylor College of Medicine

BY MAX KOZLOV

As a little girl, Gloria Echeverria didn't want to be the president or a firefighter. Instead, she promised her mother that she would one day run the US Centers for Disease Control and Prevention. Her grade-school teachers encouraged her interest in science, Echeverria tells *The Scientist*. "Perhaps it subconsciously played some kind of role, seeing these awesome women who were so knowledgeable and passionate about the subject."

Echeverria enrolled at Texas A&M University in 2004 with her sights set on a scientific research career. By her sophomore year, she was investigating how beneficial fungal organisms that grow on the roots of plants can be genetically enhanced to protect crops from pathogens (*Mol Plant Pathol*, 8:469–80, 2007). While wrapping up her undergraduate training in biochemistry and genetics, Echeverria began to apply for PhD programs, and she had a new focus—biomedical science. "I really wanted to take that knowledge from the plant pathology lab, and I wanted to apply it to something that I thought might have a nearer-term impact on human health and well-being," she says.

In 2008, she joined Thomas Cooper's lab at Baylor College of Medicine in Houston and studied how RNA splicing can go haywire in patients with the genetic mutation that causes the muscular disease myotonic dystrophy type 1 (*Nucleic Acids Res,* 39:2769–80, 2011). After completing her PhD in 2013, Echeverria moved across town to Helen Piwnica-Worms's lab at MD Anderson Cancer Center to study another disease she'd heard about in her graduate studies, triple negative breast cancer (TNBC).

Up to 15 percent of all breast cancers are TBNCs. Scientists gave the cancer that name because the malignant cells don't have estrogen or progesterone receptors and don't overexpress the HER2 protein; all three are therapeutic targets in breast cancer. TNBC is particularly hard to treat because it is "defined by what it lacks, not by what it is," Echeverria says. "One of the big challenges with triple negative is it's very heterogeneous," meaning that each tumor can have different types of malignant cells within it and that no two individuals' TNBCs are the same, she explains. "It's kind of like the leftover bucket for all the cancers that don't have the things that we understand well."

To determine how TNBC's high heterogeneity contributes to metastasis, Echeverria, Piwnica-Worms, and their colleagues inserted distinct genetic labels called DNA barcodes into the different malignant cell types of patient-derived cancer samples, and then transplanted the cells into the mammary glands of mice (*Nat Comm*, 9:5079, 2018). Using the barcodes to track how the cancer cells spread, Echeverria identified a subset that effectively metastasized and colonized other organs. "What sets great trainees apart from others are those who are fearless and willing to embrace new technologies [such as DNA barcoding] to answer key questions," Piwnica-Worms says, and Echeverria is not only fearless but has a "great intellect."

Using the same barcoding method, Echeverria next tested the cancer cells' responses to chemotherapy and found that the mice's tumors "got so small it got hard to measure them," she says. But once the team halted the treatment, the tumors' growth started skyrocketing again. "We found that we could give tons and tons of chemotherapy over and over again, and we could never prevent them from regrowing," says Echeverria.

She and her colleagues published the results in 2019, detailing how the leftover tumors survived the chemo treatment. The cancer cells entered a defensive state, the team found, in which the cells' metabolism shifted to oxidative phosphorylation rather than glycolysis, providing them with an abnormally high amount of energy. An oxidative phosphorylation inhibitor delayed regrowth of the tumors, the team found (*Sci Transl Med,* 11:eaav0936). The inhibitor is in Phase 1 clinical trials.

Echeverria started her own lab at the Baylor College of Medicine in January, and with the help of a $2 million First-Time, Tenure-Track Faculty Member Award from the Cancer Prevention and Research Institute of Texas, she will continue her study of TNBC metastasis and the cancer's resistance to treatment. "She's been working her whole life to get to this point now to be an independent PI [principal investigator] and run her own program," says Piwnica-Worms. "She picked a really important problem and a really important area to investigate, [cancer] metabolism." ■

AGAPITO SANCHEZ JR., BAYLOR COLLEGE OF MEDICINE

Space Drugs

Researching and developing drugs in microgravity could lead to better treatments. But will it ever be worth the cost?

BY KATARINA ZIMMER

On a cool December afternoon in 2018, on a viewing platform at the Kennedy Space Center at Cape Canaveral in Florida, Jordan Greco watched his research project leave planet Earth. As chief scientific officer of the Connecticut-based biotech LambdaVision, he had spent years developing a protein-based artificial retina to treat patients blinded or severely visually impaired by retinal degenerative diseases. At 1:15 PM that day, a Falcon 9 launch rocket lit up the sky as it blasted the SpaceX Dragon cargo spacecraft toward the International Space Station (ISS), carrying onboard the proteins that make up Greco's artificial retina.

