INSIDE STANFORD MEDICINE

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Researchers are building a digital model of the hippocampus.
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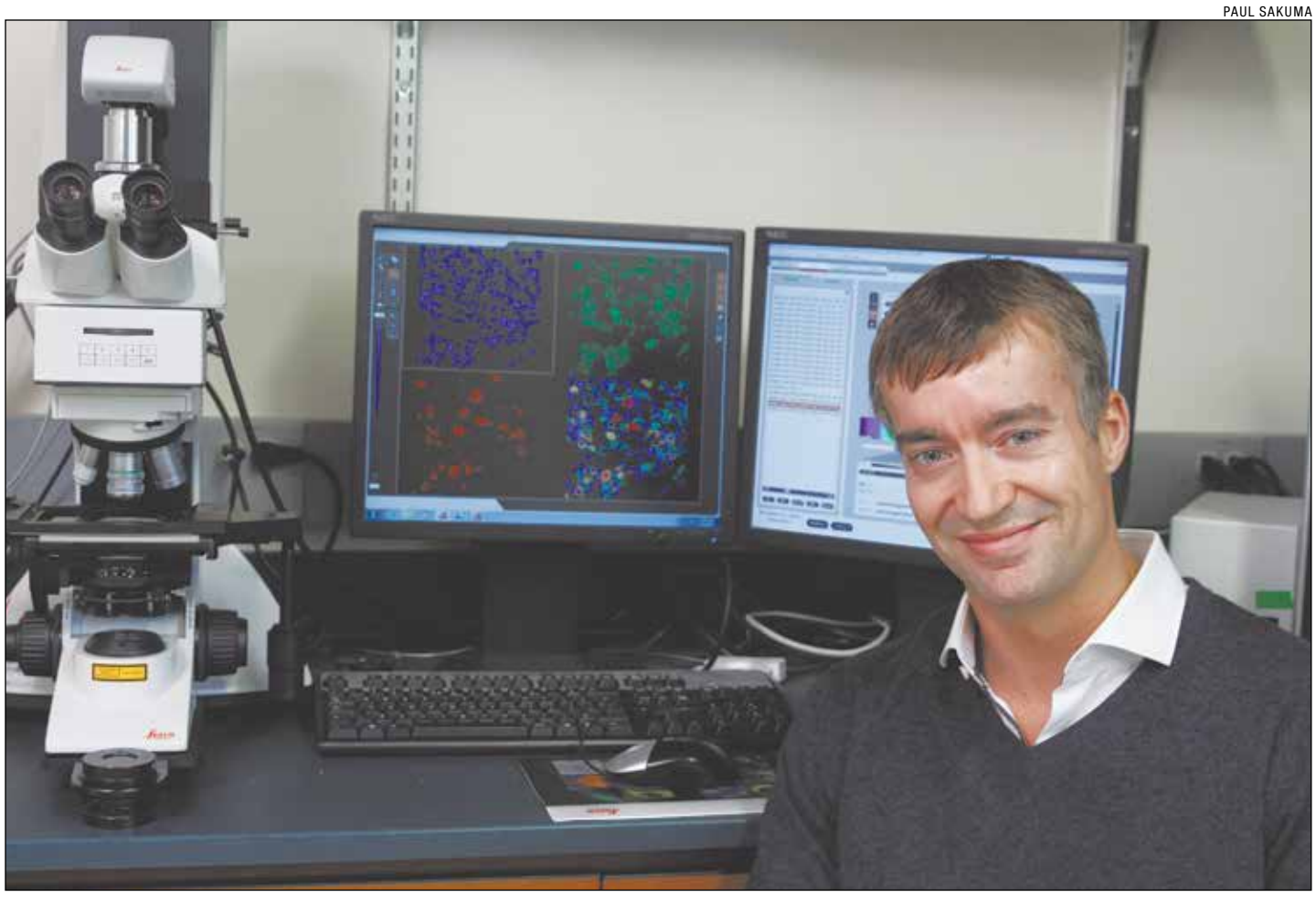
Brain cells that cause pain aversion found

By Hanae Armitage

PAUL SAKUMA



Grégory Scherrer and his collaborators have identified in mice an ensemble of cells that seems to specifically function as an on-off switch for pain aversion.

If you step on a tack, neurons in your brain will register two things: that there's a piercing physical sensation in your foot, and that it's not pleasant. Now, a team of scientists at Stanford has identified a bundle of brain cells in mice responsible for the latter — that is, the negative emotions of pain.

Pain research has traditionally focused on the neurons and molecules at the frontline of pain perception — the cells in nerves that process stings, cuts, burns and the like — and ultimately convey a physical threat message. What Grégory Scherrer, PhD, assistant professor of anesthesiology and of neurosurgery, and Mark Schnitzer, PhD, associate professor of biology and of applied physics, are studying goes one step further. "We're looking at what the brain makes of that information," Scherrer said. "While painful stimuli are detected by nerves, this information doesn't mean anything emotionally until it reaches the brain, so we set out to find the cells in the brain that are behind the unpleasantness of pain."

Peeping at pain neurons

Backed by animal-brain imaging and molecular testing, the researchers have found an ensemble of cells in the amygdala, a region of the brain classically associated with emotion and fear, that seems to specifically function as an on-off switch for pain aversion. And although the finding was made in mice, there's reason to think it could one day serve as a therapeutic target for human pain, since the mouse and human amygdala aren't so different in function. Researching this group of cells could reveal a potential treatment for chronic pain, the scientists hope.

The idea is that patients suffer from the emotional unpleasantness of pain, rather than pain sensation itself. If there's a way to dull the emotional hurt, rather than the physical sensation of pain, that could be big for chronic pain patients.

A paper describing the results of the study was published Jan. 18 in *Science*. Scherrer and Mark Schnitzer, PhD, who is also a Howard Hughes Medical Institute investigator, share senior authorship. Postdoctoral scholar Gregory Corder, PhD, and former graduate student Biafra Ahanonu, PhD, are the co-lead authors.

The amygdala seemed to the researchers a logical place to start, since it's a well-established hub for emotion in the brain. Within the amygdala, they narrowed their search by looking for neurons in mice that were active during brief pain stimulation — such as a drop of hot, but not scalding, water applied to a paw. Neurons that are active express more of a specific gene called c-Fos, and indeed, a sea of c-Fos-expressing See PAIN, page 6

Scientists find protein promotes small artery growth to damaged heart tissue in mice

By Christopher Vaughan

A collaboration between basic and clinical scientists at Stanford has revealed a protein that promotes the growth of small arteries into oxygen-starved heart tissues in mice.

Kristy Red-Horse, PhD, associate professor of biology, and Joseph Woo, MD, professor of cardiothoracic surgery, think the growth of these new arteries may help heal damage caused by heart disease or heart attack, or even help prevent that damage.

In clinical practice, Woo has observed that patients with blockages in major arteries feeding the heart often have confoundingly different outcomes. "Some patients have a blockage in one coronary artery and die; other patients have multiple blockages in multiple areas but can run marathons," said Woo, who holds the Norman E. Shumway Professorship.

