INSIDE STANFORD MEDICINE

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A scientist investigates why some pain dissipates after an injury has healed, while other pain hangs around long after.

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Bacteria, virus form diabolical partnership

By Bruce Goldman

A common bacterial pathogen called Pseudomonas aeruginosa produces a virus that substantially increases the pathogen's ability to infect us, according to a study by investigators at the School of Medicine.

P. aeruginosa weaponizes its resident virus to exploit the immune system's distinct responses to bacterial versus viral infections.

This marks the first time a bacteria-infecting virus, otherwise known as a bacteriophage or simply a phage, has been observed inducing the immune system to mount an antiviral response and, in doing so, causing it to ignore the bacterial infection. When the scientists generated a vaccine directed at the virus, they showed that it dramatically lowered the bacteria's ability to infect wounds in mice.

Detailed in a study published March 29 in *Science*, the findings could fuel new ways of preventing chronic, intractable infections by keeping antibiotic-resistant bacteria from getting a foothold in the first place. The discovery that phages foster bacterial infections also adds a previously unexpected layer of complexity to the relationship between us and the billions of bacteria inhabiting our gut and other organs.

Paul Bollyky, MD, PhD, assistant professor of infectious diseases and of microbiology and immunology, is the study's senior author. The lead author is former graduate student Johanna Sweere, PhD.

NORBERT VON DER GROEBEN

Paul Bollyky is the senior author of a study that found a possible method for reducing harm caused by a dangerous bacteria called Pseudomonas aeruginosa.

Quadrillions of phages in body

"We've long known that you've got up to 10 quadrillion phages in your body, but we just figured whatever they were doing was strictly between them and your commensal bacteria," Bollyky said. "Now we know that phages can get inside your cells, too, and make you sick."

There's currently no approved vaccine targeting P. aeruginosa, an increasingly drug-resistant pathogen that infects the lungs of most adults with cystic fibrosis and accounts for a sizeable percentage of all infections of diabetic ulcers, bedsores and burn wounds.

In 2017, the World Health Association named P. aeruginosa one of the "critical priority" pathogens posing the greatest threat to human health.

"I see this every day in my clinical practice," Bollyky said. "What starts off as a little cut can't heal as a result of a persistent, drug-resistant bacterial infection. The toll in terms of sickness, death and dollars is enormous." Infected diabetic foot ulcers are the single biggest cause of amputation, he said. **See PHAGE, page 8**

Study: Starting colorectal cancer screening at 45 would prevent deaths

But screening more older adults would have even greater benefits

By Amy Jeter Hansen

Starting routine colorectal cancer screening at age 45 rather than 50 would decrease U.S. cancer deaths by as much as 11,100 over five years, according to a new study led by researchers at the School of Medicine.

The move would also decrease the number of cancer cases nationwide by up to 29,400 over that time period. However, screening **See COLORECTAL, page 6**

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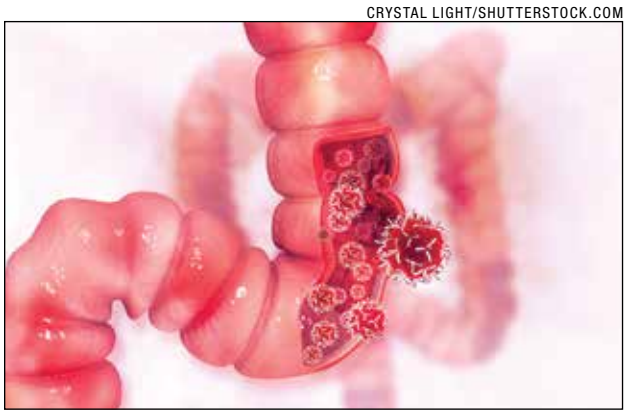

Over the next five years, initiating colorectal screening at age 45 instead of 50 could reduce the number of cancer cases by as many as 29,400 and deaths by up to 11,100, according to a new study.

Blocking protein restores mental acuity of old mice, according to researchers

By Bruce Goldman

By blocking a protein's activity with antibodies, School of Medicine investigators were able to improve cognitive behavior in aging mice.

A paper describing the finding was published online April 3 in *Nature*. Tony Wyss-Coray, PhD, professor of neurology and neurological sciences, is the senior author. The lead author is MD-PhD student John Pluvinage.

Wyss-Coray has been working for several years on the question of what causes the brain to lose its acuity with advancing age. One focus of his research has been a class of brain cells called microglia, which serve both as the brain's immune cells and its garbage crew. Among the many different things microglia do to keep the brain healthy is scarfing up bits of cellular debris and protein deposits that build up in the course of normal metabolic activity.

On average, the garbage-collecting performance of microglia diminishes in aging brains. Why this happens, and the extent to which the faulty garbage service is actually responsible for age-related cognitive losses, are unclear. But it's a decent bet that one way or another, microglial malperformance plays a role in neurodegeneration, said Wyss-Coray, the D. H. Chen Professor II and a senior research career scientist at the Veterans Affairs Palo Alto Health Care System.

"Many of the genes whose high-risk variants have recently been linked to Alzheimer's disease are known

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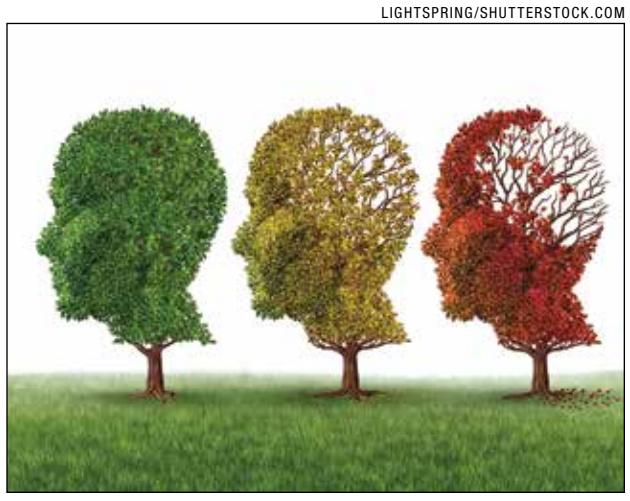

to be active in the brain only in microglia," he said. "Microglial genes' activation patterns are abnormal in Alzheimer's patients, and in other neurodegenerative disorders including Parkinson's disease and amyotrophic lateral sclerosis.

"We think we may have discovered a way to get those cells back on track and make them work the way they used to when we were young."

The ingest-then-digest procedure employed by microglia and other immune-cell types in the body is called phagocytosis. The study used laboratory techniques to identify mouse genes whose activity either impairs or enhances microglial **See MICROGLIA, page 6**

‘Free lunch’ warps inner spatial map in rat brains, study finds

By Bruce Goldman

The next time you’re offered a free sample as you’re walking past a storefront, go ahead and take it. But be aware that the more you like that little chunk of cheese or sip of herbal tea, the likelier your brain’s internal map will warp in a way that increases your ability to return to the spot where you got your freebie.

Our brains’ neural circuitry creates spatial maps as we navigate through new environments, allowing us to recall locations and directions. While it’s been known for some time that we have these internal maps, a study from the School of Medicine published online March 29 in *Science* shows how, in rats, they get redrawn when the rats learn they’ll receive a reward at a certain place on the map. This same process could play a role in addictive behavior in humans.

NORBERT VON DER GROEBEN

Lisa Giocomo and her team discovered that the internal maps in rats’ brains were redrawn to reflect where the rodents received rewards. This same process could play a role in addictive behavior in humans.

Lisa Giocomo, PhD, assistant professor of neurobiology, is the study’s senior author. Lead authorship is shared by postdoctoral scholar William Butler, PhD, and graduate student Kiah Hardcastle.

Brain area crucial to navigation

“Every time you check your Google map for a particular address or restaurant name or pair of grid coordinates, you get the same map regardless of why you’re looking at it,” Giocomo said. “The GPS that generates that map doesn’t care what you’re doing or where you’re going, or whether you’re happy, hungry or hung over. It’s always going to give you the same information.”

Scientists have assumed the brain’s internal GPS operates similarly. But it turns out that’s not quite right.

“In this study, we’ve learned your internal map changes depending on your behavior, memories and state of mind,” Giocomo said. “We pull up different maps for the same space, depending on what we’re actually trying to do in that space.”

Giocomo’s research has been focused on a brain area called the medial entorhinal cortex, which is crucial to navigation. Nestled near the center of the human brain, it integrates information from our senses to generate maps of new places.

Over the past 15 years or so, scientists have learned that various nerve cells in our medial entorhinal cortex act as compasses, speedometers, latitude and longitude coordinates, or boundary and landmark detectors. These cells have been identified in rodents, bats, monkeys and humans, suggesting that such spatial-mapping circuitry is a universal mechanism of mammalian navigation and that the findings in the study apply to humans as well.

Until now, all indications have been that it’s just that simple. But that’s because the experiments designed to capture and measure the mapping process have been deliberately kept simple, for the sake of getting decipherable results. A standard experimental setup, for example, features a spacious yet simple environment: a big, open-top box in which the floor is littered with bits of crushed cereal. No consideration is given to the test animals’ mood or intent. The animals can walk freely all around the box, foraging at their leisure for crushed Cheerios, while researchers collect data via physiological monitoring of the creatures’ brain cells. It is a relatively easy experiment to do. The scientists who thought it up won a Nobel Prize for it in 2014. (The work was done under the leadership of a pair of Norwegian researchers under whom Giocomo did a postdoctoral fellowship.)