"It didn't really hit me until we were sitting on the balcony at the NASA complex and seeing that rocket off in the distance," Greco recalls. "Our protein, our experiment that we've been working on for years, is on that thing."

Once the SpaceX capsule docked at the ISS, an astronaut in the station's near-weightless environment was to initiate an experiment that Greco hoped would help him understand how to improve the artificial retina's function. Back on Earth, he and his colleagues had been making progress with the retina—essentially a small film covered in hundreds of layers of the microbial light-activated protein bacteriorhodopsin—but were struggling to produce consistently high-quality retinas.

The team suspected that the bacteriorhodopsin proteins should be oriented the same way with respect to one another for the artificial retina to create robust electrical signals and communicate effectively with patients' neurons. But the team's process of dipping the film into protein solutions seemed to generate somewhat disordered protein arrangements. Greco suspected that gravity was negatively affecting the layering process—for instance, by causing the proteins in the solution to undergo sedimentation, he explains. To test that hypothesis, he and his colleagues sent materials to the ISS to repeat part of the experiment in microgravity.

Scientific research in space has thrived over the past decade, but it's only recently that the pharmaceutical and biotech sector has started getting in on the action, pursuing new ways to study drugs and other medical treatments. Pharma giants including Merck, AstraZeneca, Eli Lilly, and Sanofi, along with dozens of smaller companies, have all sent experiments to the ISS to reap the unique benefits of microgravity. Of the 150 or so life science research projects supported in the 2019-2020 fiscal year by the Center for the Advancement of Science in Space (CASIS)—a nonprofit that collaborates with NASA to manage the US National Laboratory on the ISS—more than a third have been led by pharmaceutical and biotechnology companies, says CASIS's interim chief scientist, Mike Roberts.

Such endeavors could one day help improve astronaut health and equip humanity for longer ventures into space, but their primary aim is to develop or improve drugs for people on Earth. That's certainly the hope of Greco and his col-

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leagues, who found out a few months after that December afternoon that, as they'd hypothesized, the proteins layered in space appeared to have more-orderly arrangements—an improvement that could benefit the artificial retina's function.

Studies such as these have yet to yield new blockbuster drugs or even significant improvements to existing ones. Research in space is slow, and the costs are sky-high. All projects are subsidized through NASA, and many rely on additional financial support through federal grants, spurring a new kind of space race—one aiming to prove that such projects are profitable enough for the private sector to fund on their own. "Overcoming that 1G gravitational pull to get rockets up to low Earth orbit or beyond is expensive still," says Roberts. But even so, "we've seen a significant uptick in interest" in conducting experiments in space.

Microgravity's perks

While microgravity can be achieved for a few moments on an aircraft rounding the top of a parabolic flight, or simulated imperfectly in bioreactors on Earth, the best way to conduct experiments under sustained microgravity is to go to the ISS. The station orbits approximately 400 km from the planet's surface and is close enough to Earth to experience about 90 percent of its gravitational pull, but astronauts aboard the station feel nearly weightless because it's in constant free fall around the planet.

The resulting microgravity conditions in this setting influence scientific experiments in many ways that appeal to drug developers. There are minimal convection currents in fluids, for instance, and hardly any sedimentation—conditions advantageous not only for LambdaVision's layering procedure but also for processes such as protein crystallization, whereby proteins form a regular array. Under near weightlessness, "you get a [higher-quality] crystal than [what you'd get through] the crystallization process on Earth," making certain proteins easier to study and more attractive as drugs, explains Marlise dos Santos, an aerospace pharmacy specialist at InnovaSpace, a UK-based think tank that promotes life science in space, among other activities related to extreme environments.

Paul Reichert, a research scientist at Schering-Plough and at Merck after their merger, was one of the first in the pharmaceutical industry to recognize the value of near weightlessness for protein crystallization. In the 1990s, before the ISS was operational, he collaborated with NASA to send interferon alfa-2b, the active ingredient in the company's antiviral and cancer drug intron A, into low Earth orbit on the Space Shuttle to see if it would crystallize in space. Upon studying the product that was returned to Earth, Reichert noticed that the protein had turned into small crystals with perfectly uniform size—the kind that would be ideal for drug delivery.