The difference, Woo said, may be that this second group of patients has collateral arteries, tiny arteries that bypass blockages in hearts' major arteries and feed areas of the heart starved of oxygen. "They are like the side streets that let you get around a traffic jam on the freeway," Woo said. Such collateral arteries could help people with atherosclerosis or people recovering from a heart attack, except that collateral arteries are only seen in a minority of patients.

Kristy Red-Horse

Joseph Woo

Now Woo, Red-Horse and their colleagues have discovered how these collateral arteries are formed and a signaling molecule that promotes their growth in adult mice, offering hope that collateral arteries may be coaxed to grow in human patients.

Their findings were published Jan. 24 in *Cell*. Red-Horse, a member of the Stanford Institute for Stem Cell Biology and Regenerative Medicine, and Woo, a member of the Stanford Cardiovascular Institute, share senior authorship of the paper. Postdoctoral scholars Soumyashree Das, PhD, and Andrew Goldstone, MD, PhD, are co-lead authors. See ARTERY, page 6

Short-term hospital readmissions for gunshot wounds cost $86 million each year, study says

By Amy Jeter Hansen

Hospital readmissions of patients within six months of suffering a firearm injury cost taxpayers, private insurers and uninsured families an average of $86 million a year from 2010 through 2015, according to new estimates from School of Medicine researchers.

During that six-year period, the annual cost of hospitalizations for gun injuries averaged $911 million, with the government shouldering See GUN, page 7

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Engineered immune cells target range of pediatric tumors in mice

By Erin Digitale

Immune cells engineered to attack childhood cancers were able to eradicate different types of pediatric tumors in mice, according to a new study from the School of Medicine.

The study, which was published online Jan. 17 in *Clinical Cancer Research*, provides evidence that these engineered cells can target many types of pediatric solid tumors, including brain tumors. Better treatments are badly needed for children with these tumors, particularly when traditional therapies fail.

"The prognosis for children with relapsed brain tumors or solid tumors or metastatic disease generally is dismal," said Robbie Majzner, MD, the lead author of the new study and an instructor in pediatrics at Stanford. "We're excited that we have a potential therapeutic representing a completely new modality to treat these children." The study's senior author is Crystal Mackall, MD, the Ernest and Amelia Gallo Family Professor and a professor of pediatrics and of medicine.

Immunotherapies that work well for adult cancers do not always succeed against childhood cancers, Majzner noted. One approach, called checkpoint inhibition, targets gene mutations that are limited in most pediatric cancers.

Another immunotherapy method, using chimeric antigen receptor T cells, or CAR-T cells, is the basis of a treatment for one form of relapsed childhood leukemia. Leukemia is a type of blood cancer. That therapy, tisagenlecleucel (brand name Kymriah), employs synthetic biology to make immune cells that react to a surface marker found on the leukemia cells.

CAR-T for pediatric solid tumors

Majzner and his colleagues decided to try to make CAR-T cells for pediatric brain tumors and solid tumors, including tumors found in bone and muscle. These cancers do not carry the same surface markers as leukemia, so the scientists' first step was to look for another marker that engineered immune cells could target.

"You need high amounts of the target on the tumor cells, and you may need the target to be on every cell in a tumor," Majzner said. The ideal surface marker must not be highly expressed on healthy tissue, to prevent engineered immune cells from attacking normal tissues.

The researchers screened 388 pediatric tumor samples for expression of a surface marker called B7-H3, which prior studies suggested might be a good candidate. B7-H3 was found on 84 percent of the samples, and it was present at high levels in 70 percent of samples. Many types of pediatric cancer were found to express high levels of B7-H3, including Ewing sarcoma (bone), rhabdomyosarcoma (muscle), Wilms tumor (kidneys), neuroblastoma (nerve cells) and medulloblastoma (brain).

The fact that the same marker exists across so many tumor types increases the chance that it could serve as the basis for a commercially viable therapy, Majzner said. Each tumor is fairly rare, with a few hundred children affected across the United States each year, but together they form a larger patient population.

STEVE FISCH

Robbie Majzner and his collaborators are working to engineer immune cells that can attack a variety of childhood cancers.

'The tumor just goes away'

The scientists then developed six types of CAR-T cells to target B7-H3 and tested them in a dish. The type of B7-H3 CAR-T cells that performed best was used for further studies.

The researchers tested these B7-H3 CAR-T cells against several xenograft models of pediatric cancer, in which human tumors were implanted in mice. In mice with osteosarcoma or Ewing sarcoma — both bone tumors — B7-H3 CAR-T cells eradicated the tumors. The treated mice lived significantly longer than animals that received a control treatment.

"The tumor just goes away," Majzner said. "It's very consistent. It happened in all the mice, and that's exciting."

A group of mice with osteosarcoma had their initial tumors surgically removed and then received B7-H3 CAR-T cells to test whether the cells could treat cancer cells that had spread to the lungs. Again, the CAR-T cells worked; the treated mice lived significantly longer than those in a control group.

The researchers also tested B7-H3 CAR-T cells in mice implanted with a pediatric brain tumor called medulloblastoma. The CAR-T cells were injected into the blood and were able to cross the blood-brain barrier and eradicate the tumors.

The researchers showed that B7-H3 CAR-T cells do not attack cells expressing low levels of B7-H3, a reassuring finding since some healthy cells produce low levels of the marker.

"We're hopeful that there may be a therapeutic window of B7-H3 levels between tumor tissue and normal tissue," Majzner said. "The only way to find out is to test our new CAR-T cells in clinical trials."

Clinical trials planned

The team is now planning a series of phase-1 clinical trials for the B7-H3 CAR-T cells, starting with adult brain tumor patients. B7-H3 is not expressed on healthy tissues in the central nervous system, making it a good starting point for human trials.

The risk exists that the treatment may leave behind a few rare cancer cells that do not carry B7-H3, which could cause a relapse, Majzner noted. "We're already making combination CAR cells that combine several targets and optimizing those for future clinical trials," he said.

The study's other Stanford co-authors are postdoctoral scholars Johanna Theruvath, MD, and Sabine Heitzeneder, MD; MD-PhD student Christopher Mount; life science researchers Skyler Rietberg and Peng Xu; graduate students Miles Linde and Louai Labanieh; senior research scientist Elena Sotillo, PhD; former senior research scientist Siddhartha Mitra, PhD; Ravindra Majeti, MD, PhD, professor of medicine; and Michelle Monje, MD, PhD, associate professor of neurology.

Majzner is a member of the Stanford Cancer Institute. Majeti, Monje and Mackall are members of Stanford Bio-X, the Stanford Maternal & Child Health Research Institute and the Stanford Cancer Institute. Majeti and Monje are also members of the Stanford Institute for Stem Cell Biology and Regenerative Medicine, and Monje is a member of the Wu Tsai Neurosciences Institute at Stanford. Mackall is director of the Parker Institute for Cancer Immunotherapy at Stanford and founding director of the Stanford Center for Cancer Cell Therapy.

