INSIDE STANFORD MEDICINE

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A genome-sequencing technique has revealed the stunning diversity of bacteria in the human gut.

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Fish offers possible clue to evolution of human toes

By Krista Conger

Consider the engineering marvel that is your foot. Be it hairy or homely, without its solid support you'd be hard-pressed to walk or jump normally.

Now, researchers at the School of Medicine and the HudsonAlpha Institute for Biotechnology in Huntsville, Alabama, have identified a change in gene expression between humans and primates that may have helped give us this edge when it comes to walking upright. And they did it by studying a tiny fish called the threespine stickleback that has evolved radically different skeletal structures to match environments around the world.

"It's somewhat unusual to have a research project that spans from fish all the way to humans, but it's clear that tweaking the expression levels of molecules called bone morphogenetic proteins can result in significant changes not just in the skeletal armor of the stickleback, but also in the hind-limb development of humans and primates," said David Kingsley, PhD, professor of developmental

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LEVENTEGYORI / SHUTTERSTOCK

Microscope rapidly measures mechanical properties of cells

NORBERT VON DER GROEBEN

Manish Butte and his colleagues developed a way to probe and get detailed measurements of living cells through an advancement in atomic force microscopy.

By Andrew Myers

In his role as a pediatrician, Manish Butte, MD, PhD, will often push and prod a patient's abdomen, feeling for abnormalities — a swollen spleen, a hardened lymph node or an unusual lump in the intestines or liver. There are still some things that can only be gleaned by touch, and Butte believes this notion applies to individual cells as well.

Yet researchers' ability to probe and measure the features of living cells has been almost nonexistent. Recently, a team of Stanford scientists and engineers set out to right that imbalance with a new technique for rapidly mapping cells. They succeeded by engineering a major advancement in a technology known as atomic force microscopy, or AFM, which itself was invented at Stanford in 1986.

A paper describing the work was published online Nov. 11 in *ACS Nano*. Butte, an assistant professor of pediatric immunology, is the senior author. Lead authorship is shared by Andrew Wang, PhD, a former postdoctoral scholar in Butte's lab, and Karthik Vijayraghavan, PhD, who was a graduate student and member of the microphotonics lab led by Olav Solgaard, PhD, a professor of electrical engineering.

"What a cell feels like — its mechanical properties that affect how it makes contact with other cells and tissues — is much more important than what it looks like, but the technology just wasn't there to allow us to examine it," Butte said. "There is a lot to be learned from studying the mechanics of a cell and its structures just beneath the surface."

The way Butte and his colleagues use AFM to measure the mechanical properties of cells is akin to the way a builder taps her knuckles along a drywall, listening for the change in pitch that will tell her a wooden stud is on the other side. When an

See MICROSCOPE, page 6

Ambien improves stroke recovery in mice

By Bruce Goldman

Mice that had strokes rebounded significantly faster if they received low doses of a popular sleeping aid, according to researchers at the School of Medicine.

Zolpidem, better known by the trade name Ambien, has long been approved by the U.S. Food and Drug Administration for treating insomnia. But it has never before been definitively shown to enhance recovery from stroke, said Gary Steinberg, MD, PhD, professor and chair of neurosurgery. Steinberg shares senior authorship of the study, which was published online Dec. 18 in *Brain*, with senior research scientist Tonya Bliss, PhD.

Steinberg, the Bernard and Ronni Lacroute-William Randolph Hearst Professor in Neurosurgery and the Neurosciences, cautioned that the study's results need to be independently replicated in other laboratories before clinical trials of the drug's capacity as a stroke-recovery agent can begin.

SEBASTIAN KAULITZKI / SHUTTERSTOCK

Every year, Americans incur about 800,000 strokes, the nation's largest single cause of neurologic disability, exacting an annual tab of about $74 billion in medical costs and lost productivity.

A stroke's initial damage, which arises when the blood supply to part of the brain is blocked, occurs within the first several hours. Drugs and mechanical devices for clearing the blockage are available, but to be effective they must be initiated within several hours of the stroke's onset. As a result, fewer than 10 percent of stroke patients benefit from them.

After a few days during which tissue death continues to spread to adjacent brain regions due to repercussions from the initial damage, the brain begins slowly rewiring itself and substituting new neural connections

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Test could indicate whether infections are viral or bacterial

By Jennie Dusheck

Purvesh Khatri

A team of immunologists and informatics experts at the School of Medicine has identified a distinctive pattern of gene expression that distinguishes people with a viral infection from those with a bacterial infection. The team also identified a second pattern of gene expression that is more specific: It can distinguish the flu from other respiratory infections.

When pathogens infect the cells of the body, the infection sets off a chain reaction involving the immune system that changes the expression of hundreds of genes. Gene expression is the process by which cells extract information from

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Study: Overprescribing opioids not limited to a few bad apples

By Beth Duff-Brown

Most prescriptions for opioid painkillers are made by the broad swath of U.S. general practitioners, not by a limited group of specialists, according to a study by researchers at the School of Medicine.

This finding contrasts with previous studies by others that indicated the U.S. opioid epidemic is stoked by a small population of prolific prescribers operating out of corrupt "pill mills."

The study, which examined Medicare prescription drug claims data for 2013, appeared in a research letter published online Dec. 14 in *JAMA Internal Medicine*.

"The bulk of opioid prescriptions are distributed by the large population of general practitioners," said lead author Jonathan Chen, MD, PhD, an instructor of medicine and Stanford Health Policy VA Medical Informatics Fellow.

Prescribing patterns

The researchers found that the top 10 percent of opioid prescribers account for 57 percent of opioid prescriptions. This prescribing pattern is comparable to that found in the Medicare data for prescribers of all drugs: The top 10 percent of all drug prescribers account for 63 percent of all drug prescriptions.

The specialties that prescribed the most Schedule II opioids in 2013 were family practice (15.3 million prescriptions), internal medicine (12.8 million), nurse practitioner (4.1 million) and physician assistant (3.1 million prescriptions), according to the study. Schedule II drugs are substances approved by the Food and Drug Administration for medical use and recognized as carrying a high potential of abuse.

SERIALCODER / SHUTTERSTOCK

"These findings indicate law enforcement efforts to shut down pill-mill prescribers are insufficient to address the widespread overprescribing of opioids," Chen said. "Efforts to curtail national opioid overprescribing must address a broad swath of prescribers to be effective."

> "The bulk of opioid prescriptions are distributed by the large population of general practitioners."

He added, "Being a physician myself, I am acutely aware of the emotional angst that can occur when deciding whether to prescribe opioids to a patient who may have simultaneously developed a chronic-pain and substance-dependence problem. The public health epidemic of opioid overuse is perhaps not surprising given the tenfold increase in volume over the past 20 years."

Different findings from different data set

In 2011, a study by the California Workers' Compensation Institute found that 1 percent of prescribers accounted for one-third of opioid prescriptions, and that the top 10 percent accounted for 80 percent of prescriptions.

The new Stanford study used a different data set: Instead of California Workers' Compensation prescriptions, it looked at prescriber data from the 2013 Medicare prescription drug coverage claims and investigated whether such disproportionate prescribing of opioids occurs in the national Medicare population.

Both studies looked at Schedule II opioids, which include the commonly abused drugs hydrocodone, codeine and fentanyl.

The data set created by the Centers for Medicare and Medicaid Services included all prescribers and represented all Medicare prescription drug coverage claims for 2013: 808,020 prescribers and 1.18 billion claims. The researchers focused on the data for Schedule II opioids: 381,575 prescribers and 56.5 million claims.

"This data set indicates no special distinctions in the concentration of opioid prescribing among Medicare prescribers," said Chen. "The earlier study suggests potentially aberrant behavior among those extreme outlier prescribers, while implying the remaining majority do not contribute much to the problem — and now we know this is not the case."

The authors attribute the difference in the California Workers' Compensation data to the traits of that specific population, which perhaps has a greater prevalence of multiple illnesses or employment in jobs more prone to injury, while the Medicare population is more generally representative of the population at large.

They found that opioid prescriptions per prescriber were concentrated among specialty services for interventional pain management (1,124.9 prescriptions, on average, per prescriber), pain management (921.1), anesthesiology (484.2) and physical medicine and rehabilitation (348.2). By sheer volume, however, there are so many more general practitioners that they dominated the total quantity of prescriptions.

Anna Lembke, MD, is the study's senior author. Other Stanford co-authors are professor of psychiatry and behavioral sciences Keith Humphreys, PhD, and associate professor of medicine Nigam Shah, MBBS, PhD.

The research was supported in part by the U.S. Department of Veterans Affairs Office of Academic Affiliations, the VA Health Services Research and Development Service, the National Institute of General Medical Sciences and the Peter F. McManus Charitable Trust.

Stanford's Department of Medicine also supported the work. **ISM**

Beth Duff-Brown is the communications manager for Stanford Health Policy.

Interaction of attention networks weaker in kids with ADHD

By Erin Digitale

Interactions between three brain networks that help people pay attention are weaker than normal in children with attention-deficit hyperactivity disorder, according to a new study from the School of Medicine.