“But animals don’t typically walk around inside big black boxes in the hope of hoovering up Cheerios dust,” Giocomo said. “You usually have a goal. So we decided to design a situation that would stimulate that goal-directedness but would also be able to relate whatever we found to what had been studied for the past 15 years.”

That meant shifting experimental animals between two alternate environments, one encouraging random meandering and the other one fostering goal-driven behavior.

A new box

To test goal-driven behavior, Giocomo and her colleagues designed a big box that was exactly the same size and shape as the one in the traditional experimental setup. Both boxes had floors with randomly scattered, crushed Cheerios. During experiments in either box, animals could forage freely and eat any Cheerio bits they found. But there was an important difference. The second box had an unmarked, roughly 8-by-8-inch “reward zone” in a fixed location on its Cheerios-strewn floor. The test animals were permitted to forage freely in this box, just as in the other one. But they soon learned that if, in response to an auditory cue, they navigated to the reward zone, they’d get a guaranteed, good-sized crushed-cereal reward. This reward was available only intermittently, and only for a short period after the cue.

Picture a “free lunch” counter in a supermarket. The counter is only open some of the time — but when it is, a storewide advertisement blares the news to hungry shoppers over the public address system.

The investigators implanted electrodes in several hundred nerve cells in the medial entorhinal cortex of rats that were placed in each of the two environments. The electrodes were connected to long cables, so the researchers could monitor individual nerve cells’ electrical activity as the animals roved freely inside whichever box they were put in.

“Rats’ spatial mapping system is the same as ours,” Giocomo said. “For rodents, they’re pretty smart. They like to move around. And they love Cheerios.”

Giocomo’s team collected and analyzed massive amounts of data, which enabled them to identify individual cells in each rat’s medial entorhinal cortex that served as compasses, speedometers and position detectors. They also observed that once a rat had learned enough about how the two environments differed — mainly, that one featured an occasional, well-advertised “free lunch” — several types of spatial-map-related cells in its medial entorhinal cortex changed their firing patterns whenever the animal got within about a foot of the “lunch counter.” For example, as the rats came within about a foot of the center of the reward zone, whether or not the free-lunch counter was open, its position-signifying cells fired faster, and the position-signifying cells that were firing were spaced closer together, indicating higher spatial resolution.

“This tells us the rat’s brains are making a new map of space, in response to their experience of a reward, that reflects the importance of the place where they got it by providing a more accurate representation of its position,” Giocomo said. If the reward is a drug of abuse, she said, the improved accuracy at the center of this reward-based map could enable an addict’s habit.

So the next time you find yourself wending your way through some nondescript side street in a strange neighborhood in search of a parking spot, remind yourself to gobble a chunk of chocolate as you’re getting out of your car. It might make it easier for you to remember where you parked. **ISM**

Some with PTSD unresponsive to behavioral therapy due to biology

By Helen Santoro

How well-connected a particular brain network is, and how successfully memories are formed, may determine which patients with post-traumatic stress disorder benefit from behavioral therapy, researchers at the School of Medicine have found.

The finding could indicate a biological subtype of PTSD whose clinical relevance only becomes obvious when patients undergo treatment, the researchers said. Furthermore, by replicating their results across a diverse range of patients, the researchers were able to clearly and objectively characterize a biological signature in PTSD patients who differ in their response to behavioral therapy.

Amit Etkin

Ruth O’Hara

The gold standard of behavioral therapy for PTSD is called prolonged exposure therapy. This treatment involves gradually exposing patients to trauma-related fears, such as loud noises or crowds. Through repeated exposures, patients become accustomed to these triggers and suffer less distress from them.

“However, exposure therapy does not work for everyone with PTSD. By better understanding the biological components of the disease, we hope to be able to develop more personalized treatments for patients who are struggling to recover,” said Amit Etkin, MD, PhD, professor of psychiatry and behavioral sciences and lead author of the study.

For the study, the researchers turned their attention to a brain network called the ventral attention network. The VAN is critical in cognition and memory. Abnormalities in this network have been linked to a range of psychiatric conditions, including schizophrenia, depression and anxiety. The scientists found that some individuals with PTSD have both reduced VAN function and diminished ability to recall lists of words during a during a memory test. Those with both traits were not responsive to exposure therapy.

This was the first study to use biology to identify two **See PTSD, page 3**

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STANFORD MEDICINE

Stanford acquires archive of palliative care pioneer Elisabeth Kübler-Ross

The archive of Elisabeth Kübler-Ross, MD, the late hospice and palliative care pioneer and author of some two-dozen books, including the 1969 *On Death & Dying*, has been acquired by Stanford Libraries Department of Special Collections.

The Swiss-American psychiatrist is best known for having developed the theory of the five stages of grief.

"The Kübler-Ross Archive is a wonderful addition to our Special Collections and offers tremendous opportunity for interdisciplinary investigation and exploration across law, medicine and sociology, to name only a few," said Matt Marostica, PhD, JD, associate university librarian for public service and collection development.

David Magnus, PhD, the Thomas A. Raffin Professor in Medicine and Biomedical Ethics and director of the Stanford Center for Biomedical Ethics, identified unpublished lectures and essays by Kübler-Ross from the late 1970s and early 1980s. He proposes to edit them for publication. Among the many documents in the archive, he highlighted a trove of thousands of letters from dozens of countries reflecting her widespread influence around the world.

Maren Monsen, director of the Program in Bioethics and Film at the Stanford Center for Biomedical Ethics, plans to make use of video components of the Kübler-Ross archive in documentary films. After the video content is transferred to a high-quality digital format, she intends to create educational videos for distribution to multidisciplinary trainees in the health professions.

The children of Kübler-Ross — Ken Ross, president of the Elisabeth Kübler-Ross Foundation, and Barbara Rothweiler, PhD — chose to give the archive to Stanford Libraries after meetings in Phoenix with faculty and with Benjamin Stone, curator for American and British history at the libraries.

Like most modern archives, the collection runs the gamut from manuscript and printed materials to correspondence, photographs, video and audio recordings. Most important, it contains archived, not-yet-made-public work by the pioneering thinker and practitioner in the palliative care movement.

The earliest item in the archive is a family scrapbook from the 1930s depicting Kübler-Ross' childhood in Switzerland. The bulk of the archive includes material from the 1980s to 2000s, a period in which her ideas gained greater traction. Of special note are complete runs of newsletters from the Shanti Nilaya Healing Center, which she founded in Escondido, California, as well as manuscript drafts of her memoir, *The Wheel of Life*. **ISM**

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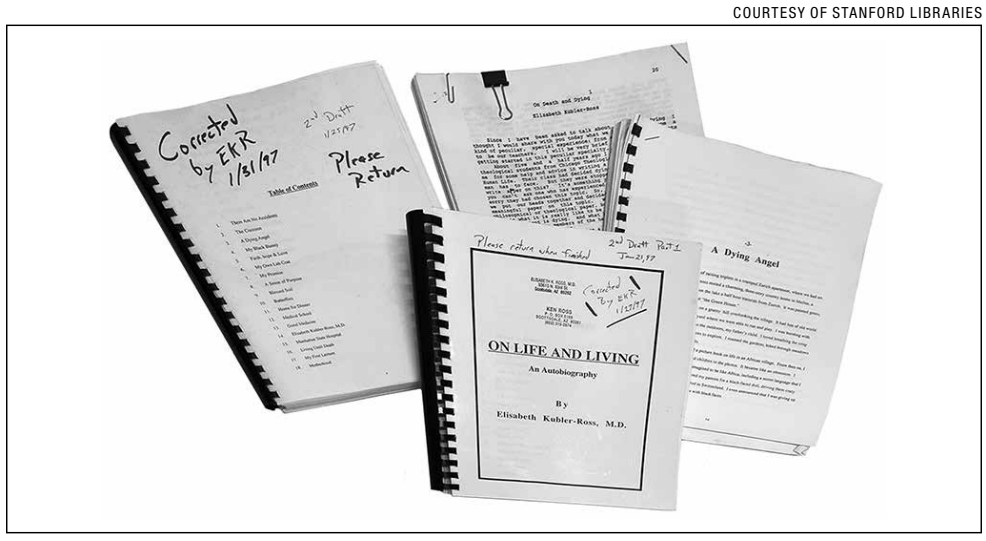


(Top) Elisabeth Kübler-Ross, the palliative-care pioneer and author of the 1969 book *On Death and Dying*. (Bottom) Manuscripts from the Kübler-Ross archive, housed in Stanford Libraries' Department of Special Collections.

PTSD

continued from page 2

types of people with PTSD, thereby transcending diagnosis based on clinical grounds alone, Etkin said. The researchers identified a clear brain-function marker that could be pinpointed regardless of whether the study participants were civilians or veterans, or how their clinical diagnosis was made.

"We really have to start utilizing these kinds of biological markers in order to get at a more fundamental understanding of the disorder and a far greater prediction of treatment response," said Ruth O'Hara, PhD, the study's senior author and a professor of psychiatry and behavioral sciences.

The study was published April 3 in *Science Translational Medicine*.