Although the crystallized interferon alfa-2b was never commercialized, Reichert has conducted similar experiments on the ISS with the monoclonal antibody pembrolizumab, the key ingredient in Merck's popular oncology drug Keytruda. Because antibodies aren't very soluble under standard conditions, treatments such as Keytruda tend to form viscous solutions at high concentrations and need to be delivered in burdensome, lengthy, and regular intravenous infusions. If pembrolizumab took the form of a compact crystalline suspension, however, it could be deliverable as an injection, Reichert explains. In his most recent experiment, published in *npj Microgravity*, he and his colleagues found that cooling pembrolizumab on the ISS yielded "a uniform population of particles [that] actually gave a better injectability profile than the heterogeneous population of crystals that we got on Earth," Reichert says.

Eli Lilly has also sent its products to the ISS to be crystallized, in this case to make them easier to study structurally using analytical techniques such as X-ray diffraction. The company has also flown mice to the ISS to test an experimental drug that boosts muscle growth. Under microgravity, the loss of physical strain on bone and muscle accelerates the natural onset of common musculoskeletal diseases in rodents, making them ideal models of such human conditions, explains Jeremy Hinds, a senior research scientist at Lilly. In addition, Hinds is studying whether near weightlessness affects the process of freeze-drying materials, a common step in drug distribution and storage. Microgravity "could have positive outcomes on the physical properties and resulting drug product performance," he explains in an email to *The Scientist*.

Microgravity influences scientific experiments in many ways that appeal to drug developers.

CASIS, which selects the research projects that go to the US national lab on the ISS and provides companies with logistical support, is also working with a number of smaller companies studying everything from treatments for rare diseases to medical devices. One such company is MIT spinout MakerHealth, which has spent nearly a decade creating a device that can produce a number of personalized pharmaceuticals on demand. A mission is slated for 2021 to carry the device's mechanical reactors to the ISS, where they'll produce some simple compounds in space. Engineer Jose Gomez-Marquez of MIT's Little Devices Lab who helped develop the device says the experiment could not only show that it's possible to make drugs in space—a prerequisite for humanity's future ventures into outer space—but also help his team understand the typical gravitational constraints on the device's function and how they can improve it further: "It's a fundamental physics question."