The degree of weakness was correlated to the severity of the children's inattention symptoms, the researchers found.

The study was published online Dec. 15 in *Biological Psychiatry*.

The researchers focused on the salience network, which is a set of brain regions that work together through well-synchronized neural activity to help decide where one's attention should be directed. In most children, this network can assess the importance of internal and external events, and then regulate other thoughts to focus attention in the right place.

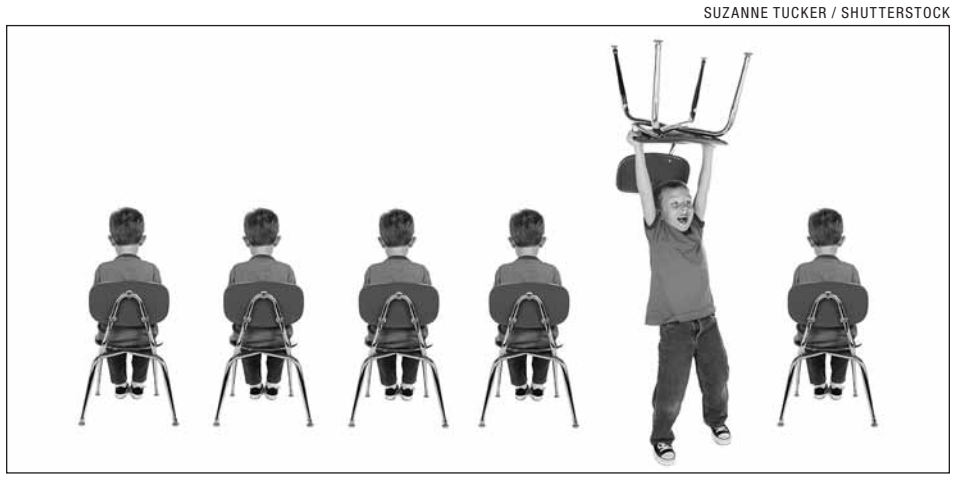
"A lot of things may be happening in one's environment, but only some grab our attention," said Vinod Menon, PhD, a professor of psychiatry and behavioral sciences and the study's senior author. "The salience network helps us stop daydreaming or thinking about something that happened yesterday so we can focus on the task at hand. We found that this network's ability to regulate interactions with other brain systems is weaker in kids with ADHD."

More than 6 million children in the United States, or 11 percent of children aged 4 to 17, have received ADHD diagnoses. The disorder is characterized by impulsiveness, hyperactivity and difficulty paying attention. Kids with ADHD tend to struggle in school, have trouble with friendships and be more prone to injury than other children their age.

Subjectivity of diagnoses

At present, diagnosing ADHD is quite subjective, with different thresholds of behavior used to make the diagnosis in different places. For instance, according to the U.S. Centers for Disease Control and Prevention, in 2011, 7.3 percent of California children had at some point been diagnosed with ADHD, making the state one of five nationwide with diagnosis rates below 8 percent among children. At the other end of the spectrum, six states had rates above 15 percent.

SUZANNE TUCKER / SHUTTERSTOCK



"It would be very beneficial to have a diagnostic measure that uses more objective and reliable measures, not just clinical and parental assessments of behavior," said Weidong Cai, PhD, an instructor in psychiatry and behavioral sciences and the study's lead author. "This study also demonstrates that we can develop a very robust biomarker based on functional neuroimaging to reliably differentiate children with ADHD from other kids."

Menon's team studied functional magnetic resonance imaging brain scans from 180 children, half with ADHD and half without. The scans were taken when the children were awake but resting quietly. The children were also assessed for ADHD symptoms using traditional diagnostic methods. All study data were obtained from the ADHD-200 Consortium, an open-source database of fMRI scans and other clinical characteristics of hundreds of children with or without ADHD. The new results are noteworthy in part because they were replicated in independent data sets from three different sites in New York, Portland and Beijing that contributed to the consortium.

Discerning ADHD from non-ADHD

The team scored each brain scan according to the synchronization between the salience network and two other related brain networks: the default mode network, a set of brain regions that directs self-referential activities such as daydreaming; and the central executive network, which manipulates information in working memory. To focus one's attention, the salience network must turn down the activity of the default mode network while turning up the activity of the central executive network.

Menon and col- **See ADHD, page 8**

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STANFORD MEDICINE

Toenail trim saves lab mice from life-threatening skin condition

By Ruthann Richter

In a new study, School of Medicine researchers report finding an easy method to cure laboratory mice of a common, life-threatening skin disease: A pedicure.

Millions of laboratory mice suffer from a skin condition known as ulcerative dermatitis — a major scourge for these animals and the most common reason for unplanned euthanasia. Between 4 and 21 percent (depending on factors like age and genetics) of laboratory mice experience the condition, in which they develop deep, ulcerated lesions that become progressively worse with repeated scratching, said Sean Adams, DVM, PhD, a third-year resident in laboratory animal medicine at Stanford and lead author of the new study.

To address the problem, which has long bedeviled veterinarians, Adams and his colleagues designed a simple plastic device that briefly immobilizes the animals so that caregivers can quickly trim their daggerlike claws. Some 93 percent of the mice whose toenails were trimmed were permanently cured of the condition, as they were unable to continue self-traumatizing the affected area despite still scratching, the researchers found. And the results held up even after the animals' toenails had regrown, as mice with clipped nails lived more than three times longer than their counterparts who were treated with topical ointments**.**

"This is a simple, cheap, effective means of treating ulcerative dermatitis, which represents the single most preventable reason for euthanasia," Adams said. "I think it's a very surprising finding in how simple this technique is."

The study, the first to systematically look at the impact of toenail trims, was published online Jan. 6 in *PLOS ONE*.

Saves mice, simplifies care

Adams said the technique not only saves mice from suffering and having to be euthanized as a humane necessity, but also simplifies their care. The Stanford veterinarians were able to clip the animals' nails in two minutes or less, saving them the time and expense of applying daily anti-inflammatory ointments, which were only minimally effective in curbing inflammation, he said.

The technique could also help preserve the integrity of mouse studies, avoiding the need for pharmacologic treatments that can compromise study results, Adams said. Most importantly, researchers need fewer mice for the same study, as they expect fewer losses, so it's an excellent example of how "good welfare is good science," he said.

At Stanford, "we don't euthanize many mice anymore due to ulcerative dermatitis because we use the toenail trim," he said.

Adams said the cause of ulcerative dermatitis in mice is unknown, with researchers speculating that it is related to genetics, diet, environment, behavior or a combination of these factors. The disease typically shows up on the nape of the animals' neck as a red, inflamed area. Because the lesions itch, animals begin scratching the area with their sharp hind claws as many as 20 to 25 times a minute, he said. With the repeated irritation, the condition spreads, often to the face, flank and back, with animals literally self-destructing over time.

"Now we have this mouse with just shreds of fur on the body. They rip themselves apart," he said.

Veterinarians have tried many approaches to treatment, typically involving application of a topical anti-inflammatory ointment. These have produced variable results, though studies have never shown them to be more than 65 percent effective, the researchers report. Moreover, these ointments have to be applied daily, causing a major burden for animal care providers.

Study methods and results

In 2013, the researchers started giving veterinarians at Stanford the freedom to apply either the topical anti-inflammatory Tresaderm to mice with ulcerative dermatitis or to trim their toenails under anesthesia. The toe-trimmed mice also got an application of Vetericyn, a form of bleach that inhibits bacterial growth and helps calm inflammation. The mice were of different strains and were housed in five facilities.

After a year, Adams and his colleagues went back and examined the records for 137 animals, including 98 who had been treated with Tresaderm and 39 who had their toenails trimmed, to see how well they did. They found that animals with clipped nails did significantly better, with 93.3 percent healing within 14 days. Among mice receiving Tresaderm, 25.4 percent were cured during the same time period.

To determine whether the results held up over time, the researchers followed another 54 animals over a six-week period, both trimming their toenails and applying Vetericyn to soothe the affected area. Mice toenails begin to regrow within a few days, so the researchers wondered if the animals would begin the destructive scratching cycle again. But to their surprise, the animals refrained from scratching their wounds, which continued to heal.

"It's a curative treatment. It's not just palliative," Adams said. "This really does break the cycle to allow a cure to occur. It is completely different from the other treatments out there."

He said the toenail treatment was not effective in healing animals who had lesions on their flank, as the mice sought relief from their discomfort by chewing the wounds in this area.