Researchers at the University of Colorado-Denver, the National Cancer Institute, the University of Virginia, the University of Washington, the University of Pittsburgh, the University of British Columbia, the British Columbia Cancer Research Center, the British Columbia Cancer Agency, MacroGenics Inc., the Children's Hospital of Philadelphia and the University of Pennsylvania also contributed to the study.

Mackall and some of the co-authors at other institutions hold patents on the use of B7-H3 CAR-T cells for cancer immunotherapy, as well as on anti-B7-H3 antibodies and single chain variable fragments. Mackall is a founder of and holds equity in Lyell Immunopharma.

The research was funded by the National Cancer Institute, the Sarcoma Alliance for Research Through Collaboration, Hyundai Hope on Wheels, the St. Baldrick's Foundation and Stand Up 2 Cancer.

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

Researchers develop sensitive urine test for bladder cancer

By Christopher Vaughan

Researchers at the School of Medicine have developed a highly sensitive urine test for diagnosing and monitoring bladder cancer.

The test involves looking for fragments of cancer DNA in urine samples. "This study describes a new diagnostic approach to bladder cancer focused on analysis of urine samples," said Maximilian Diehn, MD, PhD, associate professor of radiation oncology. "Urine is in direct contact with bladder tumors, which shed some of their DNA into it."

The findings were published online Dec. 21 in *Cancer Discovery*. Diehn shares senior authorship with Ash Alizadeh, MD, PhD, associate professor of medicine. Postdoctoral scholars Jonathan Dudley, MD, and Joseph Schroers-Martin, MD, are the lead authors.

Sixth most common cancer

Bladder cancer is the sixth most common cancer. More than 80,000 people are diagnosed with it every year in the United States. Currently, the most accurate method of diagnosing bladder cancer is through cystoscopy, an invasive method to visualize the bladder and take tissue samples. Another method is to look for cancer cells in the urine via a cytology test. Although noninvasive, this approach has suboptimal sensitivity, Diehn said.

> "Urine is in direct contact with bladder tumors, which shed some of their DNA into it."

The research builds on earlier studies co-authored by Diehn and Alizedah in which they showed that they could detect certain cancers by looking for DNA fragments of tumors circulating in the bloodstream using a method called CAPP-Seq, an abbreviation for cancer personalized profiling by deep sequencing. In the new study, the researchers modified molecular and bioinformatics aspects of this technique to apply to bladder cancer DNA fragments found in urine. They analyzed a total of 67 healthy adults and 118 patients with early stage bladder cancer who either had urine collected prior to treatment or during surveillance.

The researchers found that by testing for bladder cancer in urine, they could detect cancer in the early stages of development, when it can be treated more easily. Their approach correctly identified the presence of bladder cancer in 83 percent of patients with early stage bladder cancer, compared with only 14 percent for the clinically available urine cytology test.

One of the greatest benefits of the new approach may be its ability to detect the recurrence of bladder cancer after someone has been treated for the disease. "In our test samples, we were able to detect bladder cancer recurrence an average of 2.7 months ear-

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Paul Costello
Chief communications officer
Susan Ipaktchian
Director of print & Web communications
John Sanford
Editor
Robin Weiss
Graphic designer

Send letters, comments and story ideas to John Sanford at 723-8309 or at jsanford@stanford.edu. Please also contact him to receive an e-mail version of *Inside Stanford Medicine*.

STANFORD MEDICINE

Activated 'social' brain circuits inhibit feeding behavior in mice

By Bruce Goldman

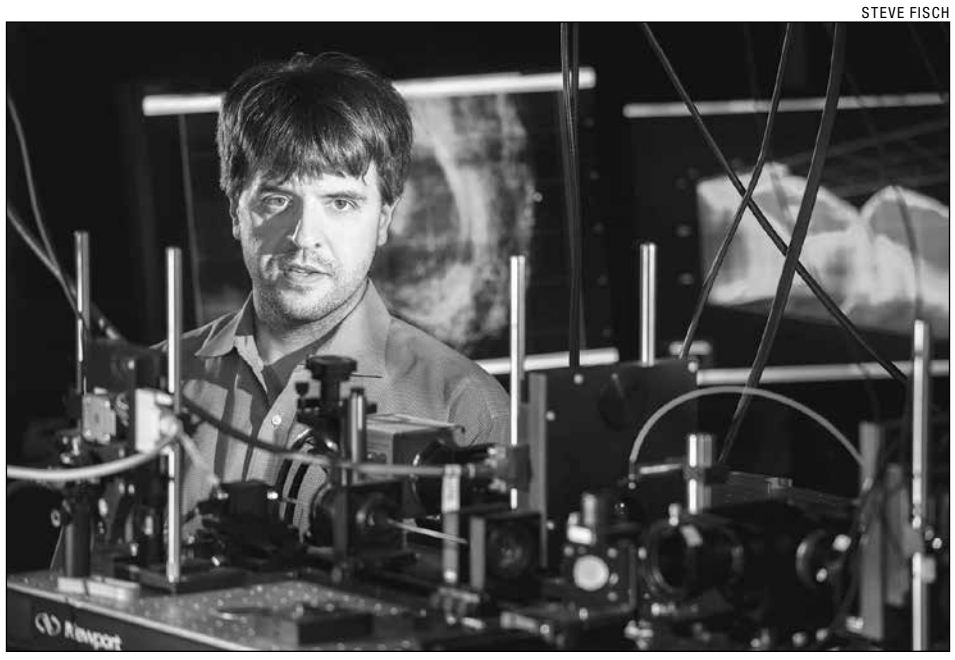
Feeding behavior and social stimulation activate intermingled but distinct brain circuits, and activating one circuit can inhibit the other, according to a new study by researchers at Stanford.

The researchers demonstrated in mice that direct stimulation of fewer than two dozen nerve cells, or neurons, linked to social interaction was enough to suppress the animals' drive to feed themselves — a finding with potential clinical significance for understanding and treating eating disorders such as anorexia.

The researchers made these findings by developing a technique for teasing apart separate but closely intertwined sets of neurons in the brain.

A paper detailing the findings and the method used to obtain them was published online Jan. 16 in *Nature*. The senior author is Karl Deisseroth, MD, PhD, the D.H. Chen Professor and professor of bioengineering and of psychiatry and behavioral sciences and a Howard Hughes Medical Institute investigator. Lead authorship is shared by postdoctoral scholars Joshua Jennings, PhD, and Christina Kim, PhD, along with staff scientist James Marshel, PhD.

STEVE FISCH



Karl Deisseroth and his lab team demonstrated that direct stimulation of fewer than two-dozen neurons linked to social interaction was enough to suppress a mouse's drive to feed itself.

Social curbs on eating behavior

"We know social situations can inhibit the urge to eat," Deisseroth said. "One example is the behavior of people at different levels of dominance in a social hierarchy. You're not going to dive into that plate of ribs when you're dining in the presence of royalty."

Anorexia is another example. "People with anorexia report that a powerful driver, at the disorder's onset, was feedback from others indicating they'd be rewarded for restricting their food intake," Deisseroth said.