Watching the brain communicate

Among people who experience or witness a traumatic event, most will be faced with temporary difficulties in the short term but will recover over time. Others may develop PTSD. Symptoms, such as nightmares, heightened anxiety and intrusive memories, can persist for months or even years.

Such individuals typically go on to receive exposure therapy. Yet some patients do not benefit from it, and about a third drop out of treatment, according to O'Hara.

"There is a critical problem in the treatment of psychiatric disorders," she said. "Diagnoses are all based on psychiatric symptoms, which for the most part are self-reported. And there is a huge amount of heterogeneity in how people self-report."

To develop more personalized treatments, the researchers examined PTSD patients' behavior and brains to understand why some patients don't respond to exposure therapy.

They conducted two studies with demographically and clinically distinct patient populations. In the first study, 112 participants, 76 of whom had been diagnosed with PTSD, underwent behavioral and clinical assessments, as well as functional magnetic resonance imaging to measure brain activity. They also completed a test of their ability to recall words. In the test, they were asked to memorize blocks of 20 words and then recall them immediately and again 15 minutes later. Sixty-six of the participants with PTSD continued on to a second part of the trial, in which they received either prolonged exposure therapy or no treatment. The majority of those participants then completed a clinical assessment of their PTSD symptoms.

In a second study, the researchers considered 245 combat veterans, 117 of whom were trauma-exposed but healthy and 128 of whom had been diagnosed with PTSD. They underwent behavioral and clinical assessments, including the verbal memory test and an fMRI scan.

In both studies, the findings revealed that a subgroup of participants with PTSD had both reduced verbal memory and VAN functioning compared with the rest of the PTSD participants and healthy control subjects. The researchers observed that participants with PTSD who had reduced verbal memory and VAN functioning did not respond to therapy, whereas the other participants with PTSD did respond.

The finding emphasizes the importance of considering a patient's biology in developing a treatment for their PTSD, O'Hara said. "Our study shows that this specific combination of a deficit in VAN communication and poor memory leads to a failure to respond to exposure therapy, above and beyond their specific symptoms," she said.

A deeper look into the VAN

In another study reported in the same paper, the veterans also underwent a direct brain activity test that used simultaneous transcranial magnetic stimulation and electroencephalography, a test that measures brain waves.

TMS is used to noninvasively manipulate activity in a targeted brain region and the networks to which it's connected. Unlike fMRI, which shows a correlation between a certain behavior and brain activity, TMS allows scientists to temporarily disrupt information processing in a particular brain region through magnetic pulses and see exactly which behavior or brain function is affected. To detect these effects, participants were also hooked up to an EEG machine, which picks up the communication between neurons using sensors that are placed on a patient's scalp.

The TMS and EEG tests revealed that the brains of veterans with poor connectivity in their ventral attention network took twice as long to recover from the TMS magnetic pulse than the brains of patients with good communication in their VAN, indicating a deficiency in information flow within this brain network.

Toward a more personalized treatment

Etkin hopes to use this information as a means to develop more specialized mental health care. Down the road, clinicians could potentially perform repetitive TMS — a therapeutic tool that is currently used to help treat depression — to stimulate the VAN and ease PTSD symptoms.

A consistent challenge in creating personalized treatments for psychiatric conditions is that the Diagnostic and Statistical Manual of Mental Disorders, which classifies all mental disorders, is constantly evolving. But if clinicians can one day use biological measures instead, they will no longer have to deal with these varying definitions of disease, Etkin said.

Etkin and his team aim to translate their findings into treatments for PTSD. Brain-imaging studies of psychiatric disorders often stop short of that translation, he said. "To me, that's not satisfying," he said. "If you were to take these findings, how would you set up a study that would allow you to get to an FDA-approved biomarker quickly — not in 20 years, but in five years? That's what we are now focused on."

The 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.

O'Hara and Etkin are members of the Wu Tsai Neurosciences Institute at Stanford and investigators at the Mental Illness Research, Education and Clinical Center of the Sierra Pacific at the Veterans Affairs Palo Alto Health Care System. O'Hara is also a member of the Stanford Maternal & Child Health Research Institute.

Other Stanford co-authors of the study are postdoctoral scholars Adi Maron-Katz, PhD, Gregory Fonzo, PhD, Corey Keller, MD, PhD, and Julia Huemer, PhD; instructor Wei Wu, PhD; software engineer Brian Patenaude, PhD; research specialist Yevgeniya Zaiko; research coordinators Kathy Peng, Emmanuel Shpigel, Parker Longwell, Raleigh Edelstein and Irene Akingbade; graduate student Russ Toll; associate professors of psychiatry and behavioral sciences Allison Thompson, PhD, and Steven Lindley, PhD; Sanno Zack, PhD, clinical associate professor of psychiatry and behavioral sciences; clinical psychologist Elizabeth Weiss, PsyD; program manager Jillian Autea; and professors of psychiatry and behavioral sciences Bruce Arnow, PhD, and Joachim Hallmayer, MD.

Researchers from the VA-Palo Alto; Sierra Pacific Mental Illness, Research, Education and Clinical Center; South China University of Technology; New York University Langone School of Medicine; University of Cambridge; Cambridgeshire and Peterborough NHS Foundation Trust; Lausanne University Hospital; Siemens Healthcare; New Mexico Veterans Affairs Healthcare System; University of New Mexico; University of Pennsylvania; Pontificia Universidad Católica de Chile; and Emory University School of Medicine also contributed to this work. **ISM**

Researcher investigates why some pain becomes agonizing a

By Nicoletta Lanese

Stacey Morris remembers being roused in the emergency room one summer night in 2008. "I took too many pills," she told the hospital staff. "I don't know what I took."

Morris, whose name is changed for this article to protect her privacy, was kept overnight on a psychiatric hold because the doctors thought she may have attempted suicide. But that wasn't the case, she insists.

Morris said she had accidentally overdosed on her prescribed medications for chronic pain, sent to the ER by a combination of gabapentin, Ambien and a small glass of wine. She was nearly a statistic, illustrative of a disturbing trend: More than 6,600 American women died of prescription painkiller overdose in 2010 — more than five times as many as in 1999. In 2016, women died from prescription opioid overdoses at a rate of 4.3 per 100,000.

Six months before Morris' overdose, surgeons removed a spattering of calcium deposits from her right shoulder. A relentless ache flared up in their place. After seven prescriptions, countless medical appointments and that fateful trip to the ER, her pain was finally diagnosed as complex regional pain syndrome, or CRPS — a condition in which pain festers in a limb long after an injury, causing swelling, discoloration and changes in sensation.

Pain specialist Vivianne Tawfik, MD, PhD, diagnosed Morris with the syndrome and has treated her at Stanford Hospital for the past five years. Tawfik's work helping patients like Morris manage their pain is increasingly important: More than 8 percent of American adults report being in severe pain every day, and pain medications rank as the second-most dispensed prescription.

Overall, an estimated 1 in 3 American adults suffer from chronic pain, meaning it has persisted for longer than three months. Morris' pain syndrome is a relatively rare form of chronic pain, with about 55,000 newly diagnosed cases each year. The pain subsides for some and persists for years in others.

Tawfik aims to help her chronic pain patients with a variety of treatments, including physical therapy, sessions in pain psychology, pain-relieving drugs and procedures such as nerve block injections.

But many of Tawfik's patients tick straight through that list and remain wracked with pain. They tote around packed pillboxes; swallow their empty promises of freedom from pain; and are left exhausted, foggy and constipated, rather than relieved. The stakes are even higher for women between the ages of 45 and 54, who have the highest risk of dying from a prescription painkiller overdose.

At the heart of the issue is a question that has plagued medicine for many years: Why does some pain dissipate after an injury has healed, while other pain hangs around long after the fact? If pain physicians knew that, they could prevent the onset of chronic pain, rather than trying to numb patients once it takes hold.

Tawfik, an assistant professor of anesthesiology, perioperative and pain medicine at the School of Medicine, hopes to someday figure that out. In addition to caring for patients, she is studying the transition from normal, short-term pain to chronic pain, with mice as her subjects.