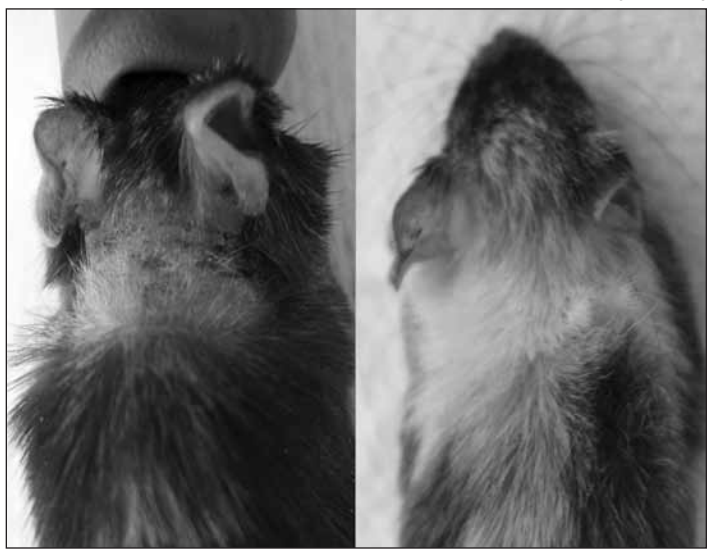
Finally, to make sure that the topical treatments were not confounding the results, the research team tested three different ointments — Tresaderm, Vetericyn and the antibiotic Bacitracin — together with toenail trimming, and found no difference in results between the three.

Immobilizing mice

While toenail trimming was clearly the superior treatment, Adams said the veterinarians recognized it wasn't entirely practical when done under general anesthesia. So he devised a simple trimming device, modifying a plastic tube to create two small cutouts for the animals' feet. When the mice are fitted into the tube, they are temporarily immobilized with their feet outstretched, making nail trimming simple and relatively stress-free for both caregivers and animals, he said.

He said the mice don't struggle or resist, and after a day or two of practice, technicians could clip the nails in as little as 30 seconds. "You just give a little pedicure and it changes everything," Adams said.

SEAN ADAMS



A lab mouse with ulcerative dermatitis (left) was given a toenail trim. Fourteen days later (right), the lesions had healed and fur had regrown in the affected area.

"I think we'll start seeing more people in other labs pick up this technique because it's very easy to do," he added. "There is definitely interest in finding good techniques for the problem because this is an issue for every institution that employs mice."

He said it's especially important to laboratories that use unusual strains, such as transgenic models, that can be very valuable.

Some institutions, including Massachusetts General Hospital and the University of Colorado, also perform toenail trims, while veterinarians at UC-Davis and UCSF have shown an interest in the technique, he said.

David Chu, DVM, a staff veterinarian, is senior author of the study. Other Stanford-affiliated co-authors of the study are Joseph Garner, PhD, associate professor of comparative medicine; Stephen Felt, DVM, MPH, associate professor of comparative medicine; and Jerome Geronimo, a research assistant.

The study was funded in part by the National Institutes of Health, which helps support Adams' residency, and by private contributions to Garner's mouse welfare work. **ISM**

Stanford Medicine, writers earn top honors from AAMC

By Susan Ipaktchian

Stanford Medicine magazine and three of the stories published in the magazine in 2015 earned the highest possible awards in the annual competition sponsored by the American Association of Medical Colleges.

Stanford Medicine, which is produced by the medical school's Office of Communication & Public Affairs, received an Award of Excellence in the external-audience periodical category. Judges for the competition praised the magazine's "beautiful design and elegant layout," and also noted that the "writing was very good and keeps the reader engaged." The magazine's editor is Rosanne Spector.

The magazine also earned the top award in each of the three AAMC writing categories.

Science writer Krista Conger received an Award of Excellence in the general staff writing category for "The butterfly effect." The article, published in the summer 2015 issue, focused on the quest for a treatment for what might be the most painful skin disease of all: the blistering disease epidermolysis bullosa. It highlighted the experiences of two patients and the testing of a stem cell gene therapy technique aimed at easing the blistering. "This story was engrossing; you made an immediate connection to the patients, and that made you want to understand more about this disease. Very well done," wrote one judge.

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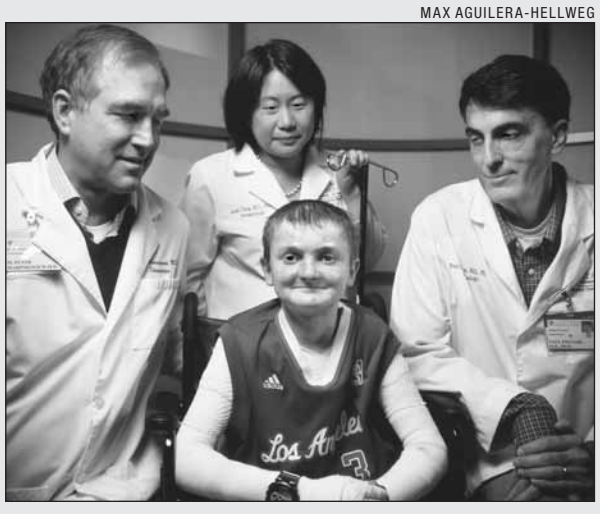


Epidermolysis bullosa patient Garrett Spaulding and his Stanford doctors were featured in "The butterfly effect," a story published in the spring 2015 issue of *Stanford Medicine* magazine.

Conger also received an Award of Excellence in the basic-science staff writing category for "The time of your life." In the article, published in the spring issue of the magazine, Conger recounted the efforts of researchers Anne Brunet, PhD, Thomas Rando, MD, PhD, and others who are trying to understand the aging process. The judges said the story was well-researched and included a variety of angles. "I was genuinely curious to learn more about the research and had several 'wow' moments throughout," one judge wrote.

The late Paul Kalanithi, MD, received an Award of Excellence in the solicited articles category for his essay "Before I go." In the piece, Kalanithi described how his perception of time changed as a neurosurgeon-turned-patient facing a terminal diagnosis. It was published in the spring issue of the magazine just a few weeks before he died of lung cancer. One judge wrote, "I was blown away by this simple, yet profound piece of writing. It tugged at my heartstrings and pulled me into the intimate thoughts of a man who is a surgeon, father and patient."

The awards are given by the AAMC's Group on Institutional Advancement, which includes communications, development and alumni relations staff at academic medical centers. This year's awards will be presented April 14 at the group's annual meeting in Phoenix. **ISM**

Researchers invent process to accelerate protein evolution

By Ramin Skibba

All living things require proteins, members of a vast family of molecules that nature "makes to order" according to the blueprints in DNA.

Through the natural process of evolution, DNA mutations generate new or more effective proteins. Humans have found so many alternative uses for these molecules — as foods, industrial enzymes, anti-cancer drugs — that scientists are eager to better understand how to engineer protein variants designed for specific uses.

Now, Stanford researchers have invented a technique to dramatically accelerate protein evolution for this purpose. This technology, described in a paper published online Dec. 7 in *Nature Chemical Biology*, allows researchers to test millions of variants of a given protein, choose the best for some task and determine the DNA sequence that creates this variant.

"Evolution, the survival of the fittest, takes place over a span of thousands of years, but we can now direct proteins to evolve in hours or days," said Jennifer Cochran, PhD, an associate professor of bioengineering, who shares senior authorship of the paper with Thomas Baer, PhD, executive director of the Stanford Photonics Research Center. The lead author is Bob Chen, a graduate student in bioengineering.

"This is a practical, versatile system with broad applications that researchers will find easy to use," Baer said.

By combining Cochran's protein engineering know-how with Baer's expertise in laser-based instrumentation, the team created a tool that can test millions of protein variants in a matter of hours.

"The demonstrations are impressive, and I look forward to seeing this technology more widely adopted," said Frances Arnold, PhD, a professor of chemical engineering at Caltech who was not affiliated with the study.

Making a million mutants

The researchers call their tool μSCALE, for Single Cell Analysis and Laser Extraction.

The "μ" stands for the microcapillary glass slide that holds the protein samples. The slide is roughly the size and thickness of a penny, yet in that space a million capillary tubes are arrayed like straws, open on the top and bottom.

The power of μSCALE is how it enables researchers to build upon current biochemical techniques to run a million protein experiments simultaneously, then extract and further analyze the most promising results.

The researchers first employ a process termed "mutagenesis" to create random variations in a specific gene. These mutations are inserted into batches of yeast or bacterial cells, which express the altered gene and produce millions of random protein variants.

A μSCALE user mixes millions of tiny opaque glass beads into a sample containing millions of yeast or bacteria and spreads the mixture on a microcapillary slide. Tiny amounts of fluid trickle into each tube, carrying individual cells. Surface tension traps the liquid and the cell in each capillary.

The slide bearing these million yeast or bacteria, and the protein variants they produce, is inserted into the μSCALE device. A software-controlled microscope peers into each capillary and takes images of the biochemical reaction occurring therein.

Once a μSCALE user identifies a capillary of interest, the researcher can direct the laser to extract the contents of that tube without disrupting its neighbors, using an ingenious method devised by Baer.