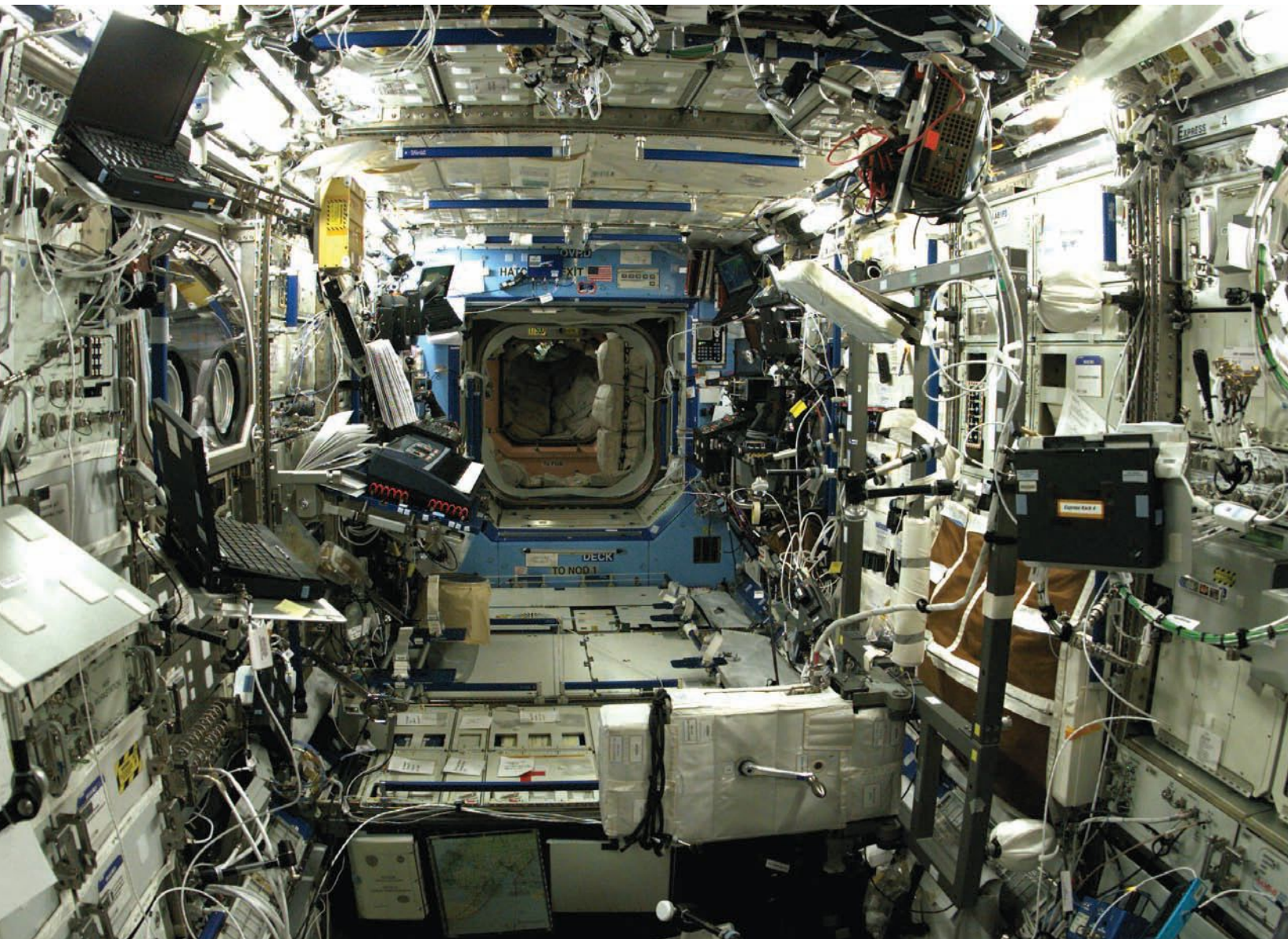
Challenges in space research

While research and development in space is well underway, progress has been slow, says Reichert. "We're still in the infancy of doing this kind of work."

Many of the challenges are logistical. Only six astronauts are stationed on the ISS; their time for experimental work is limited, and basic laboratory tasks such as pipetting and moving reagents around are challenging in microgravity. That's in part why pharma entities and biotechs typically contract companies that specialize in automating scientific experiments and packing them into flight-ready "cube labs," which astronauts simply need to activate to have the experiments conduct themselves. LambdaVision, for instance, worked with the microgravity research company Space Tango to turn their 2018 layering experiment and a more recent study of how bacteriorhodopsin functions under microgravity into miniature labs.

The downside of such arrangements is that researchers are often limited to one experiment at a time, and results can be a long time coming, Reichert says. "The astronaut just activates the experiment that sits there for two to three weeks, and then it comes back on a Dragon SpaceX module a month later, and then we first see what the results are."

Doing research in space comes with a host of other challenges as well, such as organizing simultaneous control experiments on the ground, and adapting research methods to the nonstandard laboratory equipment on the ISS. For Paul Jaminet, founder and president of the Massachusetts-based oncology startup Angiex, which undertook an experiment on the ISS in 2018, the endeavor "turned out to be significantly more work than we thought it would be." The company's experiment



EXTRATERRESTRIAL LAB: The Destiny Lab on the International Space Station allows researchers to carry out experiments in microgravity.

COURTESY OF NASA

showed that endothelial cells' response to one of the company's cancer drugs changed over the course of their time on the ISS, and that the cells generally grew and behaved differently in space than on Earth. In particular, the cells displayed unique characteristics that Angiex founder and head of research Shou-Ching Jaminet tells *The Scientist* could mimic certain features of cardiovascular conditions afflicting humans on Earth. The husband-and-wife team is interested in continuing that line of research, but due to the amount of labor, time, and money involved, it's taken a backseat to the company's work on drug candidates and other projects that are further along.

The biggest challenge is indeed the sheer cost of space experiments. Getting a single experiment to and back from the ISS can cost some $7.5 million, according to CASIS. Currently, flights to and from the ISS and astronaut time are covered by NASA, and the hardware and research costs of such experiments are sometimes partially funded through federal grants. Some smaller companies, including MakerHealth, LambdaVision, and Angiex, financed their endeavors with six-figure microgravity research grants awarded by a partnership between CASIS and Boeing through the Boston-based business accelerator program MassChallenge.

These generous subsidies and incentives are part of a long-term effort by NASA to coax private companies to recognize the value of R&D in space. In addition to bringing benefits to people on Earth, companies ideally would ultimately pay for their own research and help the US National Laboratory on the ISS become self-supporting. However, a 2018 report by NASA's Office of the Inspector General criticized CASIS for failing to recruit enough commercial users to the space station, and "question[ed] whether a sufficient business case exists under which private companies will be able to develop a self-sustaining and profit-making business [on the ISS]."