Studies offer hope for relief

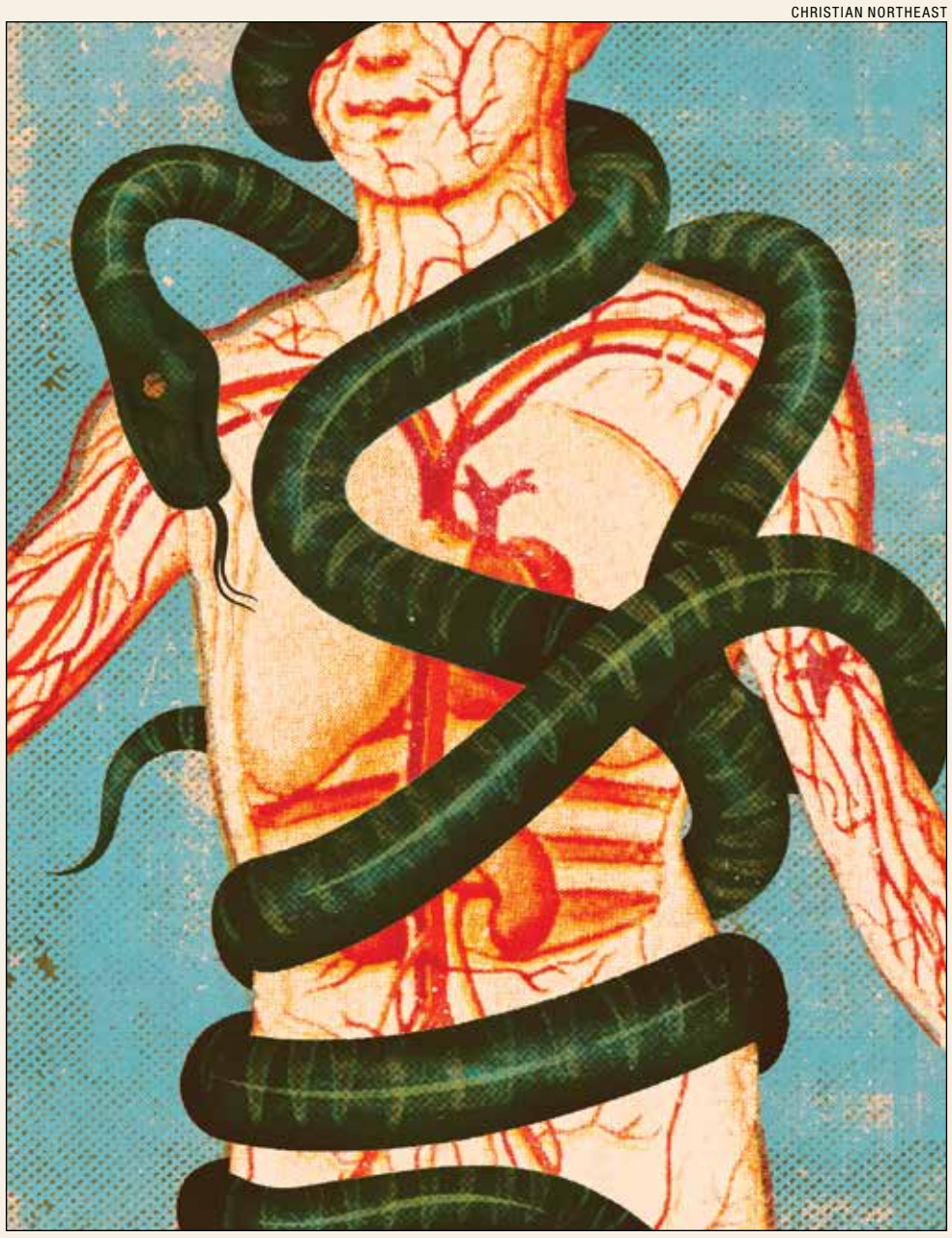
Neuroscientists have known that cells called microglia amplify pain signals on their way to the brain. If this boost persists after the painful injury has healed, it may lead to chronic pain. If this is the case, Tawfik hopes she might be able to alter the activity of microglia, tone down the incoming pain signals and turn off that prolonged pain.

Tawfik's challenge will be moving her research from mice to humans. Pain researchers have been under fire — often friendly fire — as some scientists increasingly argue that pain experiments in mice have little relevance in human disease. While pain signals move through mice and humans similarly, it's not possible to re-create the suite of emotional, psychological and physical aspects of human pain in a rodent.

Still, animal studies are irreplaceable pieces in the pain research jigsaw, Tawfik said. For patients like Morris, these studies offer some hope for relief. In the 10 years since Morris' shoulder surgery, her pain remains a moving target and a given in her daily existence. It has migrated to her left shoulder, and she compares the sensation to the pounding throb you feel after being hit with a hammer. It weighs down her body and mind like an invisible sandbag.

"I keep trying to find ways to be optimistic — that's the hard part," Morris said. "I don't want to think that I won't get better."

CHRISTIAN NORTHEAST



Tawfik is striving to improve upon pain studies of old and to achieve results in a field infamous for its shortcomings. Her largest ongoing study hints at a remedy for chronic pain.

The seed of Tawfik's current research took root in the early 1990s. Before then, scientists assumed that neurons — the excitable messenger cells of the nervous system — were wholly responsible for relaying pain signals through the body.

Neurons send electrical signals down an output cable, known as an axon, which releases chemical messages to neighboring cells. Neurons are surrounded by cells that lack axons, called glia. Glia means "glue" in Greek, and glia were once thought only to bind neurons together, providing them with insulation and structural support.

Vivianne Tawfik

Turning up the volume on pain

But as technologies were developed to better study glia, scientists found evidence that the cells are more than just brain glue. Many neuroscientists dismissed the idea at first, but now years of extensive research have provided too much evidence to ignore.

Linda Watkins, PhD, a behavioral neuroscientist at the University of Colorado-Boulder, was among those pioneering scientists who got glia into today's textbooks. Her early studies of influenza-related pain helped define the broader role for glia. "It turns out that all the symptoms of the flu are created by glia," she said. "And pain is part of that." Though they have no axon or other direct line of communication with the brain or spinal cord, glia appear to contribute to that pain signal.

"You can think of them as turning up the volume on pain," Watkins said. "If they become activated, they start spewing out substances that make pain neurons go wild."

For example, substances called proinflammatory cytokines call immune cells to assemble at an injury site and ignite inflammation to fight infection. Such chemicals make neurons more sensitive to incoming pain signals, influencing how intense pain feels down the line. After "turning up" pain for a certain length of time, glia can become prone to faster, stronger and longer activation, said Watkins.

Research suggests this prolonged glial activation might push short-term pain over the edge so that it becomes chronic. But no one knows exactly how. By studying glial cells found only in the brain and spinal cord, called microglia, Tawfik hopes to understand how these cells contribute to chronic pain and how to stop it.

Making do with mice

Pain is a complex interaction of physical, emotional and psychological factors, and scientists studying mice have yet to figure out how to ask a mouse how it's feeling, emotionally. Researchers can only study painlike behavior and nociception — how the nervous system reacts to painful stimuli — in animals. For instance, a researcher may prod a mouse's injured paw and note whether it pulls away and how quickly.

Compounding the problem, the vast majority of pain research has been done in male mice from the same genetic strain, even though chronic pain affects far more women than men.

Tawfik has tried to deal with these concerns by building an animal study that closely replicates what she sees in her patients, most of whom are women. Many of them first fracture a bone; then, after the cast comes off, the fracture pain lingers and develops into complex regional pain syndrome. Tawfik reproduces this scenario in her mouse experiments to study symptoms she sees in humans.

Manipulating microglia

In her Stanford lab, Tawfik is using genetic engineering technology to disable different genes along the pain pathway in mice that are bred to lack an essential microglial protein. She also is experimenting with disabling this component with an injectable drug to investigate how different levels of microglial activation correspond to the intensity of pain symptoms.

The classic symptom of complex regional pain syndrome is long-lasting pain that is stronger than expected given the injury that triggered it. Other symptoms include muscle tremors and weakness, brittle nails, slow-growing hair, swelling, redness or unexplained warmth in the affected limb. Those with the syndrome may become hypersensitive: A minor cut or bruise might cause severe pain while normally painless sensations, such as feeling clothing against their skin, can become excruciating. For instance, when Morris walks on pebbles with bare feet, it can feel as if she's walking on jagged shards of glass.

Tawfik studies these symptoms by observing whether her genetically altered mice are more sensitive to touch and heat after injury, or exhibit other symptoms that mimic those of her patients.

"If mice have some sort of injury, they tend to respond at a very low threshold," she said. The same goes for the mice's sensitivity to heat.

Tawfik also uses an imaging technology called pos-

and chronic

itron emission tomography to scan the brains and spinal cords of her mice. She plans to use the same technology in human patients to take a snapshot of their own nervous systems. Tawfik uses the scanner to measure how active her mice's microglia are before and after injury. She hopes the scans will tell her whether injuries cause microglia to become more active in her mice, and how that activation might be paralleled in humans.

Tawfik has run six groups of mice through her experiments. Time and time again, the same results have come back: Manipulating microglia, even temporarily or to a moderate degree, can completely change the trajectory of pain. By disabling or deleting 25 percent or more of a mouse's microglia, Tawfik can block their abnormally strong pain response before it takes hold. When she allows the mice's microglia to increase back to a normal level, they're still fine. It seems Tawfik may be flipping pain's off switch.

These are promising results, but the mechanisms that cause microglia to prolong pain remain a mystery. Tawfik wants to solve that so other scientists might develop medications to interfere with microglia and perhaps provide new treatments for chronic pain.

A pain-free tomorrow

Such drugs could give patients like Morris a new lease on life. From the outside, you'd never guess Morris was living with debilitating pain. Her fashionable outfits, sparkling nail polish and sleek, sandy hair seem incongruent with someone who's had an accidental drug overdose. If you overheard her joking at a coffee counter, asking for a unicorn drawn in her cappuccino foam, you wouldn't guess that years of relentless pain have left her clinically depressed.

"I can't even clean the dishes in my sink. I can't even put the toilet paper rolls in the bathroom, or make my bed in the morning, because I just don't want to do anything," she said, describing her worst days. "And that's so unlike me."

Morris said she has always had a "get-up-and-go" personality. She worked for years as a hospital marketing representative and raised two daughters who are now in college. She volunteers with local foster children at a Santa Cruz County nonprofit organization. In her free time, her ideal day would be filled with mountain biking, paddle boarding and canyoning, followed by beach volleyball at sunset. Tawfik notes that Morris remains extremely active — traveling, working and volunteering — in spite of her pain.