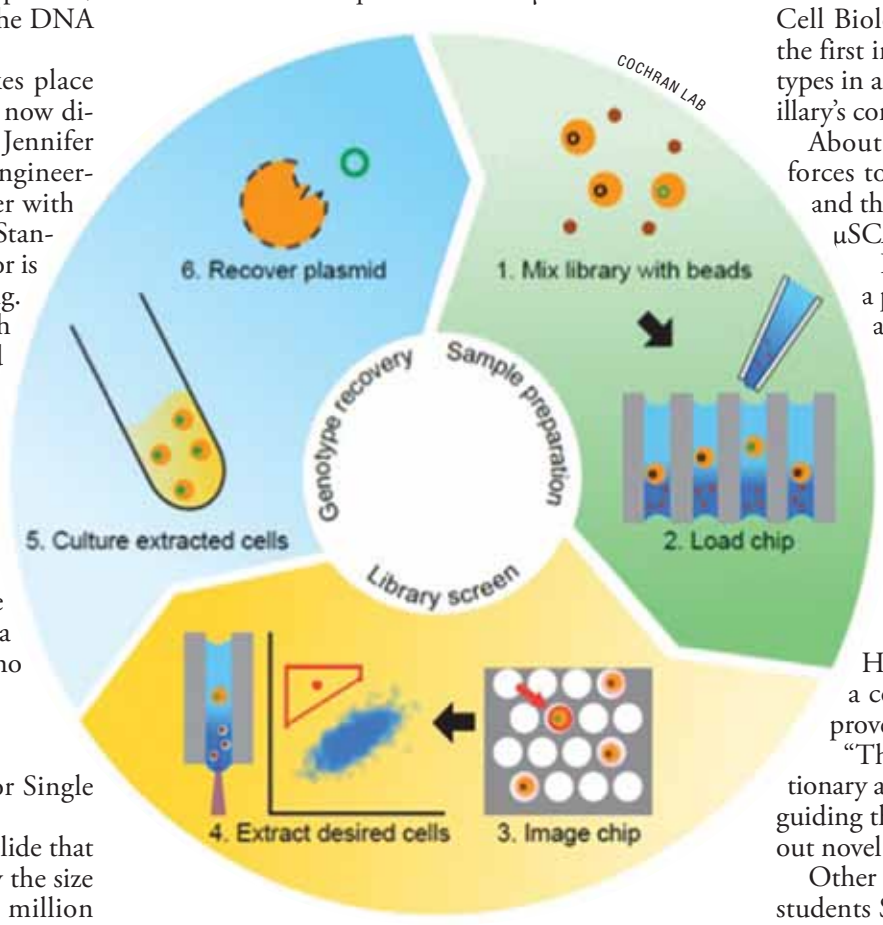
"The beads are what enable extraction," Baer said. "The laser supplies energy to move the beads, which breaks the surface tension and releases the sample from the capillary."

Thus, μSCALE empties the contents of a single capillary onto a collector plate, where the DNA of the isolated cell can be sequenced and the gene variant responsible for the protein of interest can be identified.

"One of the unique features of μSCALE is that it allows researchers to rapidly isolate a single desired cell from hundreds of thousands of other cells," said Chen, who wrote the software to examine and detect signs of interesting protein activity within the test tubes.

Promising variants can be collected and reprocessed through μSCALE to further evolve and optimize the protein.

"This is an exciting new tool to answer important questions about proteins," Cochran said, likening μSCALE to the way that high-throughput tools for gene analysis have allowed researchers to unlock key features of biology underlying human disease.



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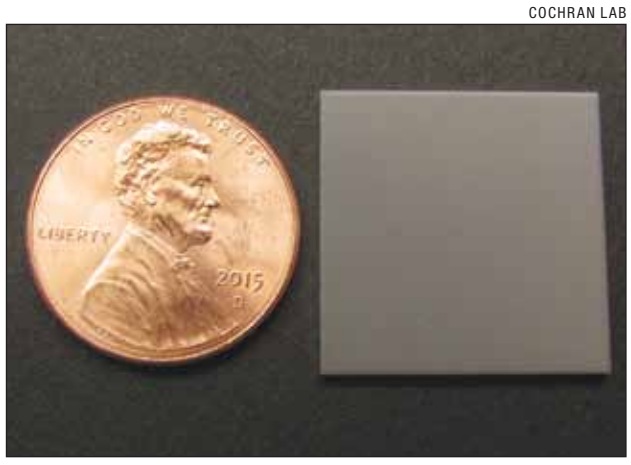


Top: An overview of the directed evolution process using μSCALE. Bottom: The microcapillary glass slide, roughly the size and thickness of a penny, holds the protein samples.

Genesis and proofs

The project began five years ago when Baer and study co-author Ivan Dimov, PhD, a visiting instructor and Siebel Fellow at the Stanford Institute for Stem Cell Biology and Regenerative Medicine, developed the first instrument. They showed how to identify cell types in a microcapillary array and extract a single capillary's contents using glass beads and a focused laser.

About three years ago, Cochran and Baer joined forces to develop μSCALE for protein engineering, and the team devised three experiments to showcase μSCALE's utility and flexibility.

In one experiment, researchers sifted through a protein library produced in yeast cells to select antibodies that bound most tightly to a cancer target. Antibodies with a high target-binding affinity are known to be effective against cancer.

In a second example, they engineered a bright orange fluorescent protein biosensor. Using μSCALE, they did this almost 10 times faster than previous methods. Such biosensors are often used as tags in a wide variety of biology experiments.

A third experiment, carried out with Daniel Herschlag, PhD, professor of biochemistry and a co-author of the study, used μSCALE to improve upon a model enzyme.

"This system will allow us to explore the evolutionary and functional relationships between enzymes, guiding the engineering of new enzymes that can carry out novel beneficial reactions," Herschlag said.

Other Stanford co-authors of the paper are graduate students Sungwon Lim and Arvind Kannan, postdoctoral scholar Spencer Alford, PhD, and research assistant Fanny Sunden.

This project was supported by the Stanford-Wallace H. Coulter Translational Partnership Award Program, the Siebel Stem Cell Institute and the Thomas and Stacey Siebel Foundation, the Stanford Photonics Research Center, a Hitachi America Faculty Scholar Award, the National Institutes of Health, the National Science Foundation Graduate Fellowship Program, the Howard Hughes Medical Institute International Student Research Program, a Fannie and John Hertz Foundation Graduate Fellowship, the Stanford Bio-X Fellowship Program, the Stanford Graduate Fellowship Program and the Stanford Dean's Fellowship Program.

ISM

Ramin Skibba is a former science-writing intern for Stanford Engineering.

SRI Biosciences, Stanford Cancer Institute launch drug discovery program

A new collaborative program between scientists at SRI Biosciences, a division of SRI International, and physician-researchers from the Stanford Cancer Institute will pursue development of novel compounds to treat multiple forms of cancer and other conditions.

The SRI Biosciences-Stanford Drug Discovery and Development Program was created in response to a significant drop in the early pipeline of innovative new drugs, and builds on a history of partnerships among investigators from both institutions. The combined basic research, drug discovery and drug development expertise of researchers from SCI and SRI Biosciences has successfully advanced numerous projects, and the new program adds structure, support and coordination to such efforts.

DELIVERANCE / SHUTTERSTOCK

Previous collaborations have yielded therapeutic candidates, including Tirapazamine, an experimental anticancer drug discovered by SRI and SCI investigators and brought to phase-3 clinical trials. Several other SRI-SCI developed compounds are undergoing preclinical testing.

Recently, Tony Wyss-Coray, PhD, professor of neurology and neurological science at Stanford, and Mary Tanga, PhD, SRI Biosciences director of medicinal and synthetic chemistry, jointly discovered and developed a small molecule agonist of the TGF-beta signaling pathway for Alzheimer's disease. The new agent has moved through preclinical development and is continuing into clinical trials.

"The SCI-SRI Biosciences collaboration provides a fully integrated engine for taking ideas to the investigational new drug (IND) stage and beyond," said Nathan Collins, PhD, executive director of the pharmaceutical and chemical technologies section of SRI Biosciences. "Our focus is on developing 'first-in-class' drugs and delivering improved outcomes for patients."

The program brings together teams of multidisciplinary scientists in both discovery and refinement of novel compounds and targets, and it provides access to the critical scientific infrastructure necessary for disease mechanism understanding and target discovery, and drug discovery and development through clinical safety and proof of concept.

"Advances in genomic and molecular analysis of individual patients and their cancers are creating new therapeutic opportunities," said Beverly Mitchell, M.D, director of the Stanford Cancer Institute. "We are excited to work with the skilled SRI Biosciences researchers to enhance our drug development efforts."

The program will be co-led by Sanjay Malhotra, PhD, associate professor of radiation oncology at Stanford, and Collins. Together they will coordinate and support a diverse and evolving group of investigators and technical experts to advance promising projects. **ISM**

Stunning diversity of gut bacteria revealed through technique

By Jennie Dusheck

A collaboration between computer scientists and geneticists at Stanford has produced a novel technique for mapping the diversity of bacteria living in the human gut.

The new approach revealed a far more diverse community than the researchers had anticipated. "The bacteria are genetically much more heterogeneous than we thought," said Michael Snyder, PhD, professor and chair of genetics.