Virtually nothing is known about the neural underpinnings of this inhibition, he said. "We sought to understand, at the level of individual neurons, how these potentially competing drives may negotiate with each other, and how the brain circuits associated with feeding versus social behavior may interact."

Deisseroth's group focused on a part of the brain called the orbitofrontal cortex, a sheet of cells that, in both mice and humans, lies on the brain's outer surface toward the front of the organ. This brain region, which is similar in the two species, has been shown in human imaging studies to be active when subjects are wishing for, seeking, obtaining and consuming food, or when they're socially engaged.

Exploring the interactions of feeding and social drives was guaranteed to be tricky.

"It's not as if there is a cluster of 'feeding' neurons' and another cluster of 'social' neurons sitting in two neatly labeled clumps in the orbitofrontal cortex, so you can just position an electrode in one or the other cluster and find out all you need to know," Deisseroth said. The neurons driving and responding to these different activities are interspersed, scarce and scattered throughout the orbitofrontal cortex like sprinkles on a cupcake. Plus, they all look pretty much the same.

So the researchers designed a sophisticated system for simultaneously stimulating and monitoring activity in multiple designated neurons. This let them determine which orbitofrontal-cortex neurons were active during feeding-associated or social activities, or both, or neither. The technology also allowed them to stimulate on the order of 20 neurons identified as dedicated to one or the other activity and watch what behavior resulted.

> "You're not going to dive into that plate of ribs when you're dining in the presence of royalty."

Over the past decade and a half, Deisseroth has pioneered the development of an experimental approach called optogenetics, in which a gene for a light-sensitive protein called an opsin is inserted into neurons so they can be activated by pulses of laser light reaching them via an implanted optical fiber. Recent advances in his lab have optimized one such opsin to the point where his team can stimulate numerous selected, behaviorally categorized neurons at a time in a mammal.

"This study builds on our initial demonstration in mammals of single cell control with optogenetics in 2012, but now marks the first demonstration of control of mammalian behavior by the manipulation of multiple, individually specified neurons," he said.

The scientists inserted the gene for this improved opsin into the orbitofrontal cortex of mice, along with another gene that causes neurons to fluoresce in proportion to their activity. A tiny lens at the tip of the optical fiber guided light across numerous targeted neurons with near simultaneity, causing as many as two dozen designated neurons to fire together.

Food vs. friends

During the experiments that followed, the mice were constrained by an apparatus that kept their heads comfortably fixed in place. In one set of experiments, mice were exposed to a spout that occasionally issued a drop of a high-calorie solution, which could be readily licked up. For each mouse, Deisseroth's colleagues recorded which orbitofrontal-cortex neurons among the several hundred in their field of view lit up during this activity.

Optogenetically stimulating just 20 feeding-responsive neurons enhanced the mice's licking activity in the presence of the high-calorie solution, tying those neurons causally to feeding behavior.

To identify social-responsive neurons in the mice's orbitofrontal cortex, the scientists introduced juvenile mice — which older mice perceive as nonthreatening potential buddies and set about sniffing — and tracked activity levels in the neurons in the field of view. They were able to identify specific neurons responsive to the exploratory social interaction.

Optogenetically stimulating social-responsive orbitofrontal neurons in the presence of a caloric reward reduced the amount of time the mice spent licking the solution. So did the natural-stimulation equivalent: exposure to juvenile mice. The more the social interaction, the less the interest in calories.

While the mice in this study weren't a disease model, Deisseroth noted the findings' potential clinical significance.

"We've been able to pinpoint otherwise indistinguishable orbitofrontal-cortex neurons involved in feeding and social drive states," he said. "A key goal was to know which neurons actually matter for behavior. Now that we do, we can examine them more closely to look for, say, surface-protein markers or wiring differences that distinguish them from one another. If there are any distinctions like that, it will deepen our understanding of how competing drives are negotiated among neuronal cell types in the cerebral cortex — and could even lead to pharmaceutical interventions that reduce social inhibition of food consumption among people with anorexia."

Deisseroth is a member of Stanford's Wu Tsai Neurosciences Institute and of Stanford Bio-X.

Other study co-authors are graduate student Misha Raffiee; postdoctoral scholar Li Ye, PhD; senior computational and optical systems engineer Sean Quirin, PhD; life science research professional Sally Pak; and life science research assistant Charu Ramakrishnan.

The study was funded by the National Institutes of Health, HHMI, the Defense Advanced Research Projects Agency, the National Science Foundation, the Wiegers Family Fund, the Nancy and James Grosfeld Foundation, the Sam and Betsy Reeves Fund and the H.L. Snyder Foundation.

Stanford's departments of Bioengineering and of Psychiatry and Behavioral Sciences also supported the work. The Department of Bioengineering is jointly managed by the School of Medicine and School of Engineering. **ISM**

Bladder

continued from page 2

lier than could be done with cystoscopy," Alizadeh said. With the new approach, they detected almost all cases of recurrent bladder cancer, nearly double the sensitivity of cystoscopy and cytology.

The researchers believe that the method of looking for cancer DNA in body fluids other than blood could be more widely applied. "It may eventually be useful for testing saliva for oral cancer, cerebrospinal fluid for neurological cancers or sputum for lung cancer," Diehn said.

Other Stanford co-authors are associate professor of urology Joseph Liao, MD; clinical assistant professor in pathology Henning Stehr, MD; pathology resident Simon Chen, MD; urology affiliate Dharati Trivedi, MD; pathology instructor Helio Costa, PhD; postdoctoral scholars Mohammad Esfahani, PhD, Mandy Sin, PhD, and Barzin Nabet, PhD; former postdoctoral scholar Aadel Chaudhuri, MD, PhD; medical students William Shi and Daniel Lazzareschi; former graduate student Jacob Chabon, PhD; research associate Chih Long Liu, PhD; cytologist Harumi Lim; and cytopathology laboratory director Marumi Lim.

This research was supported by the Stanford-National Institute of Standards and Technology Joint Initiative for Metrology in Biology, the Stanford Cancer Institute, the Albert Institute for Bladder Cancer Care and Research, the National Cancer Institute, the National Institutes of Health, the Virginia and D.K. Ludwig Fund for Cancer Research and the CRK Faculty Scholar Fund.

Stanford's departments of Pathology, of Medicine and of Radiation Oncology also supported the work. **ISM**

MARK TUSCHMAN

A study co-authored by Maximilian Diehn (left) and Ash Alizadeh found that testing for fragments of cancer DNA in urine enabled bladder cancer to be identified in its early stages of development.

Researchers work toward modeling hippocampus in silicon

By Bruce Goldman

In 1953, a 27-year-old Montreal man who'd had frequent seizures ever since getting a nasty smack in the head at age 7 underwent surgical removal of brain tissue containing the site where his seizures originated. The excised tissue included both of his hippocampi.

The hippocampus is a little horn-shaped structure found on each side of the brain's midline just above the ears. If you spend enough time staring at a cross section of it on a slide, you may eventually come to see it as resembling a seahorse, which is what the Latin terms *hippo* and *kampos* roughly translate to.