But none of this is possible without pain medications. Morris takes two tablets of the narcotic Vicodin two to three times a day, along with a nerve pain medication, Topamax, that makes her face twitch. That's in addition to injections twice a month of a nerve blocker — a medication that numbs the nerves in her neck to prevent the pain in her arm. She recently upped her Cymbalta prescription to treat her depression and nerve pain. The medications put her in a fog, make her drowsy, sap her motivation and disrupt her digestion.

On bad days, the pain still keeps her from her favorite activities and from her work with foster children.

"How can I be there for them when I'm not there myself?" Morris said.

In March of 2018, Watkins helped usher into human trials a glia-targeting drug that aims to interrupt the signals that glial cells use to turn up pain in patients with arthritis. It's already worked in rats, dogs and horses, and Watkins hopes it will prove effective in people.

"The end goal would be that a patient comes into clinic, they get a scan and we can see that their microglia are activated," Tawfik said. "Then we can say, 'You're appropriate for treatment with this drug that modulates those cells.'"

Tawfik envisions a better future for her patients — one where she can offer them permanent escape from the pain that holds them hostage. **ISM**

Researchers outline the possible role of a deep brain structure in concussion

By Taylor Kubota

SAUL BROMBERGER AND SANDRA HOOVER

David Camarillo is co-senior author of a study suggesting that impacts to the side of the head might cause harmful vibrations in a brain structure connected to the corpus collosum.

Concussion researchers have long suggested that damage to the corpus callosum, a thick bundle of nerves that connects the brain's two halves, could result in some common side effects of concussion, like dizziness or vision problems. The assumption is straightforward — that damage to the corpus callosum could affect coordination between the two halves — but difficult to prove.

Although still not proof, Stanford researchers have gathered evidence to support the idea by combining data from sensors worn by athletes, simulations of brain movement based on those measurements and brain images of people with and without concussions.

A paper describing the findings, published March 12 in *Biomechanics and Modeling in Mechanobiology*, suggests that impacts to the side of the head might cause harmful vibrations in a structure connected to the corpus collosum.

"Concussion is a big, vague term, and we need to start breaking it down," said former graduate student Fidel Hernandez, PhD, a lead author of the paper. "One way we can do that is to study individual structures that would be likely to cause traditional concussion symptoms if they were injured."

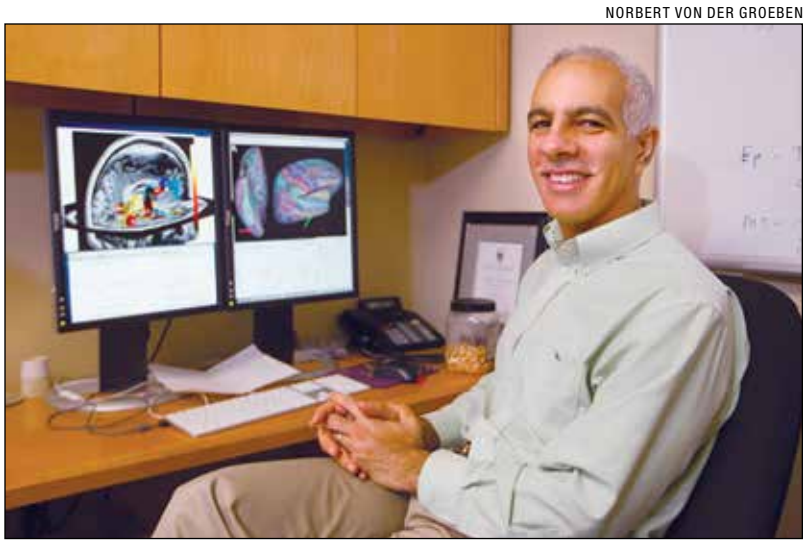
Evaluation, three ways

This research is built on data from mouth guards worn by football players. Each mouth guard records head movement and acceleration in six directions through an integrated accelerometer and gyroscope. Analyzing 115 impacts recorded by these mouth guards, the researchers found two associated with concussion diagnoses. By applying the mouth guard measurements to a simulation of the neck, head and brain, the researchers saw instances in which the corpus callosum was pulled around by a structure above it called the falx.

The falx sits like a mohawk hairstyle between the brain's two halves and is stiffer than the rest of the brain, like leather versus gelatin. Watching reproductions of the recorded impacts and additional simulations, the researchers saw that hits to the side of the head could produce vibrations in the falx, due to its stiffness. Those could then propagate down to the corpus callosum, creating the kind of tissue strain that is often implicated in concussion. Simulated strikes that made the head tilt toward the shoulder produced C-shape waves in the falx, while those that caused the head to turn produced S-shaped waves.

"Concussion is a big, vague term, and we need to start breaking it down."

NORBERT VON DER GROEBEN



Michael Zeineh, the study's other co-senior author, and his lab assessed magnetic resonance imaging scans from two athletes who had been diagnosed with concussion.

Michael Zeineh, MD, PhD, assistant professor of radiology, and his lab assessed magnetic resonance imaging scans from two athletes who had been diagnosed with concussion. The researchers looked with the most sensitive method available — diffusion imaging — and found evidence of possible damage to the corpus callosum in their both brains.

Diffusion imaging is rarely used in clinical practice and, even with this advanced technology, the researchers only saw the corpus callosum abnormalities because they knew where to look and had a comparison group — scans from athletes from the same sport and with similar years of experience who had never been diagnosed with concussion.

"The bottom line is, when we do post-concussion brain scans in clinical settings, we don't find anything. I'd say 95 percent of them are normal," said Zeineh, a senior author of the paper. "Clinically, we interpret by eye, but the kinds of changes we're showing in the paper, you can't see with your eye. Concussion cannot be diagnosed by imaging alone."

Given there were only two concussions in the data, the researchers emphasize the connection between side impacts, corpus callosum strain by the falx and concussion is still a hypothesis. A few previous studies have discounted this link, but none have combined biometric measurements, simulations and neuroimaging at this resolution. The researchers need more data to see how their hypothesis holds up and are already working with women's lacrosse players and additional football players to obtain that.

"The neuroimaging is really important for confirming the simulated models, but it's been difficult to get this combination of mouth guards and imaging," said David Camarillo, PhD, assistant professor of bioengineering and a senior author of the paper. "Now, we can prove these things out in a more rigorous and larger sample size."

Understanding what we're up against

When someone is diagnosed with a concussion, the treatment is almost always the same. The problem is that there are likely many kinds of concussions with symptoms that depend on which part of the brain was injured and how badly.

"All concussions are not created equal," Hernandez said. "We try to draw a line — a binary 'yes concussion' or 'no' — but concussions happen on a gradient."

A more detailed understanding of concussions could lead to more tailored treatments and change how we prevent them, showing us, for example, which kinds of concussions are most damaging or easiest to avoid. This topic matters beyond sports, as many concussions are the consequence of falls, particularly in the elderly or very young. Car crashes carry concussion risk as well. So, not only could research like this help improve protective gear for football players and cyclists, it could inform safety standards for cars or suggest new ways to design safer homes.

Former postdoctoral scholar Chiara Giordano, PhD, and postdoctoral scholar Maged Goubran, PhD, are also lead authors of the paper. Medical resident Sherveen Parivash, MD, and professor of neurosurgery Gerald Grant, MD, are co-authors.

Camarillo is a member of Stanford Bio-X, the Stanford Maternal & Child Health Research Institute and the Wu Tsai Neurosciences Institute at Stanford. Zeineh is a member of Bio-X and the Wu Tsai Neurosciences Institute.This work was funded by the Maternal & Child Health Research Institute, the Lucile Packard Foundation for Children's Health, the National Institutes of Health, the Radiology Society of North America and GE Healthcare.

Stanford's Department of Bioengineering, which is jointly managed by the School of Medicine and School of Engineering, and the Department of Radiology also supported the work. **ISM**

Microglia

continued from page 1

phagocytosis and whose activity levels either increase or decrease substantially with age.

Blocking genes' functionality

The investigators picked about 3,000 genes encoding proteins that they judged could be targeted by drugs or that had already been the focus of drug development. One at a time, they blocked each gene's ability to encode a protein. The goal was to learn how each blockade affected the ability of cultured mouse microglia to ingest small particles of fluorescently labeled latex. (The brighter a microglial cell glowed, the better a refuse eater it was.)

"It was like examining the books of the garbage-hauling company," Wyss-Coray said. "We wanted to know: 'Is it the garbage truck's faulty wheels? The rusty containers? An unanticipated garbage overflow? Lazy or poorly trained staff? Or is the street in bad shape?'"

In a parallel experiment, the investigators determined which of those approximately 3,000 genes are more or less active in microglia from the hippocampi of young mice versus old mice. (The hippocampus is a brain structure, one on each side of the brain, that's essential to learning and memory.)

Surprisingly, when the scientists compared the results of both experiments, they found just one gene that affected microglial phagocytosis and whose activity in microglia substantially changed with advancing age. Older microglia produced far more copies of this gene — a proxy for upregulated production of the protein for which the gene is a blueprint — than younger ones did, and knocking out its function greatly improved microglial phagocytosis.