Any two humans typically differ by about 1 in 1,000 DNA bases, whereas bacteria of the same species may differ by as many as 250 in 1,000, Snyder said. "I don't think people realized just how much diversity there was. The complexity we found was astounding," he said.

In the past, researchers could only study bacteria that would grow in the lab. But the vast majority of bacterial species will not grow on traditional culture medium. As a result, the true diversity of bacteria — not only in the human gut but throughout the living world — has remained largely unexplored.

In recent years, a genomics approach has begun to reveal diverse communities of new bacterial species growing nearly everywhere biologists have looked. Modern gene sequencing has tantalized biologists with hints of bacterial worlds as biodiverse as any tropical rain forest. Yet the limitations of current technologies have created only a blurry picture and prevented researchers from seeing all that is there.

Of particular interest are the bacteria that live in our intestines. Some communities of bacterial species in the gut have been associated with good health, others with any of a long list of conditions — including obesity, Type 2 diabetes, bowel disease and liver disease. And some are outright pathogens that can sicken and even kill, such as certain strains of *E. coli* or the bacterium that causes cholera. Given their importance to human health, the ecological communities of bacteria that live inside us and on our skin have come under increasing scrutiny.

A Stanford team has overcome some of the limitations of current sequencing technology to create a sharper picture of the bacterial community, or microbiome, of the human gut. The team used new computational approaches and "long-read" DNA sequencing to reveal the diversity of bacteria in the gut microbiome of a single male human.

A paper describing their work was published online Dec. 14 in *Nature Biotechnology*. The lead author is Volodymyr Kuleshov, a doctoral student in computer science at Stanford. Snyder, the Stanford W. Ascherman, MD, FACS, Professor in Genetics, is co-senior author with professor of computer science Serafim Batzoglou, PhD.

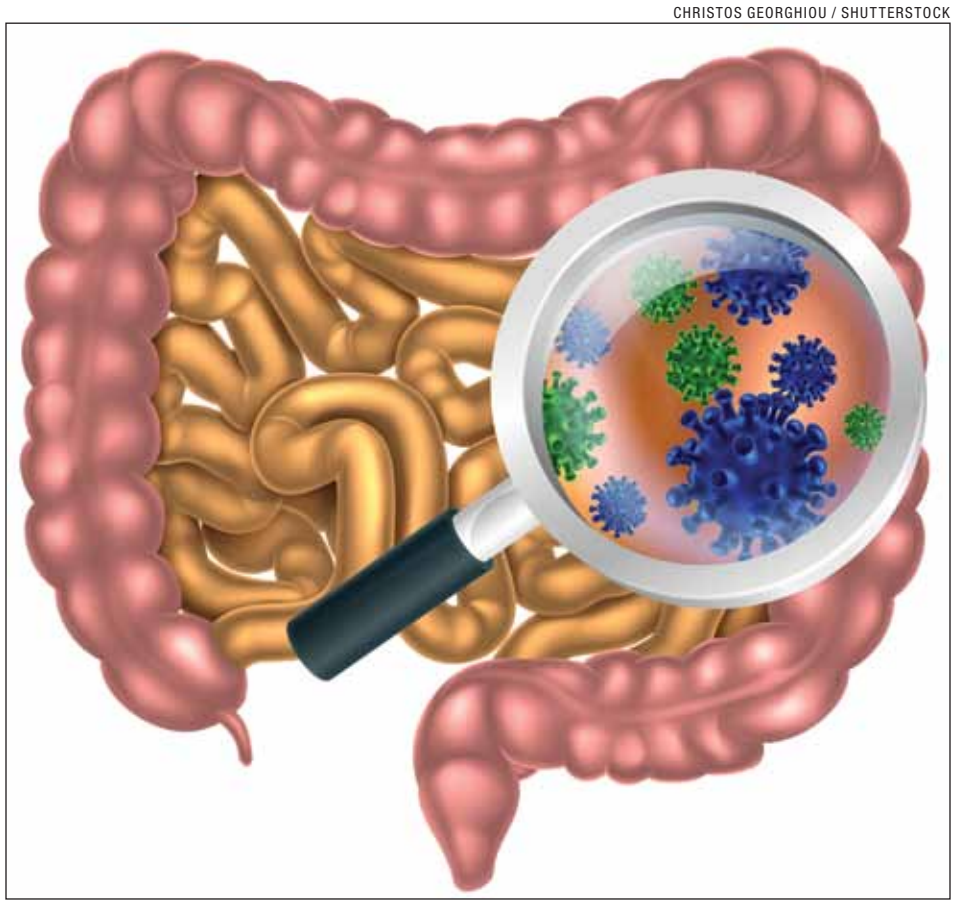
Problem posed by short snippets of DNA

Current DNA sequencing technology looks at very short snippets of DNA sequences. If you are looking at just one genome — from a bacterium or a single person, for example — you can assemble the snippets into a whole genome, much as you might painstakingly assemble a jigsaw puzzle.

But when you are looking at snippets from a mass of different bacteria from the human gut, assembling those snippets is like trying to assemble 100 jigsaw puzzles from a pile of pieces from all 100 puzzles jumbled together, explained Snyder. Any two pieces could be from completely unrelated puzzles — analogous to different species of bacteria — while others could be from multiple copies of the same puzzle — analogous to the same species of bacteria.

If that sounds difficult, the real challenge is being able to tell apart the pieces from puzzles that are almost the same but not quite. And that's what the researchers' new technique does. "We assembled one whole genome from this big gemisch, which has never been done before," said Snyder.

CHRISTOS GEORGHIOU / SHUTTERSTOCK



Researchers have overcome some of the limitations of current sequencing technology to better measure the diversity of bacteria in the human gut.

"We normally sequence 100 DNA bases off a 300-base fragment," he said. "You just get snippets of information." But using a new informatics approach, Snyder and Batzoglou's team stitched together larger segments of the genome. "We have a sophisticated algorithm that lets us put together all these pieces — first assembling the snippets into longer, 10,000-base pieces, then the 10,000-base pieces into still-longer fragments, and then those into whole genomes," Snyder said.

Michael Snyder

Great bacterial diversity

Such long sequences of DNA can span hundreds or even thousands of genes that couldn't be recovered from short-read sequencing; they can help classify bacteria and other organisms by how related they are to one another; and the long sequences also help identify rare bacteria that might be missed by current methods. "We could assemble either entire genomes or at least very, very large chunks of the genome," said Snyder.

Being able to see such long sections of the genome means being able to distinguish not only different species of bacteria, but different strains of the same species. The team tested the technique on a standardized sample of known bacteria and then took it for a spin on the gut contents of a human male. The result revealed not only lots of species, but many different strains of the same species. One bacterial species, for example, included five separate strains — all from one person.

The consequences of having so many different strains are hard to predict, but some strains may be more or less likely to make people ill. For example, many strains of *E. coli* bacteria live harmlessly and even helpfully in the human gut, while others are lethal. Being able to tell one strain from another could help researchers determine which strains are dangerous and why.

Right now, researchers who want to study virulence have to isolate that strain and then grow it in the lab. But some bacteria don't grow easily in the lab. If researchers can study the genes that contribute to virulence directly in the mixture of bacteria from a human gut sample, they don't need to isolate it and grow it in a pure culture. "When you assemble the whole genome, you have a better idea of what the pathogenic genes are. I think it's going to be very, very powerful for understanding the genetic basis of pathogenesis," said Snyder.

The new approach will make it easier to construct the evolutionary history of strains of infectious bacteria or viruses, such as Ebola. And the approach can be used in the field to study microbial diversity in healthy people and other animals, as well as in plants, water and soil. "When we put this together now, using these long reads, it's like an IMAX movie," Snyder said. "You can see the whole thing much more clearly than with what we do now, which is like an old black-and-white TV."

Other Stanford-affiliated authors of the paper are postdoctoral scholars Chao Jiang, PhD, and Wenyu Zhou, PhD, and research associate Fereshteh Jahaniani, PhD.

This work was supported by National Institutes of Health. Stanford's departments of Genetics and of Computer also supported the work. **ISM**

LARRY STRONG

Neuroscience Health Center opens to patients

The 92,000-square-foot, five-story Neuroscience Health Center opens to patients today. The center, a part of Stanford Health Care, is designed to serve people with neurological conditions or injuries such as brain tumors, movement disorders, brain aneurysms, spine deterioration, Parkinson's disease and memory disorders. Located on the Stanford Medicine campus next to Hoover Pavilion, the center offers advanced diagnostic testing and treatments, as well as support services, all available in a single, easy-to-navigate building.

Microscope

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AFM probe taps the surface of a cell, it vibrates, and the pattern of these vibrations, like the sound waves reflecting from the stud, gives mechanical information about the structures of the cell being touched.

However, existing AFM probes are relatively large and, as a result, insensitive to high frequencies, which communicate much of the key information about a cell's innards. The Stanford team's device couples a very small probe with a traditional one. This assembly allows the device to sense faster oscillations than conventional devices and, accordingly, to take more detailed and much faster measurements.