In the 1950s, not much was known about the role of the hippocampus — or any other brain structure. The plight of the patient, Henry Molaison — referred to as H.M. — is widely known among neuroscientists as both a cautionary tale and a wake-up call.

Sure enough, his seizures did subside. But for the rest of his long life (he died in 2008 at 82), he could not remember anything new — not a single thing — for more than 30 seconds. His pre-existing biographical memories were unaffected.

"You could have a perfectly lucid conversation with him, walk out of the room to get coffee, come back in and have the same exact conversation all over again," said Ivan Soltesz, PhD, professor and vice chair of neurosurgery at the Stanford School of Medicine. "For H.M., it was always as if for the first time."

H.M. could learn new motor skills just fine but couldn't remember learning them. Plus, his spatial memory was shot. He couldn't get around on his own.

From his experience and many thousands of unrelated experiments, brain scientists and brain surgeons have learned that the hippocampus is both indispensable for learning and memory and, often, the seat where epileptic seizures are initiated.

These features, along with some anatomical and physiological ones that make it easy and exciting to study, have propelled the once-mysterious hippocampus to the fore as arguably the most thoroughly researched part of the brain. Much of this learning has come about by carefully taking the hippocampus apart and analyzing the activities of its components and connections.

But Soltesz, who has devoted more than 30 years to understanding how brain circuits work (or don't), has gone a step further, taking to heart a thought attributed to the late, famed Caltech physicist and Nobel laureate Richard Feynman: If you can build it, you can understand it.

Soltesz, Stanford Medicine James R. Doty Professor of Neurosurgery and Neuroscience, and his teammates are building a full-scale virtual model of the hippocampus. But unlike a wooden airplane you might see hanging from a 12-year-old's bedroom ceiling, this model lives in a computer, in the form of mathematical depictions of neuronal types and their electrochemical components and connections that drive the reception, propagation and handoff of nerve impulses.

The resulting mathematical constructs mimic the component processes that go into a neuron firing off an impulse or its failure to fire one. As a result, the properties of any given individual virtual neuron, or its connections with other virtual neurons, are very similar to what you'd find in its biological counterpart.

"Anything we feed into the model is based on hard experimental evidence," Soltesz said. "If we're telling the computer that 'the firing frequency, strength and duration of this neuron-to-neuron connection should be *this* much,' it's because that's what we've observed in biological systems. We don't make stuff up."

They've completed two of its most important sections — about half of the entire structure — and used them to illustrate what goes on inside their flesh-and-blood counterparts. The project, funded by the National Institutes of Health and the National Science Foundation, is steaming along, with plans to complete the model within two or three years.

That, in turn, will enable neuroscientists to get a better handle on how the hippocampus does the immensely important things it does: namely, ruling over two crucial cognitive processes. The first is episodic memory (what I had for breakfast), and the second is spatial memory (where I parked the car).

The ability to run virtual experiments may also speed the much-sought understanding of why the hippocampus is so vulnerable to normal aging — leading us to forget what we ate for breakfast and where we parked the car — and is so particularly prone to the biological deterioration set in motion by Alzheimer's disease.

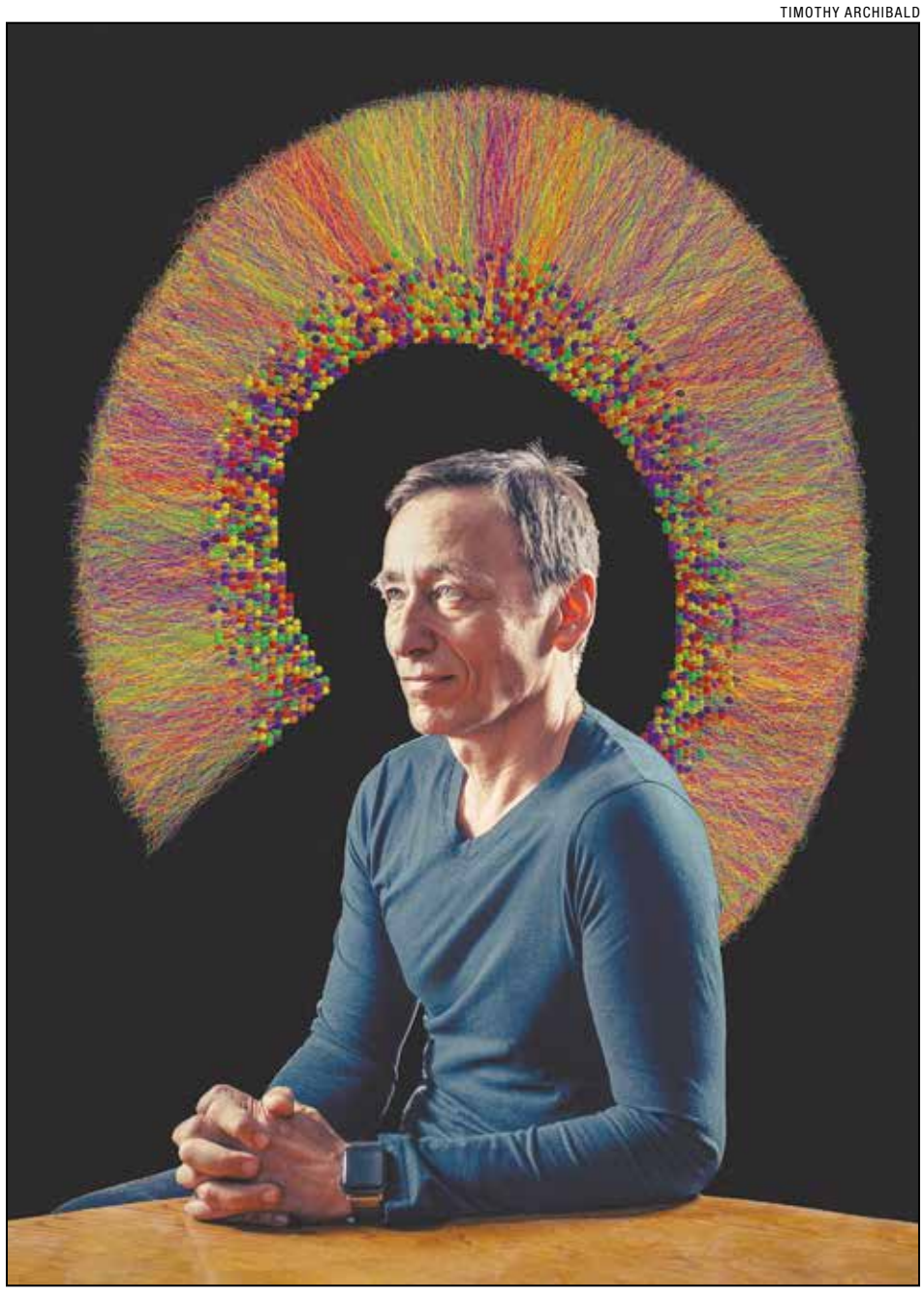
It may even lead to better understandings and treatments of a wide range of neurological conditions. And, of course, it's the next step to the logical final outcome: a virtual brain.