That's broadly in line with an analysis by Nicholas Vonortas, a microeconomist at George Washington University who received a NASA grant in 2015 to conduct a cost-benefit analysis of using protein crystallization on the ISS to get better structural information about peptides. Through economic models that considered the risk of experiments failing, among other factors, Vonortas found that the potential financial benefits of crystallizing proteins on the ISS will likely not be enough to outweigh the costs if they're shouldered by the private sector alone. "All of this together, when you do the calculations, brings a result that is not as attractive as the scientists think," he tells *The Scientist*.

Researchers are often limited to one experiment at a time, and results can be a long time coming.

Space pharmacy ahead?

Costs may decrease over time as travel to and from the ISS becomes more frequent, Vonortas says. Entrepreneur Elon Musk, for instance, has said he wants to establish a more regular service to the station than there is currently—an idea not without its skeptics. But a significant source of uncertainty is that the ISS, after more than 110,000 laps around the planet, may be nearing the end of its life. NASA and other participating space agencies plan to continue operations through 2024, but what happens after that is unclear.

Instead, pharma research of the future may take advantage of independent initiatives developed by a growing community of companies working to make conducting experiments in sustained microgravity cheaper, faster, and more accessible for life scientists. For instance, the Israeli-Swiss company SpacePharma, founded in 2011, develops autonomous research stations that can be operated from the ground. "Until now, unless you were part of NASA or some space agency, it was very difficult to initiate and perform such experiments" in space, says Guy Samburski, SpacePharma's director of chemical and pharmaceutical applications.

The company recently launched the satellite DIDO 3, carrying four experiments by Italian and Israeli researchers on board, all packed into a milk carton–size box. The satellite won't return to Earth, but is currently recording and transmitting research data back to scientists on the ground. SpacePharma's next launch will involve a larger system that will eventually return home so researchers can physically collect materials and results. British spaceflight company Virgin Galactic and Jeff Bezos's space company Blue Origin have also begun to offer such opportunities to scientists.

The emergence of an entire ecosystem devoted to bringing pharmaceutical research into space has opened up new possibilities to those in the industry. "Could we have space labs in the sky that can operate autonomously and discover new lifesaving medications for us?" Gomez-Marquez asks. And while the return on investment currently isn't ideal, many believe such research will become profitable over time. Eventually, "[it] might be financially beneficial for a company to have things produced or manufactured in space," in the same way we outsource drug production to different countries on Earth, suggests Thais Russomano, a space medicine expert and cofounder and CEO of InnovaSpace. In fact, LambdaVision is already considering launching production of its artificial retina in space, encouraged by the potential superiority of space-made products.

Whether such visions become reality, only time will tell. "If you're asking me whether this is possible—absolutely, this is technically possible," Vonortas says. But "the economics is a problem." ■

Katarina Zimmer is a freelance journalist. Find her on Twitter @katarinazimmer.

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Dreaming of Possibilities

The sleeping brain may help us explore potential solutions to waking concerns.

BY ROBERT STICKGOLD AND ANTONIO ZADRA

At the start of the 21st century, scientists had little idea why we sleep, leading J. Allan Hobson at Harvard Medical School to quip that the only known function of sleep was to cure sleepiness. But 20 years later, we know a lot more. It turns out that for every two hours a person is awake and interacting with the world, the brain on average needs to go "offline"—disconnected from the outside world— for an hour to process and contextualize those experiences.

Sleep benefits memory in myriad ways. For simple procedural skills—how to ride a bicycle, for example—a night of sleep or an afternoon nap following learning leads to a dramatic improvement in performance. Sleep also stabilizes verbal memories, reducing their susceptibility to interference and decay.

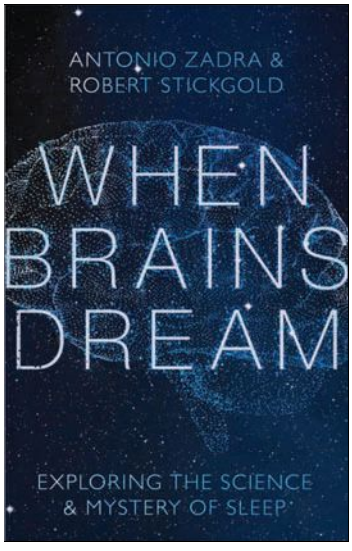
But the benefits of sleep can be more sophisticated than simply strengthening and stabilizing memories. Sleep can lead to the selective retention of emotional memories, while allowing other memories or less emotional parts of a scene to fade. And it has been shown to help infants gain language skills. Disruptions of normal sleep in neurological and psychiatric disorders can lead to a failure of these processes.