"The main difference between this and previous atomic force microscopes is that we are able to measure the impact of the probe on the cell very fast and get specific readings, whereas typical AFMs simply provide an average. This allows us to accurately measure some very soft materials for the first time," said Solgaard, who also is a co-author of the paper.

Current probes measure cellular stiffness by tapping against the cell around one or two times per second — the fastest that the large probes can make measurements. The small probe, however, can make detailed measurements easily at five to 10,000 taps per second because of its sensitivity. He likened the leap in sensitivity to the difference between driving a Cadillac Escalade down the road and pushing a Hot Wheels toy car along the same surface: "The small Hot Wheels will feel every little bump so much more than the large Cadillac."

'Beautiful solution'

AFMs measure movement of the probe by bouncing a laser off its tip. As the tip moves up and down, the laser is reflected. The Stanford invention couples the small probe with the large one by means of a fork-shaped structure called an interferometric grating. The grating produces a diffraction pattern based on the movements of the small probe, and allows the AFM to conveniently capture its measurements.

"Our tip actually produces a second signal, and that is what allows us to get much greater detail. From an engineering standpoint, it's an extremely simple, beautiful solution," Solgaard said, referring to the diffracted signals from the grating.

Best of all, the team's device can be directly attached to existing AFMs, potentially saving millions of dollars on new equipment that could otherwise be spent on research. A new AFM can run as much as $500,000, according to Solgaard.

The objective is the cellular equivalent of Butte pressing a child's abdomen.

"We want to study cell stiffness to understand what is beneath the surface and how cells are structured," Wang said.

As a demonstration, the team measured a section of a red blood cell, making approximately 4 million total measurements in about 10 minutes — all without damaging the delicate cellular exterior.

> **"The small Hot Wheels will feel every little bump so much more than the large Cadillac."**

"The same measurements would have taken more than a month to complete using conventional atomic force microscopes," said Vijayraghavan. The technology is so fast that the team was able to create a series of time-lapse images of a living cell, each taken just seven minutes apart, a previously unimaginable pace.

Potential applications

The practical applications of the device range from basic scientific understanding of cellular structure to immunology and oncology. Scientific understanding of the mechanical forces at play in cells is so lacking that the field — now being called mechanobiology — is really in its infancy, according to Butte.

The mechanical forces in the body can come from tissues, which range in stiffness from softest brain matter to stiffest bones, from gravity, and even from the pushing and pulling movements of other cells. Cancer cells make their environment mechanically rigid by secreting chemicals that stiffen up the extracellular matrix. Cancer cells likewise interpret the mechanical forces of a tissue to make decisions about growth and metastasis. Surprising feedback loops like this also appear to occur for stem cells in the bone marrow and during embryonic development. How immune cells interpret mechanical forces is still totally unknown.

"The lowest-hanging fruit is cancer. Cancers are often stiffer than normal, healthy tissues and we can use that knowledge to diagnose disease. But first, you have to have good data, which our device provides," Wang said. He has already used an early form of the new Stanford probe in pilot work on breast cancer specimens taken from mastectomies.

For his part, Butte plans to use fast AFM to study the immune system. He hopes to explore why otherwise disease-fighting T cells often remain dormant once inside a tumor. He theorizes that the mechanical stiffness of the tumorous tissue may be preventing T cells from freely making contact with cancer cells and from triggering their cancer-fighting functions. In essence, the tumor may be too crowded for the T cells to work. On the other end of the stiffness gamut, he believes that the soft mechanical properties of chronically inflamed or infected tissues provoke the immune system into over-activity, like autoimmunity.

It is a theory no one has yet explored due to technical barriers, which the fast AFM could overcome. Butte's lab has begun a broad effort to link mechanical forces with immune responses at the molecular, cellular and tissue scales. "There is so much we don't know about the mechanical properties of various cell types and diseased tissues. Almost nothing, in fact," Butte said. "The first step is to probe. Now, we can do that."

The work was funded by the National Institutes of Health, the National Science Foundation, the Stanford Center for Probing the Nanoscale, the Stanford Child Health Research Institute and Stanford Bio-X.

The departments of Pediatrics and of Electrical Engineering also supported the work. **ISM**

Andrew Myers is a freelance science writer.

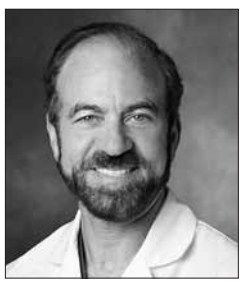
Stroke

continued from page 1

for those destroyed by the stroke. Within three to six months, at least 90 percent of all the recovery a stroke patient is likely to experience takes place. No pharmaceutical therapy has been shown to improve recovery after the stroke. In fact, no effective treatments during the recovery phase exist, other than physical therapy, which has been shown to be only marginally successful.

Nerve-cell signaling bolstered

Steinberg and Bliss attributed zolpidem's effectiveness to its enhancement of a type of nerve-cell signaling activity whose role in recovery unexpectedly appears beneficial. In the study, this signaling was bolstered even though the drug was given at doses well below those at which it exerts its hallmark sedative effect.



Gary Steinberg

Nerve cells signal to one another by means of substances called neurotransmitters. When neurotransmitters are secreted by the nerve cell sending the signal, they dock in receptors situated on abutting nerve cells' surfaces. Most of this signaling takes place at specialized junctions called synapses, which feature high concentrations of neurotransmitters from the upstream cell that activate receptors on the downstream cell.

Neurotransmitters can be excitatory, triggering propagation of an impulse in the receiving nerve cell. Or they can be inhibitory, temporarily preventing the receiving nerve cell from propagating any impulses. The roughly one-fifth of all nerve cells in the brain that are inhibitory mainly do their job by secreting a neurotransmitter called GABA.

While the bulk of GABA signaling takes place at synapses, scientists have learned that nerve cells can also feature GABA receptors elsewhere on their outer surfaces. These are called extrasynaptic receptors. In 2010, other researchers reported that extrasynaptic GABA signaling impeded stroke recovery in an animal model. But until the Stanford study, nobody had looked into the impact on stroke recovery of the far more common synaptic GABA signaling.

To do that, Steinberg, Bliss and their associates conducted a series of anatomical, physiological and behavioral experiments. Their efforts were assisted by the fact that there are small structural differences between synaptic and extrasynaptic GABA receptors, so they can be distinguished by various techniques.

Using a high-resolution visualization method, the Stanford scientists examined a region of the mouse brain near the area that had been destroyed by stroke and is known to rewire afterward. They saw a transient increase in the number of GABA synapses. This increase peaked at about a week after the stroke and subsided to baseline levels by one month after the stroke. The rise and fall of synapse-associated GABA receptors was restricted to a particular layer of the cerebral cortex that sends output to the spinal cord and to other brain areas.

Intrigued by this anatomical finding, the scientists looped in their colleague John Huguenard, PhD, professor of neurology and neurological sciences and co-author of the study. Electrophysiological experiments in Huguenard's lab confirmed that the transitory increase in GABA synapse numbers in the brain area under scrutiny was matched by an increase, followed by a decline to baseline levels, in synaptic GABA signaling, confirming that the synapses were indeed functional.

To determine whether the transient increase in post-stroke synaptic GABA signaling was beneficial — and, if so, whether it could be enhanced — the investigators turned to zolpidem, which works by enhancing synaptic GABA signaling. They induced either of two different types of strokes in mice — one type severely damages sensory ability; the other deeply impairs movement — then put the mice on a regimen of either zolpidem or a control solution that did not contain the drug.

Sub-sedative doses

The scientists administered the drug in sub-sedative doses. They wanted to see how the mice would perform on tests of sensory ability and motor coordination, so the mice needed to be fully awake. Zolpidem is known to have a much higher affinity for synapse-associated GABA receptors than for their extrasynaptic counterparts. So low doses were likely to enhance synaptic GABA signaling without having much of an effect on extrasynaptic signaling.

The team delayed zolpidem administration until three days after the stroke in order to ensure that any benefit they observed was resulting from an effect on brain recovery, rather than from the drug preventing initial tissue damage from the stroke.

The researchers subjected these mice to two kinds of tests. One measured the speed with which they removed a patch of adhesive tape from one of their paws (mice ordinarily are quick to do so). The other test gauged their ability to traverse a horizontal rotating beam.

In almost every case, zolpidem-treated mice recovered at a faster rate than control mice did. It took about a month, for example, for mice not given zolpidem to fully recover their stroke-impaired ability to notice the tape stuck to their paw. Mice given zolpidem recovered that ability within a few days of treatment.

While zolpidem dramatically improved mice's rate of recovery from stroke, its ability to increase the extent of their recovery couldn't be determined because, unlike humans, mice naturally regain most of their pre-stroke function eventually. So the Stanford researchers intend to test the drug in other animal models, as well as to experiment with different dose sizes and timing, before proceeding to clinical trials.

"Before this study, the thinking in the field was that GABA signaling after a stroke was detrimental," said Steinberg. "But now we know that if it's the right kind of GABA signaling, it's beneficial. And we've identified an FDA-approved drug that decisively promotes the beneficial signaling."