Replicating anatomy

Modeling the hippocampus at such a level of biological realism is a tall order. Like all brain regions, it's mostly a complicated tangle of individual neurons. The average neuron in the hippocampus is in communication with something like 1,000 other neurons, so the circuit diagram quickly gets crazy complicated. Making matters more daunting, not all neurons are alike.

About 80 percent of hippocampal neurons are excitatory: Their output signals have a stimulating effect on neurons they contact. Neuroscientists have divided the hippocampus into four or five serial compartments whose boundaries are roughly defined by where each compartment's excitatory neurons pass along information to their downstream partners. The first of these compartments is called the dentate gyrus, which plays a crucial role in the transfer of information. That's followed by compartments known as CA3, CA2 and CA1. (CA2 is very small and somewhat underexplored.)

TIMOTHY ARCHIBALD



Neuroscientist Ivan Soltesz in front of an image of the virtual dentate gyrus that his team created.

Most of the remaining neurons in the hippocampus are inhibitory, with their outputs exerting an impulse-stifling effect on downstream neurons. Unlike excitatory neurons, their reach is almost entirely restricted to other neurons within their own compartments. So, the hippocampal inhibitory neurons are also known as interneurons. An interneuron can impinge on both excitatory neurons and other inhibitory interneurons, forcing anyone trying to parse the logic of compartmental circuitry to cope with cascades of double and triple negatives.

> **"The result we got almost numerically mimicked what happens in the biological brain."**

Exploiting the model

"It would be valuable, from both medical and strict scientific standpoints, to know what will happen if — say, as a result of illness or trauma — the hippocampus loses 20 percent of one or another particular population of neurons," Soltesz said. But in the majority of cases, there's no known way to experimentally manipulate the activity of a specific neuronal subtype.

"That's where we come in," said Soltesz, referring to his virtual hippocampus.

In 2017, the Soltesz lab published a report in *eLife* on its virtual model of a rat's hippocampal compartment CA1 replete with 338,740 neurons, most of them excitatory. The minority of inhibitory interneurons are crucial, though. Of the many actual interneuronal subtypes known to inhabit CA1 (they differ in how hard and how quickly they inhibit and which part of their target neurons they contact), the model captures the majority of those whose characteristics and connections are well-known. The model features an astounding 5.7 billion neuron-to-neuron connections.

The numbers involved are so gigantic that a four-second simulation of CA1 activity takes four hours to run on Blue Waters, a powerful supercomputer hosted by the University of Illinois at Urbana-Champaign. Blue Waters' operating speed is measured in the number of mathematical calculations it can perform in one second: 10 to the 15th power, the equivalent of stringing a few hundred thousand high-performance laptops together to work in tandem.

Modeling the functions of a *human* hippocampus — 100 times bigger, with an accordingly astronomical number of neurons and connections among them — would crash any existing computer, however super. Besides, there's much more experimental data available about rodents because you can do experiments with them that can't be done on humans.

Soltesz's CA1 model exists in isolation. But he and his colleagues have approximated the placement, strength and timing of the inputs that this compartment's real-life biological counterpart would have received from an estimated 454,700 neurons coming into it from elsewhere.

Intriguingly, the researchers have found that this model spontaneously reproduces some important rhythmic firing patterns seen in real neurons in CA1. Soltesz thinks different phases of these rhythms serve a multiplexing function, like different channels on a TV, allowing separate streams of information to be processed in parallel and then routed to the right destination. When the timing is off, information is misrouted or loses its meaning. Researchers can maximize these rhythms by keeping the total level of input stimulation within a certain range.

Among the most prominent rhythms in the brain are so-called theta waves, which arise in the hippocampus. These occur when a mammal (including the human kind) is learning, moving around or dreaming. It turns out that the peak incoming-stimulation level for optimizing theta rhythms in Soltesz's CA1 model duplicates what's been determined using an isolated rat CA1 in a dish.

"The result we got almost numerically mimicked what happens in the biological brain," Soltesz said. "It's mind-blowing."

Soltesz's team went beyond merely recapitulating experimental findings and performed some simulations whose outcomes couldn't be predicted based on previous experimental experience. In their model, for instance, several different types of inhibitory cells needed to be transmitting impulses to recipient excitatory neurons at just the right time, frequency, speed and power or else the theta rhythm would collapse.

This finding doesn't prove that the same thing would happen in a living animal's hippocampus, but it generates a hypothesis that can in some cases be tested.

"I don't care if the model's predictions are proved true or false," Soltesz said. "The point is to make experimentally testable predictions. The model is a tool, not a be-all and end-all."

Playing around with an early, much simpler 10,000-cell model of another hippocam- **See BRAIN, page 5**

Scientists deploy worms to investigate neurological drugs

By Nathan Collins

There are drugs derived from plants to treat epilepsy, to prevent migraines and to halt manic episodes in people with bipolar disorder. But in many cases, no one knows exactly how those and other neurological drugs work — what chemical processes in the brain those drugs alter, or sometimes even what the active ingredients are.

Now, researchers at Stanford and the Carnegie Institution for Science have an unusual idea for how to better understand how those plant-derived drugs work. In the long run, that project — dubbed NeuroPlant and funded by a Big Ideas grant from the Wu Tsai Neurosciences Institute at Stanford — could also lead to new, more efficient ways to develop drugs to treat a variety of neurological and psychiatric diseases in humans.

"We're interested in finding out what the mode of action is of these compounds from plants that have known effects on the human nervous system," said Sue Rhee, PhD, a senior research scientist at the Carnegie Institution for Science and one of NeuroPlant's principal investigators. "I think it's an interesting place where we can potentially connect things like ethnobotany and plant science with neuroscience and perhaps even medicine."

Observing worm behavior

NeuroPlant is grounded in a simple observation: Plants have a powerful influence on our health and behavior. Around 80 percent of the world's population relies on herbal medicine for their primary health care, and many modern drugs, including neurological drugs, are derived from plants. That got Rhee wondering: What molecules do plants produce that affect our health and behavior, and what exactly are those molecules doing to us?

In a conversation with Miriam Goodman, PhD, professor of molecular and cellular physiology, Rhee learned that a tiny roundworm called Caenorhabditis elegans could be the perfect platform for studying these questions. For one thing, C. elegans are complete living systems, as opposed to the disembodied cells or isolated proteins researchers typically study, meaning that the NeuroPlant team can study their behavior, nervous systems and genetics all at once.

What molecules do plants produce that affect our health and behavior?

At the same time, C. elegans are much simpler than humans, while sharing a lot of genetic similarities. About 75 percent of the worms' genes have human equivalents, and their neurons are remarkably similar to humans' as well. That means what researchers learn about C. elegans stands a decent chance of applying to humans.

With Rhee's expertise in plant biology, Goodman's experience with C. elegans sensory systems and the neurobiology and genetics expertise of collaborator Thomas Clandinin, PhD, the Shooter Family Professor and a professor of neurobiology, the group thought they could pioneer a new approach to understanding the neurological effects of plant-derived compounds.