As we describe in our upcoming book *When Brains Dream*, dreams appear to be part of this ongoing memory processing, and their occurrence and content can predict subsequent memory improvement. There is a vigorous debate over whether the conscious experiencing of dreams serves a function; we believe that it does, and that it is similar to that proposed for waking consciousness. Antonio Damasio, in his 2000 book *The Feeling of What Happens*, argues that consciousness provides two critical functions to the human brain: to construct narratives and to feel one's emotional response to them. Together, these functions give humans (and presumably any other conscious animals) the ability to imagine possibilities, evaluate them, and thereby plan future actions. Our NEXTUP model of dreaming (Network Exploration to Understand Possibilities) proposes that dreaming serves a similar function.

We argue that dreaming allows the sleeping brain to enter an altered state of consciousness in which it can construct imagined narratives and respond emotionally to them. While dreaming, the brain identifies associations between recently formed memories (typically from the preceding day) and older, often only weakly related memories, and monitors whether the narrative it constructs from these memories induces an emotional response in the brain. If an emotional feeling is detected, the brain tags the association as potentially valuable, strengthening the link between the two memories and making the association available during subsequent wakefulness.

But dreaming is different from waking consciousness. First, the dreaming brain cannot access and incorporate complete episodic memories (i.e., memories of actual events in our lives), so the associative exploration of dreams is limited to semantic and nondeclarative memories (i.e., memories related to general world knowledge and those acquired and used unconsciously, respectively). In other words, while imagining and planning during wakefulness is normally based on recalled events, narrative construction during dreaming is based on semantic associations of these events, giving dreams their metaphorical quality and allowing for a more expansive investigation of associative links.

Second, the neurochemical modulation of the brain is altered during sleep, and especially during rapid eye movement (REM) sleep, when the release of norepinephrine and serotonin in the brain is shut off while levels of acetylcholine reach their peak in regions such as the hippocampus. These shifts bias memory networks toward the activation of normally weak associations, perhaps explaining the bizarreness of many dreams, especially during REM sleep.



W. W. Norton & Company, January 2021

Third, REM sleep is accompanied by a general activation of the limbic system, presumably explaining the enhanced emotionality seen in REM dreams, while also biasing the brain toward creating emotional responses to imagined dream narratives.

Finally, unlike problem solving during wakefulness, which relies on imagining and planning, dreaming stops short of offering definitive solutions to our current concerns. Instead, our dreams serve to explore the solution space, helping us to discover new possibilities. It is up to other processes, both in wakefulness and sleep, to draw conclusions and delineate our plans. Dreaming takes what has been and shows us what might be. ■

Robert Stickgold is a professor at Harvard Medical School and director of the Center for Sleep and Cognition. Antonio Zadra is a professor at the Université de Montréal and a researcher at the Center for Advanced Research in Sleep Medicine. Read an excerpt of When Brains Dream *at the-scientist.com.*

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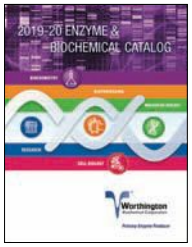

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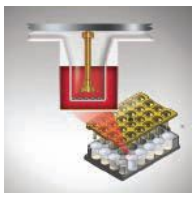

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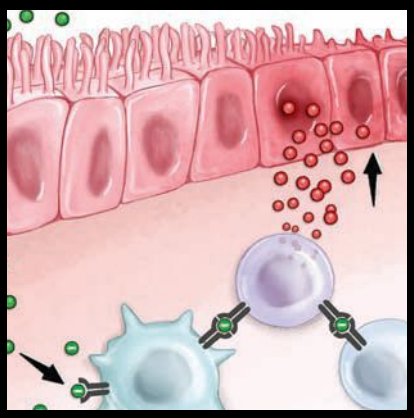
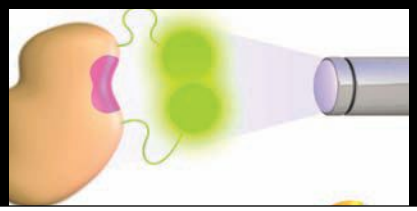
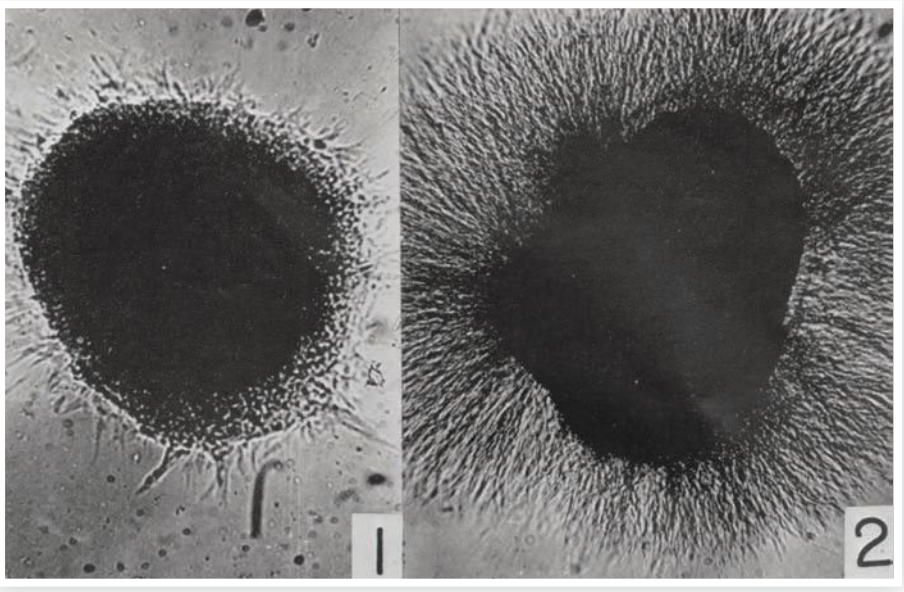