"Now we had a tentative suspect, a gene that had never before been implicated in microglial garbage removal," Wyss-Coray said. So they zeroed in on this gene, called CD22, which is found in both mice and humans.

In a follow-on experiment, the CD22 protein turned up three times as often on the surface of older mice's microglia as on those of younger mice's microglia, confirming the gene-activity finding. These proteins could be blocked by antibodies, molecules that bind to a specific protein and can be generated in the lab. Antibodies are bulky and don't easily penetrate cells, but they're excellent for targeting cell-surface proteins.

Wyss-Coray's team injected antibodies to the CD22 protein into the hippocampus on one side of mice's brains. They also injected similar antibodies that were incapable of binding to CD22 into the hippocampus on the opposite side.

Along with the antibodies, the scientists administered fluorescence-labeled bits of myelin. This substance coats numerous nerve cells, for which it provides insulation. But myelin debris accumulates in aging brains and has been shown to overwhelm microglia's ability to clear it away.

Wyss-Coray and his associates found that, 48 hours later, the myelin bits they'd injected into the mice's hippocampi were far less prevalent on the side where they had administered CD22-blocking antibodies rather than "dummy" antibodies.

"Microglia are the only cells in mice's brain that actually express the CD22 protein, so this difference is likely due to the CD22-blocking antibodies' effect on microglia," Pluvinage said.

The investigators conducted analogous experiments, substituting a protein called beta-amyloid, whose buildup in the brain is a hallmark of Alzheimer's disease, and alpha-synuclein, another protein similarly associated with Parkinson's disease. In both cases, microglia exposed to CD22-blocking antibodies outperformed their peers on the opposite side of the brain in ingesting the neurodegeneration-linked substances.

Lengthening period of exposure

Then, the researchers lengthened the period of exposure from 48 hours to a full month. They reconfigured their injection technique to provide continuous CD22-blocking antibody infusion on both sides of the brain over this period. Along with a host of findings consistent with their earlier ones, Wyss-Coray's team observed that old mice receiving these infusions outperformed control mice of the same age on two different tests of learning and memory that are commonly used to assess mice's cognitive ability.

"The mice became smarter," Wyss-Coray said. "Blocking CD22 on their microglia restored their cognitive function to the level of younger mice. CD22 is a new target we think can be exploited for treatment of neurodegenerative diseases."

Stanford's Office of Technology Licensing has filed for a patent on intellectual property associated with the study.

NORBERT VON DER GROEBEN

Tony Wyss-Coray has been working for several years on the question of what causes the brain to lose its acuity with advancing age. One focus of his research has been a class of brain cells called microglia.

Wyss-Coray is a member of the Wu Tsai Neurosciences Institute at Stanford, the Stanford Maternal & Child Health Research Institute and Stanford Bio-X and a faculty fellow of Stanford ChEM-H.

Other Stanford co-authors are postdoctoral scholars Michael Haney, PhD, Tal Iram, PhD, and David Gate, PhD; MD-PhD students Benjamin Smith and Liana Bonanno; undergraduate student Jerry Sun; medical student Madeleine Scott; graduate students David Morgens, Andrew Yang and Steven Shuken; research assistant Lulin Li; research associate Davis Lee; senior research scientist Jian Luo, MD, PhD; associate professor of bioinformatics and of biomedical data science Purvesh Khatri, PhD; professor of chemistry and ChEM-H director Carolyn Bertozzi, PhD; and assistant professor of genetics Michael Bassik, PhD. **ISM**

Colorectal

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a greater number of older and high-risk adults would avert nearly three times as many diagnoses and deaths at a lower cost, the study found.

Uri Ladabaum

The study models potential effects of a 2018 change to the American Cancer Society's screening guidelines. Following increases in the incidence of colon and rectal cancer among people in their 40s, the society lowered the recommended age for a person at average risk of colorectal cancer to begin screening from 50 to 45. Other groups, including the U.S. Preventive Services Taskforce, are studying whether their screening recommendations should also change.

The shift has concerned some physicians who worry that screening resources may be drawn away from higher-risk populations. Overall, colorectal cancer incidence remains two to 13 times higher among people over the age of 50 than in younger people.

"This is one of the most important changes to guidelines that has occurred in the colorectal cancer screening world recently, and it was very controversial," said Uri Ladabaum, MD, professor of medicine at Stanford. "Our aim was to do a traditional cost-effectiveness analysis, but then also look at the potential tradeoffs and national impact. We wanted to crystalize the qualitative issues into tangible numbers, so people could then have a productive debate about these very issues."

The study found that over the next five years, initiating testing at age 45 could reduce the number of cancer cases by as many as 29,400 and deaths by up to 11,100, at an added societal cost of $10.4 billion. An additional 10.6 million colonoscopies would be required.

By comparison, increasing screening participation to 80 percent of 50- to 75-year-olds would reduce cases by 77,500 and deaths by 31,800 at an added cost of only $3.4 billion, according to the model. The number of additional colonoscopies needed would be 12 million.

A paper describing the work was published online March 28 in *Gastroenterology*. Ladabaum is the lead author. Robert Schoen, MD, professor of medicine and epidemiology at the University of Pittsburgh, is the senior author.

Cost versus benefits

The incidence of colorectal cancer among people 50 and older decreased by 32 percent between 2000 and 2013, largely due to a broad embrace of screening. But rates for people in their 40s rose by 22 percent, according to the American Cancer Society.

Physicians haven't definitively identified what has driven the increase, but obesity and diet likely are factors, said Ladabaum, who directs the gastrointestinal cancer prevention program at Stanford Health Care.

"With obesity being such a big problem and hard to tackle, and other potently influential factors not well-defined, people turn to what we know can help in terms of colon cancer risk mitigation, and that's screening," he said. "That's what brings us to this question."

Aiming to stem the rise in colon cancer cases among younger people, the American Cancer Society's new guidelines recommend screening for an estimated 21 million additional people.

The new study compares the potential costs and benefits of this approach by modeling five screening strategies, including a colonoscopy every 10 years; annual fecal immunochemical testing; and a sigmoidoscopy at age 45 followed by other tests in subsequent years.

To assess cost-effectiveness, Ladabaum and his colleagues calculated the cost of the additional screening in relation to years gained without or with cancer, a measure known as quality-adjusted life years. An intervention is generally accepted as cost-effective if it costs less than $100,000 per quality-adjusted life-year gained.

The study found that all five strategies offered benefits at acceptable costs when started at age 45 versus age 50, with the cost per additional quality-adjusted life-year ranging from $2,500 to $55,900.

"Is screening starting at 45 cost-effective by traditional standards? The answer is yes," Ladabaum said. "But the bottom line for me is that this is nuanced. The crucial question is: Can we screen younger people and at the same time do a better job of screening older and higher-risk people?"

Navigating tradeoffs

Physicians who were hesitant to endorse the American Cancer Society's new recommendations point to the work that remains in getting higher-risk people screened. Although the vast majority of colorectal cancer cases occur in people older than 50, only about 62 percent of them participate in screening, despite the goal of the health care community to bring that number closer to 80 percent.

In their study, Ladabaum and his colleagues explored the potential results of allocating resources in different ways. According to the model, initiating colonoscopy screening at age 45 would require 758 additional colonoscopies per 1,000 people, and would lead to a reduction of four cancer cases and two deaths per 1,000 people. By comparison, those procedures instead could be used to screen 231 previously unscreened 55-year-olds or 342 previously unscreened 65-year-olds through age 75. Those options would avert 13 to 14 cases and six to seven deaths per 1,000 people. They also would save $163,700 to $445,800 on balance, due to averted cancer treatment costs.

"If we actually do face tradeoffs on the societal level, either in terms of the effort we can put into this or the supply of colonoscopies and the distribution of colonoscopies by geography, then one can debate whether the efforts should go toward now bringing in younger people or whether we should focus on older people," Ladabaum said. "If we can bring in everybody, great. But if not, screening older and higher-risk people is higher yield in terms of public health benefit. It can get emotional and passionate because death from cancer at a young age is particularly devastating."

Other Stanford co-authors are research scientist Ajitha Mannalithara, PhD, and postdoctoral scholar Reinier Meester, PhD.

A researcher from the University of California-San Diego also contributed to the study.

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

OBITUARY

Ralph Greco, former chief of general surgery, dies at 76

By Rosanne Spector

Ralph Greco, MD, a pioneer in the movement to support work-life balance for physicians and trainees and the former chief of the School of Medicine's division of general surgery and director of its general surgery residency program, died March 31 at his home on the Stanford campus, surrounded by family. He was 76.

The cause of death was prostate cancer, said his wife, Irene Wapnir, MD.

Spurred by the suicide of a former resident, Greco created the first program to promote well-being among general surgery residents in the United States and pushed successfully for changes at the national level to require residency programs to support work-life balance.

"He was fearless about taking on the surgical establishment," said Claudia Mueller, MD, a Stanford pediatric surgeon who was associate director of the Balance in Life Program Greco established when it was launched in 2011. She now directs the program. "Ralph's ideas about wellness for trainees were not popular among old-school surgeons. It's a macho culture. But he was so committed to supporting trainees and so clever and farsighted about how to establish that program that he made it happen. It was stunning."