Lead authorship of the study is shared by Takeshi Hiu, MD, PhD, a former postdoctoral scholar in the Steinberg laboratory, and Zoya Farzampour, PhD, a former graduate student in the Huguenard laboratory.

Other Stanford co-authors are former postdoctoral scholar Jeanne Paz, PhD, now at the University of California-San Francisco; former research assistants Eric Wang and Corrine Badgely; Andrew Olson, director of microscopy for the Department of Neurology; basic life science research associates Gordon Wang, PhD, and Xibin Liang; former visiting scholars Robin Lemmens, MD, PhD, and Yasuhiro Nishiyama, MD; former life science research assistant Kevin Tran, MD; Scott Hamilton, PhD, a consulting associate professor at the Stanford Stroke Center; senior research scientists Kristina Micheva, PhD, and Nancy O'Rourke, PhD; and former professor of molecular and cellular physiology Stephen Smith, PhD.

The study was supported by the National Institutes of Health, Bernard and Ronni Lacroute, Russell and Elizabeth Siegelman and the National Science Foundation.

Stanford's Department of Neurosurgery also supported the work. **ISM**

Infection

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the genes and render it as molecules of protein or RNA. Cells have the capacity to express more or less of each molecule, creating a pattern of expression that changes in response to external influences — including infection by viruses.

Purvesh Khatri, PhD, assistant professor of medicine, and a team of six other researchers at Stanford identified 396 human genes whose expression changes in a consistent pattern that reveals the presence of a viral infection. The pattern of changes, which they call the meta-virus signature, occurs in a range of viruses and is distinct from the pattern of gene expression in healthy people or in people with bacterial infections. The meta-virus signature pattern of gene expression is also present even before a person has clear symptoms of infection.

In their paper, which was published Dec. 15 in *Immunity,* the authors also described a second gene expression pattern that signals when a person is infected specifically with the flu virus. This second pattern, the influenza meta-signature, consists of a change in the expression of just 11 human genes. The influenza meta-signature pattern can distinguish flu from other viral infections, as well as from bacterial infections. It can also identify a flu infection before a person has symptoms and even reveal whether a person is building immunity after getting the flu vaccine.

Khatri, a bioinformatician, is the senior author of the paper. Lead authorship is shared by doctoral student Marta Andres-Terre and former postdoctoral scholar Helen McGuire, PhD.

Khatri said his team was motivated by the long-term goal of finding broad-spectrum antiviral drugs, much like the broad-spectrum antibiotics that have saved so many people from deadly bacterial infections. Broad-spectrum antivirals could be used against dengue fever and other killers, he said.

Waving a red 'infection' flag

The researchers' first step was to look for a general change in gene expression in response to infection by viruses generally. They began by looking at changes in gene expression in a set of publicly available data. In blood samples from 205 people infected with a flu, cold or respiratory syncytial virus, the team found 396 genes whose expression changed in the same way during all three types of infections, with an increase in the expression of 161 genes and a decrease in the expression of 235 genes.

The team then found the same pattern in a larger group of blood and tissue cell samples from 2,939 people consisting of healthy controls and those infected with a diverse array of pathogens, including viruses such as SARS coronavirus, enterovirus and adenovirus, as well as bacteria such as *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae* and Salmonella. In the larger group, the team found the same altered pattern of expression in the same 396 genes among patients with viral infections.

The meta-virus signature not only identified individuals with an active viral infection, but also those who were incubating one. By studying blood samples taken frequently — every eight hours for five days — the Stanford team discovered the meta-virus signature pattern waving a red "infection" flag up to 24 hours before the first symptoms. "An individual's gene expression signature changed before they became sick, so we could predict up to 24 hours before who was going to show symptoms," said Khatri.

The same high-frequency sampling data also revealed that the meta-virus signature signal, the one indicating any virus, began first. Then, a few hours later, the more-specific influenza meta-signature signal began in people with the flu. "It seems that when there is a viral infection, the immune system turns on a general response to all viruses, followed by a virus-specific response to the particular virus," said Khatri. "You can imagine a decision tree where the immune system asks, 'Is it bacterial or viral?' And if it's viral it turns on the meta-virus signature response. And then it asks, 'If it's viral, which virus is it?' And then it turns on a specialized response for that virus."

Theoretically, the meta-virus signature could be used clinically to distinguish viral from bacterial infections to determine if an antibiotic should be prescribed. The Khatri lab has funding to develop such a test.

The team's work is an example of Stanford Medicine's focus on precision health, the goal of which is to anticipate and prevent disease in the healthy and precisely diagnose and treat disease in the ill.

Is the vaccine working?

Another goal is to more precisely determine whether someone is responding to vaccination. "The goal of vaccination is to generate the same immune response without the symptoms," he said. "If the IMS response is truly virus-specific, we should see the same response in vaccination." And, in fact, the Khatri team found that in three independent studies of flu vaccine recipients, all those judged to have responded to vaccination by other measures also displayed the 11-gene influenza meta-signature. Likewise, nonrespondents showed no influenza meta-signature response. In short, if you see the gene expression signature, you know the person is responding to the vaccine.

Until now, said Khatri, no one has found the immune response that turns on in both the vaccination response and in actual infections. This paper demonstrates for the first time a "transcriptional signature" that can be used as a proxy for whatever immune mechanism is induced by both vaccination and infection. "We have identified the common signature that links infection and vaccination," he said.

The work on the vaccination response also added to the understanding of men's immune response, which is different from women's. Other research has suggested that men's immune response to vaccines was somehow suppressed. In previous work, researchers looked at men's and women's responses on the third day after vaccination, when women had a strong reaction and men had none. But Khatri's group found that men were responding most on the first day after vaccination. In other words, men were responding to flu vaccine sooner than women. By the third day, men's immune response returned to baseline. "The dynamics are different," he said, "and we haven't been sampling at the right time."

The Stanford paper also looked at samples from patients with acute pneumonia. In these patients, the influenza meta-signature distinguished viral pneumonia from bacterial pneumonia. As patients recovered, their influenza meta-signatures gradually returned to a healthy baseline level. "So you can also use IMS to monitor patients' progress," said Khatri.

Other Stanford-affiliated authors of the paper are doctoral student Erika Bongen; research associate Timothy Sweeney, PhD; and Cristina Tato, PhD, MPH, research and science analyst.

This work was supported by the National Institutes of Health , the La Caixa Foundation, a National Health and Medical Research Council early career fellowship and the Stanford Institute for Immunity, Transplantation and Infection.

Stanford's Department of Medicine and Department of Surgery also supported the work. **ISM**

Toes

continued from page 1

biology at Stanford. "This change is likely part of the reason why we've evolved from having a grasping hind foot like a chimp to a weight-bearing structure that allows us to walk on two legs."

Kingsley, who is also a Howard Hughes Medical Institute investigator, is the senior author of a paper describing the work that was published online Jan. 7 in *Cell*. The lead author is former Stanford postdoctoral scholar Vahan Indjeian, PhD, now head of a research group at Imperial College London.

Adapting to different environments

The threespine stickleback is remarkable in that it has evolved to have many different body structures to equip it for life in different parts of the world. It sports an exterior of bony plates and spines that act as armor to protect it from predators. In marine environments, the plates are large and thick; in freshwater, the fish have evolved to have smaller, lighter-weight plates, perhaps to enhance buoyancy, increase body flexibility and better slip out of the grasp of large, hungry insects. Kingsley and his colleagues wanted to identify the regions of the fish's genome responsible for the skeletal differences that have evolved in natural populations.

The researchers identified the area of the genome responsible for controlling armor plate size, and then looked for differences there in 11 pairs of marine and freshwater fish with varying armor-plate sizes. They homed in on a region that includes the gene for a bone morphogenetic protein family member called GDF6. Due to changes in the regulatory DNA sequence near this gene, freshwater sticklebacks express higher levels of GDF6, while their saltwater cousins express less. Strikingly, marine fish genetically engineered to contain the regulatory sequence of freshwater fish expressed higher levels of GDF6 and developed smaller armor plates, the researchers found.

Regulatory regions in humans vs. chimps

Kingsley and his colleagues wondered whether changes in GDF6 expression levels might also have contributed to critical skeletal modifications during human evolution. The possibility was not as far-fetched as it might seem. Other studies by evolutionary biologists, including Kingsley, have shown that small changes in the regulatory regions of key developmental genes can have profound effects in many vertebrates.

They began by working with colleagues in the laboratory of Gill Bejerano, PhD, Stanford associate professor of developmental biology, of computer science and of pediatrics, to compare differences in the genomes of chimps and humans. In previous surveys, they found over 500 places in which humans have lost regulatory regions that are conserved from chimps and many other mammals. Two of these occur near the GDF6 gene. They homed in on one in particular.

STEVE FISCH

A study of stickleback fish led David Kingsley and his colleagues to identify a genomic region possibly linked to modifications in human toes and feet that enable upright walking.