The team will start with a large collection of C. elegans worms containing a range of genetic mutations and a plant called valerian, extracts of which are now used in drugs to treat epilepsy and which have been used for thousands of years to treat mild anxiety.

When the team exposes their worms to valerian extracts they'll watch to see which ones wiggle closer and which flee. As the worms move toward and away from valerian extracts, they will sort themselves according to their genetically encoded responses to those extracts. Once they've self-sorted, the researchers can look to see which genes are responsible for that behavior — revealing, in the process, the genetic basis of valerian's effects on the worms. The team will also study how the nervous systems of the two groups of worms differ from each other. Because humans and roundworms have so many genes in common, these studies should offer hints about how molecules derived from valerian affect humans.

If the experiments with valerian work, the group will branch out to other plants that are the source of neurologic drugs.

A goal: Discover new drugs

The ultimate hope is to use these experiments not just to understand the genetic and neural pathways through which plant extracts and plant-derived drugs work, but also to discover new drugs. Right now, Goodman said, most drugs are developed by screening one small molecule at a time and focusing on just one genetic pathway at a time. If NeuroPlant's approach works, it could allow researchers to screen many potential drugs at a time and look at how those drugs affect a plethora of genetic pathways all at once, potentially speeding the discovery of new treatments.

For now, the aims are more focused. In a year, they hope to have identified a few plant molecules and a few target pathways for those molecules that could explain how a plant like valerian can help treat disease and shape behavior.

"I'm not expecting those chemicals to be drugs" that could actually treat disease, Goodman said. "But if we can identify targets, if we can identify chemicals, it becomes a productive entry point" for a new way to develop new drugs.

Clandinin and Goodman are members of the Wu Tsai Neurosciences Institute and Stanford Bio-X. ISM

L.A. CICERO/STANFORD NEWS SERVICE

Sue Rhee, Thomas Clandinin and Miriam Goodman are team members on the NeuroPlant project, which is using microscopic worms to better understand how plant molecules shape behavior.

Brain

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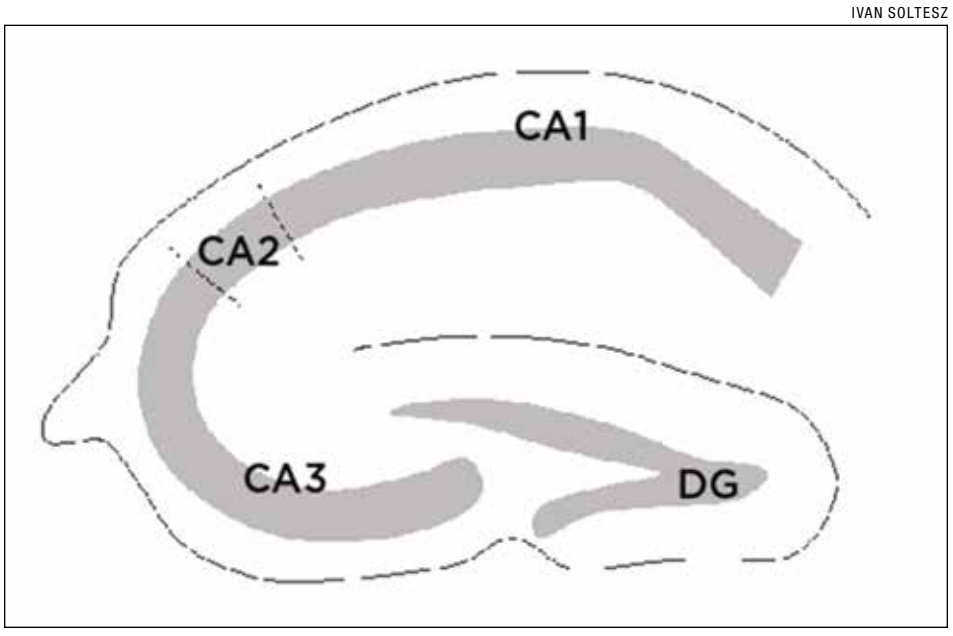
pal compartment, the dentate gyrus, generated an intriguing hypothesis that not only was borne out but also could have huge implications for people with epileptic seizures.

"We became intrigued by a small population of cells in the dentate gyrus, called mossy cells," Soltesz said. If neurons were airports, mossy cells would be JFK or LAX, he said. "These mossy neurons were especially hublike — each connects with many thousands of other neurons in the dentate gyrus." His team wondered if the loss of some significant fraction of these sparse but densely connected neurons might increase the likelihood of symptomatic epileptic seizures.

Because mossy cells, which are inhibitory, hook up with both excitatory and other inhibitory neurons, trying to predict what will happen when you silence some of them is a toss-up. In a study published in *Science* in early 2018, Soltesz's group succeeded in getting a clear answer. Their experiments in live mice showed that a die-off of mossy cells did trigger a rise in the spread of seizures originating in the dentate gyrus throughout the mice's brains. It also reduced the mice's ability to recall spatial information.

IVAN SOLTESZ



The hippocampus is divided into compartments that are roughly defined by where excitatory neurons — starting with the dentate gyrus — transfer information that is essential to forming memories.

Mossy cells are especially delicate and vulnerable to pressure as well as to decreased oxygen supply, which occur in brain injury or stroke. Concussions and stroke are known risk factors for increased seizure susceptibility. A drug aimed at protecting mossy cells could have major clinical possibilities.

"The point is to make experimentally testable predictions."

Soltesz's models, which he makes freely available, have been used by labs all over the world for research on memory storage and retrieval, antiepileptic drug effects, genetic models of disease and more. He makes available all the experimental data his team has fed into the model, so the data can be funneled into any new, improved model that comes along. He's even giving away software to investigators inclined to tinker with the model.

"What's most impressive about Ivan's CA1 model," said Angus Silver, PhD, a professor of neuroscience at University College London and a fellow of the Royal Society, "is that it's full-scale. He and his team have combed the literature for all the information available about this part of the hippocampus, they've put it all together and, amazingly, it exhibits several key properties of the real CA1, without your having to tweak it. It certainly gives you hope that this approach is useful."

Meanwhile, Soltesz and his team are forging ahead with a full-scale model of the rodent dentate gyrus (1 million cells in all, three times as many as in CA1), which Soltesz expects to complete in less than a year. Next on the to-do list are CA3, then models of nearby structures that send inputs to the hippocampus. In the longer term, he wants to model the entire brain region that includes the hippocampus — the temporal lobe.

But why stop there? Other well-funded groups, such as the Allen Institute for Brain Science in Seattle and the Human Brain Project in Switzerland, are also building representations of various parts of the brain.

"We're not working in a vacuum," Soltesz said. "I can imagine that in 20 years or so we'll have full-scale models of mouse and human brains at single-cell resolution." It may someday be possible, Soltesz speculated, to build customized, patient-specific models of the entire brain based on advanced noninvasive imaging and recording techniques, so the effects on any patient's brain of a given drug or other intervention can be tested.

When a model has as much resolution as the thing it's modeling, is it still a model? Or is it a working copy?