Greco was a product of that same macho culture. "You are expected to work all the time and never complain," Mueller said. "You're the captain of the ship, and anything that goes wrong is your responsibility. That's how Ralph was trained."

And in his early years as director of Stanford's general surgery residency program, that's what he expected of his trainees.

"He was a great advocate for us. We knew he had our back, and that engendered a lot of loyalty, but he didn't seem to be emphasizing wellness when I was a resident," said Marc Melcher, MD, who now directs the residency program.

"His attitude was more, 'No whining.' But then he had this major conversion," said Melcher, associate professor of surgery. "He dove in full force."

The conversion

This conversion occurred in 2010 after a well-respected former Stanford surgical resident, Greg Feldman, MD, killed himself just four months into a vascular surgery fellowship at another medical center.

Feldman's suicide spurred Greco to develop a plan to change not only Stanford's general surgery residency but the trainee experience nationally. "He pushed with the zeal of a reformer," Mueller said, pointing out that when Greco presented his plan to the medical school's leadership, he identified himself as "Dr. Ralph S. Greco, formerly the director of a malignant program" and as a "repentant sinner."

He gained the support of the then-chair of surgery, Thomas Krummel, MD, to create the Balance in Life Program, which provides general surgery residents with regular group therapy with a psychologist, mentoring partnerships between junior and senior residents, group activities planned and coordinated with input from the residents themselves, and a seemingly small but crucial element — a ready supply of healthy snacks.

"It was especially influential to build such a program in a surgical program, where the culture tended not to support the notion that physicians should ever be in need of help," said Bryan Bohman, MD, associate chief medical officer at Stanford Health Care and senior adviser to the WellMD Center, Stanford Medicine's wellness program for physicians. "To help overcome the 'iron person' culture of medicine at the time, Ralph and Tom built their program as a performance-enhancement tool, aimed at deriving the highest performance from the trainee surgeons. This was very effective framing, and the program has been quite popular with the house officers."

Greco also successfully advocated for the residency program accrediting agency to require well-being support in all residency programs.

"Ralph Greco was a visionary force behind pioneering efforts at Stanford to support physician well-being," said Lloyd Minor, MD, dean of the School of Medicine. "His efforts have benefited not only physicians trained at Stanford, but trainees throughout the country. As the leaders of other training programs have come to recognize the value of work-life balance for surgeons, many have emulated the program he created here."

"We get calls every month asking how we do it. They think we have something golden," said Mueller, who is one of several Stanford physicians who have been invited to other academic medical centers to discuss how the program works and its impact.

Bronx native

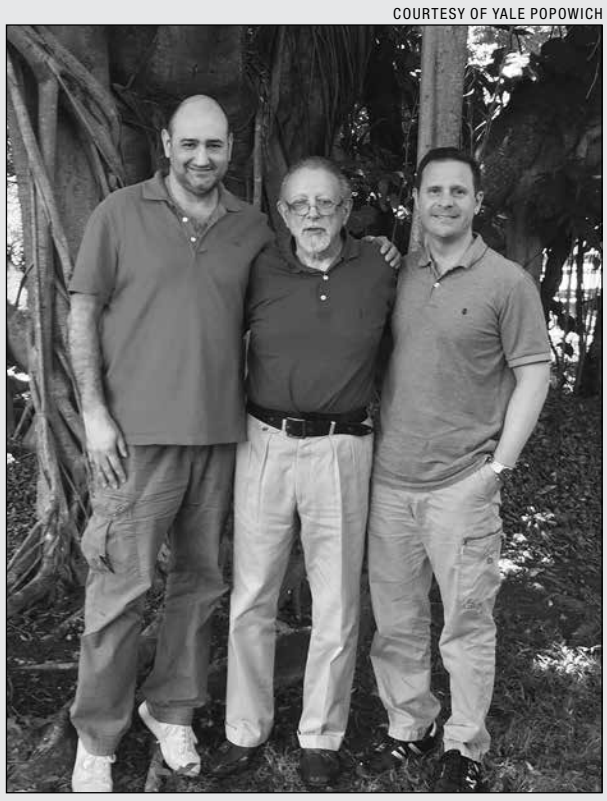
Greco was born and raised in the Bronx, a borough of New York City. His mother was an elementary school teacher, and his father designed women's clothing. He went to college at Fordham University, graduating cum laude, and then earned a medical degree at Yale in 1968.

Ralph Greco

Greco completed his internship and residency at Yale New Haven Hospital, where he was chief resident in 1972-73; served as a staff surgeon in the military from 1973-1975 — at the U.S. Army Hospital in Seoul, South Korea, and at Kimbrough Army Hospital in Fort Meade, Maryland; and joined the faculty of Rutgers Medical School (now the Robert Wood Johnson Medical School), where he specialized in surgical oncology and became chief of general surgery in 1982. He became chief of surgery at Robert Wood Johnson University Hospital in 1997.

While at Rutgers, Greco began research on the clinical use of biomaterials, which he continued at Stanford. Over his career, he co-authored more than 100 papers in the scientific literature. He met Wapnir, now a professor of surgery at Stanford, during her fellowship in breast surgery at Robert Wood Johnson Medical School. They married in 1991.

COURTESY OF YALE POPOWICH



Ralph Greco with former Stanford surgical residents Yale Popowich (left) and Geoffrey Nadzam in Haiti in 2014. Greco established an elective rotation in Haiti for surgical residents.

In 2000, Greco was recruited to Stanford for what he described in a 2011 article in the *Journal of Graduate Education* as a "job of a lifetime," with an endowed chair — the Johnson & Johnson Professor of Surgery — and the position of chief of general surgery and director of the general surgery residency program. As the program's director, he developed a new curriculum for the residents, restructured the program in expectation of new national guidelines limiting residents' hours on duty, established an elective rotation in Haiti for residents and created the well-being program. He served as chief of general surgery from 2000-2006 and as director of the residency program from 2000-2009.

In recognition of his accomplishments, the Accreditation Council for Graduate Medical Education bestowed on him in 2012 the organization's highest honor, the John C. Gienapp Award, for his work as a surgery program director, humanitarian and innovator of a surgery curriculum focused on resident well-being and wellness.

Though Greco didn't begin advocating balance in life until he was decades into his career, his life had long been an exemplar of the concept.

"He was very doting with the kids, and quite easy-going — letting them be themselves," Wapnir said. "He loved sharing his love of sports, especially baseball — Yankees — with the boys and always indulged them. He was a poor disciplinarian and the opposite of a helicopter parent."

'The pursuit of beauty'

Alongside his work in surgery and his involvement with his family, he immersed himself in the world of art. Greco began learning to sculpt in 1987, studying briefly with sculptor Lilli Gettinger, and then pursuing it on his own. "It is the pursuit of beauty — nothing more, nothing less," he told MEDdebate, a medical humanitarian organization, in a 2015 interview.

He went on to create dozens of sculptures, working in many types of stone, wood and terra cotta, creating both representational and abstract pieces. His sculptures can be found in the collections of Johnson & Johnson and the Robert Wood Johnson University Hospital, as well as in the homes of individual collectors and on the Stanford campus.

He also had a longtime connection to Haiti, where he traveled frequently for working visits at Hôpital Albert Schweitzer. In a 2002 article in *Stanford Report*, he said he fell in love with the country when he first visited as a resident in the 1970s. He later took surgery residents with him and established a month-long Stanford rotation there.

"The Haiti experience was a lesson in humility," said Yale Popowich, MD, a former resident who went to Haiti with Greco three times, including Greco's last trip, in 2014. "I learned so much about life, survival and the human spirit on each trip. For surgery residents, it was an opportunity to hone skills, diagnose and treat under some very difficult situations. For Dr. Greco, Haiti was a lifeline. It was a place that captivated his soul; the people, the artwork, their passion."

'Sarcastic but funny'

Greco was deeply serious about his responsibilities as a surgeon and teacher, but laughter was an important part of his life, too. "He was very irreverent," Mueller said. "There was nobody who was a god or who was unassailable. He liked and respected his colleagues, but he would call them out for their silliness. There were meetings when I laughed constantly."

"He was very sarcastic but funny, and you really had to be sharp to catch it. Not all can," said Nicole Delgado, his administrative associate for many years.

"To someone who doesn't know him, I would say he looks intimidating, but in reality he is a big softy with an amazing heart," Delgado said. "He truly cared about his patients and those around him."

"A lot of my fondness for the Stanford general surgery residency is because of Dr. Greco," said Arghavan Salles, MD, PhD, a former Stanford general surgery chief resident, now at Washington University School of Medicine, who helped Greco develop the Balance in Life Program.

"He was always willing to listen and give advice," Salles said. "He never hesitated to offer assistance because he was committed to helping residents succeed."

At a graduation dinner for chief surgical residents in 2009, he received a 400-pound marble boulder as a gift. From this he sculpted an abstract letter S, representing "Surgery." It is displayed in the complex of offices just outside the chair's office in the Department of Surgery.

Greco was the recipient of many awards over his career. In addition to ACGME's Gienapp award, he especially valued the council's 2006 Parker J. Palmer Courage to Teach Award, given to outstanding program directors annually, and Stanford's 2016 Shumway Society Lifetime Achievement Award.

He retired from Stanford in August 2017 but continued his involvement with the Balance in Life Program.