Action at a Distance, Circa Early 1950s

BY DIANA KWON

What do nerves need in order to grow? That question first caught Rita Levi-Montalcini's attention in the 1930s, when she came across a recent paper by embryologist Viktor Hamburger. After observing that clipping the wing bud off chicken embryos stunted the growth of spinal nerves and ganglia on the side of the body with the excision, Hamburger reported that signals from the limb drove the growth and differentiation of immature cells in the central nervous system. Levi-Montalcini was intrigued. But after repeating the embryo experiments and finding that the chick's nerve cells continued to develop after amputation and died later—just before reaching their target tissue—she came to a different conclusion. Rather than failing to initiate nerve growth, she hypothesized, the animals were unable to sustain the growing cells, causing a degenerative process that limited their proliferation.



VENOMOUS GROWTH: Rita Levi-Montalcini and Stanley Cohen found that when nerve tissue from chicken embryos is cultured alongside snake venom, a rich source of nerve growth factor (NGF), it grows a dense halo of nerve fibers (right). Without NGF (left), fewer nerve fibers develop, and those that do grow are smaller.

Levi-Montalcini began these experiments at the University of Turin in Italy, but as a Jewish scientist, she was forced to leave in 1938 when Mussolini's Fascist government made it illegal for her to work at state universities. She continued the work from a secret, makeshift laboratory in her bedroom until the end of World War II.

Levi-Montalcini sent reports to her former advisor, histologist Giuseppe Levi (no relation to Levi-Montalcini), then in Belgium, who published their coauthored manuscripts in academic journals. In 1946, Hamburger invited Levi-Montalcini to his lab at Washington University in St. Louis. Together, they found that many nerve cells die during normal development, and that limb amputations heighten this loss. Soon after, Levi-Montalcini followed up on the findings of Hamburger's former graduate student Elmer Bueker, who had observed that, like the developing limb, a rapidly growing malignant tumor could also promote the growth of nerve cells in chicken embryos. Levi-Montalcini transplanted tumors onto the membrane around an embryo, where they were only connected to the developing animal by a common blood supply, and demonstrated that the tumors could still encourage neural growth. It seemed there was a diffusible agent that was influencing nervous system development.

In the early 1950s, Levi-Montalcini began collaborating with biochemist Stanley Cohen, who had just joined Hamburger's lab. The pair isolated and characterized the mystery molecule, nerve growth factor (NGF), which turned out to be crucial for the development and survival of cells in the nervous system. Cohen later identified another factor, epidermal growth factor (EGF), which stimulates the growth of epithelial cells. And in 1986, Levi-Montalcini and Cohen shared the Nobel Prize in Physiology or Medicine for their discoveries of NGF and EGF, respectively. The discovery of these first growth factors was a "breakthrough in the field of extracellular messengers," a category that also includes vitamins and hormones, says Pietro Calissano, a neuroscientist and vice president of the European Brain Research Institute in Italy, which Levi-Montalcini founded. "[NGF and EGF] brought to light the existence of an entirely new category of diffusible substances."