"This regulatory information was shared through about 100 million years of evolution," said Kingsley. "And yet, surprisingly, this region is missing in humans."

To learn more about what the GDF6 regulatory region might be controlling, the researchers used the chimp regulatory DNA to control the production of a protein that is easy to visualize in mice. Laboratory mice with the chimp regulatory DNA coupled to the reporter protein strongly and specifically expressed the protein in their hind limbs, but not their forelimbs, and in their lateral toes, but not the big toes of the hind limbs. Mice genetically engineered to lack the ability to produce GDF6 in any part of their bodies had skull bones that were smaller than normal and their toes were shorter than those of their peers. Together, these findings gave the researchers a clue that GDF6 might play a critical role in limb development and evolution.

The big toe: an explanation

The fact that humans are missing the hind-limb-regulatory region probably means that we express less of the gene in our legs and feet during development, but comparable amounts in our nascent arms, hands and skulls. Loss of this particular regulatory sequence would also shorten lateral toes but not the first toe of feet. This may help explain why the big toe is aligned with other short, lateral toes in humans. Such a modification would create a more sturdy foot with which to walk upright.

"These bone morphogenetic proteins are strong signals for bone and cartilage growth in all types of animals," said Kingsley. "You can evolve new skeletal structures by changing where and when the signals are expressed, and it's very satisfying to see similar regulatory principles in action whether you are changing the armor of a stickleback, or changing specific hind-limb structures during human evolution."

Other Stanford co-authors of the study are graduate student Garrett Kingman, former postdoctoral scholar Felicity Jones, PhD, and research specialist Catherine Guenther, PhD.

The research was supported by the National Institutes of Health and the Howard Hughes Medical Institute.

Stanford's Department of Developmental Biology also supported the work. **ISM**

Stanley Falkow to be awarded National Medal of Science

By Krista Conger

Stanley Falkow, PhD, the Robert W. and Vivian K. Cahill Professor in Cancer Research, Emeritus, at the School of Medicine, has been awarded the 2015 National Medal of Science. The honor was announced Dec. 22 by the White House.

Falkow is being recognized for his pioneering work in studying how bacteria can cause human disease and how antibiotic resistance spreads.

"It was a total surprise," said Falkow, who learned of the award on Dec. 19 in an email from John Holdren, PhD, the president's chief science adviser. "I always say, 'In science, it's not "I," it's "we." And it's so true. There are hundreds of students and colleagues around the world with whom I'd like to share this honor."

"Dr. Falkow is deeply deserving of this award," said Lloyd Minor, MD, the dean of the School of Medicine. "He has made invaluable contributions to the field of microbiology and understanding the effect of bacteria on human health. We at Stanford Medicine are extremely proud and honored he has been recognized in this way."

Falkow, 81, is an emeritus professor of microbiology and immunology and a member of the Stanford Cancer Institute. The award will be presented in a ceremony at the White House later this month.

Falkow is well-known for his work on extrachromosomal elements called plasmids and their role in antibiotic resistance and pathogenicity in humans and animals. As a graduate student in the early 1960s, first at the University of Michigan and later at Brown University, and then as an independent researcher at Georgetown University, he learned the biochemical and microbiological techniques necessary to deduce how bacteria transmit antibiotic resistance to one another. In particular, he found that some bacteria were resistant to antibiotics to which they had never been exposed, which at first confounded researchers. Falkow subsequently discovered that bacteria gained their resistance by sharing their genes much more promiscuously than had been thought possible.

When Falkow arrived at Stanford in 1981, he set aside his study of plasmids to concentrate on how organisms as diverse as cholera, plague and whooping cough cause disease in humans.

Falkow is one of nine recipients of the 2015 National Medal of Science, which recognizes individuals for outstanding contributions to the fields of several scientific disciplines. He is one of two Stanford recipients; the other is psychologist Albert Bandura, PhD, the David Starr Jordan Professor, Emeritus.

L.A. CICERO

Stanley Falkow will receive a 2015 National Medal of Science at a White House ceremony.

"We congratulate both emeriti professors Stanley Falkow and Albert Bandura on this extremely well-deserved honor. We are so proud that they have been recognized for their contributions not just to our country, but to humanity," said Stanford President John Hennessy, PhD. "Their lifetime of work in preventing infectious disease, and in learning how we can understand and change behavior, has been instrumental in helping people around the world lead healthier, more productive and more peaceful lives."

Falkow's previous honors include the 2008 Lasker-Koshland Award for Special Achievement in Medical Science; the 2000 Robert-Koch Award from the Robert-Koch Foundation in Germany; election to the Institute of Medicine; membership in the National Academy of Sciences and the Royal Society; and a former presidency of the American Society of Microbiology. ISM

OF NOTE

reports on significant honors and awards for faculty, staff and students

MICHELE BERK, PhD, was appointed assistant professor of psychiatry and behavioral sciences, effective Sept. 1. She specializes in researching and preventing adolescent suicide. Her most recent project investigates the effectiveness of dialectical behavior therapy on decreasing repeat suicide attempts among at-risk adolescents.

HELEN BLAU, PhD, was elected to the council of the American Academy of Arts and Sciences for a four-year term. The council oversees the academy's membership selection process and provides oversight of its academic efforts. She is the Donald E. and Delia B. Baxter Foundation Professor, director of the Baxter Laboratory for Stem Cell Biology and professor of microbiology and immunology.

SANJIV SAM GAMBHIR, MD, PhD, and **BRIAN KOBILKA**, MD, were named 2015 fellows of the National Academy of Inventors. Gambhir, the Virginia and D.K. Ludwig Professor for Clinical Investigation in Cancer Research and professor and chair of radiology, develops strategies for integrated diagnostics and molecular imaging of living subjects. Kobilka, a professor of molecular and cellular physiology who holds the Hélène Irwin Fagan Chair in Cardiology and who won the 2012 Nobel Prize in chemistry, investigates G-protein-coupled receptors.

SIGURDIS HARALDSDOTTIR, MD, was appointed assistant professor of medicine, effective Oct. 1. She specializes in gastrointestinal cancers with a clinical focus on colorectal cancer. Her research focuses on mismatch repair deficiency and the inherited cancer disorder Lynch syndrome. She is characterizing drivers of colorectal cancer by analyzing population-based cohorts, with the goal of identifying new drug targets.

ANNE MUEHE, MD, a postdoctoral fellow in radiology, received a $1,000 research trainee prize for her studies on the safety of ferumoxytol nanoparticles as an MRI contrast agent in children at the annual meeting of the Radiological Society of North America in December. She studies clinical imaging technologies for cancer staging in children and young adults.

ZARA PATEL, MD, was appointed assistant professor of otolaryngology-head and neck surgery, effective Sept. 1. She specializes in endoscopic sinus and skull-base surgery. Her research interests include treatment of refractory sinusitis, racial disparities in sinonasal cancer survival and curing olfactory loss.

DOLORES GALLAGHER THOMPSON, PhD, professor of psychiatry and behavioral sciences, was awarded an honorary doctorate in education by the Hong Kong Institute of Education in November. She directs the Stanford Geriatric Education Center and outreach, recruitment and education for the School of Medicine's new Alzheimer's Disease Research Center.

JOSEPH WU, MD, PhD, director of the Stanford Cardiovascular Institute, received the inaugural Joseph A. Vita Award at the 2015 American Heart Association meeting in Orlando, Florida. This award is given to an investigator whose published work in the past five years has had a transformative impact on cardiovascular research. Wu is the Simon H. Stertzer Professor and professor of medicine and of radiology. ISM

Michele Berk

Helen Blau

Sanjiv Gambhir

Brian Kobilka

Sigurdis Haraldsdottir

Anne Muehe

Zara Patel

Dolores Gallagher Thompson

Joseph Wu

ADHD

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leagues have previously proposed that poor coordination between these three brain networks could underlie a variety of psychiatric and neurologic problems, including depression, schizophrenia, brain injury, autism and drug addiction.

The children with ADHD had weaker interactions between these networks than children without the condition. The difference was large enough that brain scans could distinguish kids who had ADHD from those who did not. Among children with ADHD, worse scores on clinical tests of inattentiveness were linked with weaker interactions between the three brain networks.

"These three brain networks come up over and over in pretty much every cognitive task we ask subjects to do," said Menon, who holds the Rachael L. and Walter F. Nichols, MD, Professorship. "They are critical for information processing and attending to stimuli in the environment."

Future research is needed to explore whether fMRI can also differentiate between the brains of children with ADHD and those with other psychiatric or neurodevelopmental conditions, the researchers said. Answering that question is an important aspect of determining whether brain scans could become a practical component of ADHD diagnosis.

Other Stanford co-authors on the study are research associates Tianwen Chen, PhD, and Kaustubh Supekar, PhD; and research assistant Luca Szgletes.

The research was supported by the National Institutes of Health and a Big Data for Human Health Seed Grant from the Li Ka Shing Foundation.

Stanford's Department of Psychiatry and Behavioral Sciences also supported the work. ISM