In addition to his wife, Greco is survived by a brother, Ronnie Greco of New Jersey and Florida; a daughter, Ilana Greco of Stanford; two sons, Eric Greco of San Francisco and Justin Greco of New Haven, Connecticut; and three nieces and nephews.

In lieu of flowers, the family requests that donations be made to the Balance in Life Program. For information on giving to the program, contact Stephanie Edelman at (650) 725-6493 or at sedelman@stanford.edu, or give online at http://surgery.stanford.edu/gift.html. (In the special instructions box, enter "Stanford General Surgery, Balance in Life.") ISM

Faculty elected to American Institute for Medical and Biological Engineering

Several faculty members at the School of Medicine have been elected to the college of fellows of the American Institute for Medical and Biological Engineering.

An induction ceremony was held March 25 during the institute's annual meeting at the National Academy of Sciences in Washington, D.C. The college of fellows class of 2019 comprises 157 inductees, of whom four are faculty at the medical school:

Helen Blau

Markus Covert

Brian Hargreaves

Shreyas Vasanawala

HELEN BLAU, PhD, the Donald E. and Delia B. Baxter Foundation Professor, professor of microbiology and immunology and director of the Baxter Laboratory for Stem Cell Biology, was recognized for her contributions in the use of bioengineered materials to advance stem cell biology and regenerative medicine.

MARKUS COVERT, PhD, associate professor of bioengineering, was recognized for his contributions to systems biology, including constructing the first "whole-cell" computational model.

BRIAN HARGREAVES, PhD, professor of radiology, was recognized for his contributions to body and orthopaedic magnetic resonance imaging, including MRI near metal implants and MRI of breast cancer.

SHREYAS VASANAWALA, MD, PhD, professor of pediatric radiology, was recognized for his contributions to the fields of fast, quantitative body, cardiovascular and pediatric MRI. **ISM**

Phage

continued from page 1

P. aeruginosa is itself frequently infected with a phage called Pf. This phage lives inside the bacteria but can be shed from the bacterial surface into the surrounding environment (such as a wound), much like the virus herpes lives in our cells and is shed from cold sores. In the study, Bollyky's team showed Pf was common in wounds infected with P. aeruginosa. The researchers examined 111 patients with microbially infected, non-healing wounds and found that 37 of them were infected with P. aeruginosa. Two-thirds of those wounds infected with P. aeruginosa were carrying Pf — a fraction that grew the longer a wound persisted.

To prove Pf actually promotes P. aeruginosa infections rather than merely co-exists with them, the scientists inoculated small wounds in the skin of mice with P. aeruginosa strains that either did or didn't contain Pf. They observed that the two strains differed greatly in their ability to establish wound infections. The inoculation dose necessary to result in a reliable P. aeruginosa infection was 50 times larger if it lacked Pf.

Next, the scientists looked to see what Pf might be doing to immune cells that could affect P. aeruginosa's ability to sustain an infection. In a lab dish, they found that the presence of the phage in P. aeruginosa reduced by 10-fold the number of invading bacteria that were engulfed by either mouse or human phagocytes — immune cells that ingest, then digest, invading bacteria.

"The phagocytes lost their appetite," Bollyky said.

Tripping molecular detectors

Bollyky's team determined that stretches of the phage's genomic material trigger molecular detectors in the phagocytes, steering the immune system's response from an antibacterial to an antiviral one.

When a phagocyte encounters bacteria, the appropriate response is to gobble them up, chew them up and call in more troops. But phagocytes' response to a virus is different, Bollyky said. "If you're an immune cell, ingesting a virus is absolutely the worst thing you can do, because now you've let it get inside of you — you're infected by it."

So it's only sensible for a phagocyte that comes in contact with a virus to shut down phagocytosis. The appropriate antiviral immune response involves the generation of antibodies to tag virally infected cells and to signal other types of immune cells to home in on and destroy any virus-carrying cell they come across.

What Pf does inside phagocytes, Bollyky said, is like somebody pulling the fire alarm when they should have called the police. "If 20 fire engines pull up to the scene of the crime, it makes it easier for the thief to get away," he said.

The investigators generated a vaccine containing a component of a Pf protein and noted that it cut the incidence of wounds infected with Pf-positive P. aeruginosa by half. They also generated antibodies that specifically target the same protein component and showed that they worked at least as well as the vaccine.

Bollyky and his colleagues have filed for a patent on intellectual property associated with the vaccine, and they plan to test it in large animals as a step toward eventual clinical trials.

The findings could fuel new ways of preventing chronic, intractable infections.

Bollyky's vision is to vaccinate people against Pf when they're first diagnosed with cystic fibrosis or diabetes, as well as people in nursing homes and hospitals, in order to protect them from P. aeruginosa infections. Since a vaccine takes time to arouse the immune system, he suggested that Pf-targeting antibodies (which can be produced in bulk and stored for long periods) could be useful in burn cases, when there's no advance warning.

The Pf vaccine might turn out to be effective against other pathogenic bacteria, such as E. coli and Klebsiella pneumoniae, which can also carry Pf and tend to co-infect wounds colonized by P. aeruginosa, Bollyky said.

Other Stanford co-authors of the study are postdoctoral scholar Jonas Belleghem, PhD; former postdoctoral scholars Vivekananda Sunkari, PhD, Xiou Cao, PhD, and Christiaan de Vries, MD; research scientists Gernot Kaber, PhD, and Robert Manasherob, PhD; Stanford Health Care affiliate physician Gina Suh, MD; technician Dung Lam; lab manager Heather Ishak; graduate students Michelle Bach and Medeea Popescu; medical student Payton Marshall; the late MD-PhD student Maria Birukova; former undergraduate Ethan Katznelson; and former medical student Daniel Lazzareschi, MD.

Researchers at Baylor College of Medicine in Houston, the University of Washington and the University of Montana also contributed to the study.

Bollyky is a member of Stanford's Bio-X, Maternal & Child Health Research Institute and Wu Tsai Neurosciences Institute.

The work was funded by the National Institutes of Health, Stanford SPARK, the Falk Medical Research Trust and the Cystic Fibrosis Foundation and Bio-X.

Stanford's departments of Medicine and of Microbiology and Immunology also supported the work. **ISM**

OF NOTE

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

GOZDE DURMUS, PhD, was appointed assistant professor (research) of radiology, effective Feb. 1. Her research focuses on developing tools to detect and investigate circulating biomarkers and rare cells from biological fluids for precision medicine.

AARON GITLER, PhD, professor of genetics, will receive the 2019 Sheila Essey Award from the American Academy of Neurology, the ALS Association and the American Brain Foundation. The $50,000 prize recognizes significant contributions in the search for the causes of amyotrophic lateral sclerosis, also known as Lou Gehrig's disease, as well as ways to prevent and cure the disease. His research uses genetic screening to focus on mechanisms of neurodegenerative diseases, including Parkinson's, Alzheimer's and ALS.

JAIME LOPEZ, MD, was promoted to professor of neurology and neurological sciences, effective Feb. 1. He founded and directs the Stanford Intraoperative Neurophysiologic Monitoring Program. His research focuses on developing techniques for monitoring the nervous system during surgical and endovascular procedures, and identifying how these techniques alter surgical management and patient outcomes.

JUNO OBEDIN-MALIVER, MD, MPH, MAS, was appointed assistant professor of obstetrics and gynecology, effective Feb. 1. She specializes in gynecological and reproductive health care needs of sexual and gender minority people, and she co-directs the PRIDE Study, a national longitudinal study of sexual and gender minority adults.

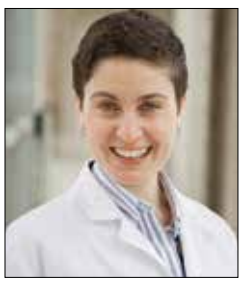
CAROLYN RODRIGUEZ, MD, PhD, was promoted to associate professor of psychiatry and behavioral sciences, effective March 1. Her research investigates the neurobiology of obsessive-compulsive disorder and related mental health conditions, with the aim of developing targeted, rapid-acting treatments.

YUANJIA ZHU, MD, resident in cardiothoracic surgery, received a $60,000 research fellowship award from the Thoracic Surgery Foundation, the charitable arm of the Society of Thoracic Surgeons. The award will support her effort to engineer blood vessels that are similar to arteries and that have the potential to promote the growth of additional vessels. **ISM**

Gozde Durmus

Aaron Gitler

Jaime Lopez



Juno Obedin-Maliver

Carolyn Rodriguez

Yuanjia Zhu

Inaugural gathering set for Stanford Medicine Abilities Coalition

The Stanford Medicine Abilities Coalition will hold its inaugural event, a mixer that will include members of the Medical Students with Disability and Chronic Illness group, at 5:30 p.m. April 15 in Room 308 of the Li Ka Shing Center for Learning and Knowledge.

The debut gathering, sponsored by the Office of Faculty Development and Diversity, is free and open all members of the Stanford community. It will feature an informational session on disability initiatives at the medical school and university. Refreshments will be served.

The Stanford Medicine Abilities Coalition is an initiative started by Peter Poullos, MD, clinical associate professor of radiology and of medicine. Its goals are to foster equal treatment; advocate for accessibility, resources and services; and promote diversity and inclusivity at the medical school and hospitals.

Register for the event at http://bit.ly/abilitiescoalition. **ISM**