Following the discovery of NGF, Levi-Montalcini spent much of the rest of her career investigating the role of the growth factor in the developing nervous system. Before passing away in 2012 at the age of 103, she also penned several books and set up a foundation to provide guidance and financial support to young students seeking higher education. Calissano, who worked with Levi-Montalcini for more than 40 years, remembers her as "a brilliant scientist and charming woman who liked to approach research with imagination." ■

PNAS, 42:571–74, 1956

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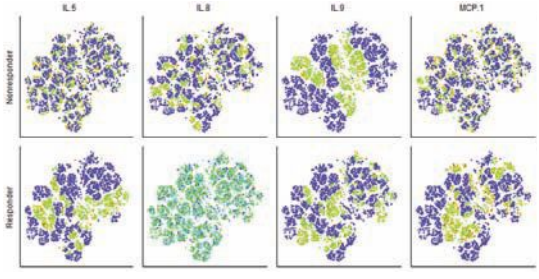

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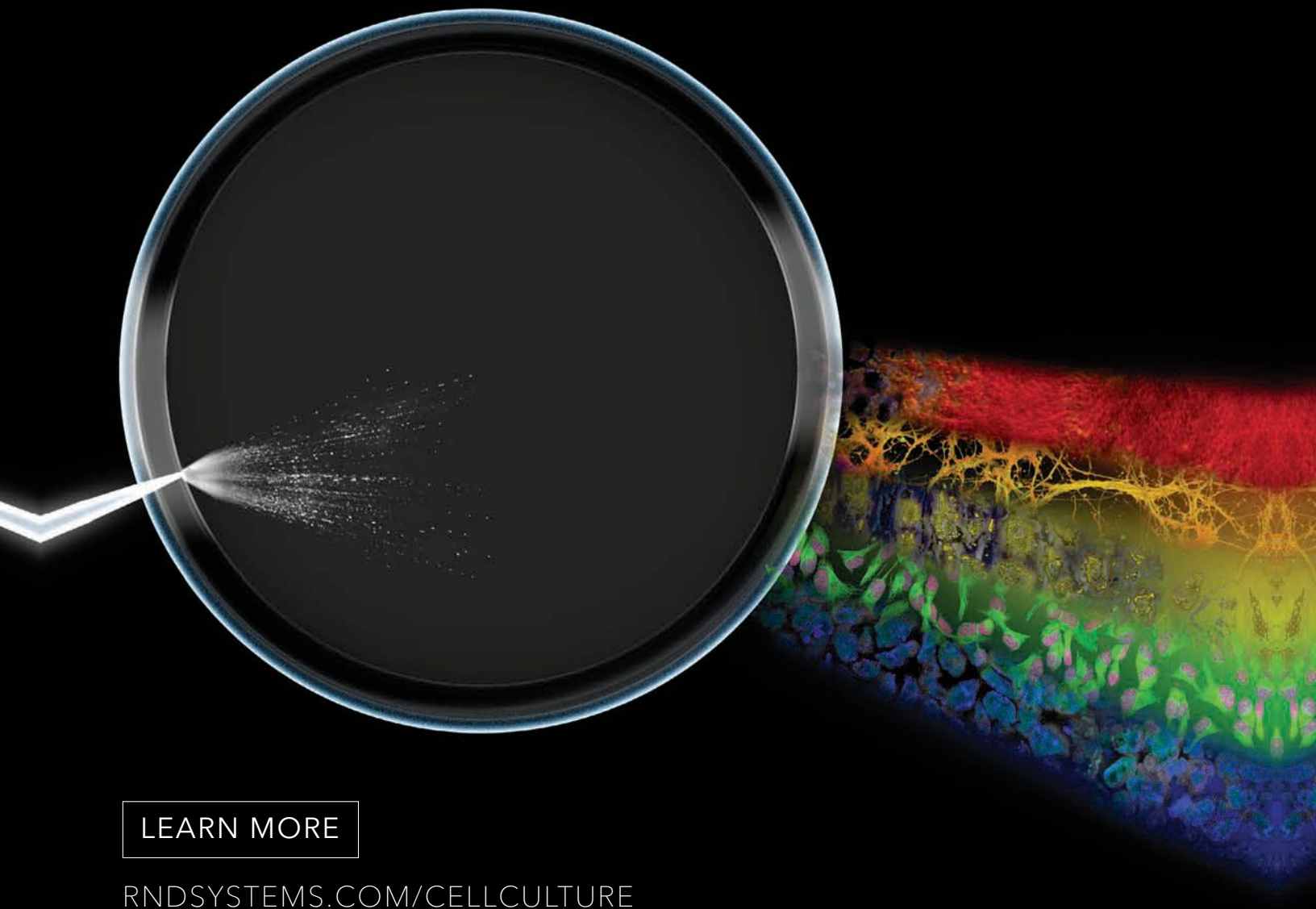

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