"That's an excellent question," Soltesz said. "Luckily, we're nowhere near being able to answer it. It's pure fantasy right now. But we humans are busily working our way toward that quandary. We'll deal with it when we get there." ISM

Pain

continued from page 1

Mark Schnitzer

neurons flared after this stimulus.

"But that really only tells you that those neurons were active at some point, and it's not specific enough," Scherrer said. "What we wanted was to look at the neurons of freely moving animals."

To observe the deep-seated wiring of a mouse's brain, Scherrer partnered with Schnitzer, who had developed a "miniscope" — a microscope about the length of a small paper clip, which could be affixed to a mouse's head to record activity in its brain. They positioned the device strategically to visualize the amygdala. The mouse, alive and well, could stroll as it pleased, while the miniscope recorded calcium flux in the neurons, a proxy for cell activity.

The scientists monitored the mouse brains with the microscope, watched the mice detect something uncomfortable, observed the aversive reactions and then checked which neurons were active. "With this setup, we identified a set of neurons in the amygdala that selectively encodes signals related to the emotional aspects of a painful experience," Schnitzer said.

When the mice touched a drop of uncomfortably hot or cold water (neither of which were severe enough to injure the mice), they withdrew, signaling to the scientists that the rodents were not pleased. Upon this withdrawal, the microscope's recording showed a bundle of neurons firing in the amygdala — specifically in the basolateral region — suggesting that these neurons were specifically responsible for the emotion of pain.

It was, however, still possible that this basolateral ensemble was simply firing to relay general emotion, rather than the unpleasantness of pain specifically. So, the researchers fed the mice sugar water — a sweet treat known to bring joy to any mouse — and kept an eye on the collection of neurons suspected to relay displeasure. As expected, those neurons stayed silent.

"There's also a difference between experiencing pain and experiencing something annoying, so we further wanted to test if the amygdala neurons active during pain were also associated with overall negative emotion, rather than pain particularly," Scherrer said.

What miffs a mouse? The same things that might bother a sibling: tiny puffs of air to the face, an unappetizingly bitter taste or a very bad smell. While bothering the mice, the researchers again monitored the basolateral amygdala pain ensemble, and here, too, the neurons remained subdued.

Tracking the perception of pain

"After all of that, we concluded that this ensemble of neurons selectively responds during pain," Scherrer said. "But it still didn't fully demonstrate that they underpinned the emotional response."

To investigate that question more deeply, the researchers set up a walking track with three invisible lanes: On the far left was a cold strip; on the right, a hot one; and in between the two was a temperate middle ground. (For context, walking in the two outer lanes was comparable to walking barefoot on pavement during winter or summer, respectively — uncomfortable, but not permanently damaging.)

Normal mice that walked on the track gradually learned that the middle lane was tolerable, while the outer two were unpleasant. But in a select group of mice, the researchers temporarily disabled the bundle of amygdala pain neurons thought to relay feelings of physical discomfort. These mice — free of pain-incited unpleasantness — skittered around the outer regions, undeterred by the extreme temperatures.

What's intriguing about this, Scherrer said, was that these mice weren't bereft of physical feeling. "Pain was just no longer unpleasant for them," he said. The rodents could still feel and respond to physical sensations, but the stimuli they once perceived as unpleasant (hot or cold drops of water) were no longer bothersome. When exposed to a drop of hot water, for example, the mice with a muted basolateral neural ensemble would move their paw away from the dropper, signaling that they felt the stimulus — but they would move their paw back to its original position, something that normal mice did not do. This is a crucial part of harnessing the ensemble as a tool in pain therapy, Scherrer said, as an animal, or human, without the ability to physically feel anything at all leaves them vulnerable to injury.

Long term, Scherrer aims to confirm that the function of the basolateral ensemble in mice is the same as it is in people, and then down the line, find a safe and effective way to silence the ensemble's function without interfering with other neurons.

"There's really no good treatment for chronic pain in humans, and that's a major driver of the opioid epidemic," Scherrer said. "But you'll notice, patients who take opioids for pain report that they can still feel the sensation of pain but say it's less bothersome — the emotions of pain are different. Our big future hope is that the cells in the basolateral ensemble could be a tactic to curb the ailment of pain without causing addiction and thus, ideally, act as a possible substitute for opioid treatment."

Other Stanford co-authors of the study are former Stanford postdoctoral scholar Benjamin Grewe, PhD, and research scientist Dong Wang, PhD.

The study was funded by the National Institutes of Health, the New York Stem Cell Foundation, the Rita Allen Foundation, the American Pain Society, the National Science Foundation, the Howard Hughes Medical Institute, the Bill and Melinda Gates Foundation and the Swiss National Science Foundation.

Stanford's departments of Anesthesiology, Perioperative and Pain Medicine, of Neurosurgery, of Biology and of Applied Physics also supported the work. ISM

Artery

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The researchers began by looking at newborn mice. "Neonatal mice have a robust ability to heal injured heart tissue, but they no longer have that ability in adulthood," Red-Horse said. "Understanding why could identify ways of reigniting regeneration in adults."

They documented that the young mice's healing was due in part to the growth of new collateral arteries into the injured area. Through advanced imaging that let them look at the intact newborn hearts at the cellular level, the researchers showed that this happened because arterial endothelial cells exited the artery, migrated along existing capillaries that extended into injured heart tissue and reassembled to form collateral arteries.

> "They are like the side streets that let you get around a traffic jam on the freeway."

Then the researchers investigated how the cells knew to do this. Red-Horse and Woo knew that the molecule CXCL12 is an important signal during embryonic development of arterial cells, and has been shown to improve cardiac recovery and function after heart attacks. The scientists wondered if this molecule had a beneficial effect by promoting collateral artery growth in injured heart tissue. They found that CXCL12 was mostly restricted to arterial endothelial cells in uninjured neonatal mouse hearts. In newborn mice with heart injuries, it shows up in the capillaries of the injured area. The researchers found evidence that low oxygen levels in the injured area turned on genes that create CXCL12, signaling the areas to which arterial endothelial cells should migrate.

Testing CXCL12 in adult mice

Next they investigated whether CXCL12 could help adult heart tissue grow collateral arteries. "Our studies showed that adult hearts do not form collateral arteries in the way newborns do after injury," Red-Horse said. After inducing heart attacks in adult mice, they injected CXCL12 into the injured areas. Sure enough, 15 days after the injuries, there were numerous new collateral arteries formed by the detaching and migrating artery cells. Almost none were present in control mice.

Red-Horse and Woo think the complete story is not this simple. "We speculate that there is a whole suite of proteins that support cell migration out of arteries and promotes cell proliferation among the injured cells," Red-Horse said. Nonetheless, they hope that this discovery can become the basis for a new therapy.

"The question now is whether this mechanism we have discovered can be manipulated therapeutically to generate collateral arteries in human patients," Woo said.

Other Stanford co-authors of the study are associate professor of medicine Vinicio de Jesus Perez, MD; postdoctoral scholars Hanjay Wang, MD, Michael Paulsen, MD, Gaetano Amato, PhD, and Siyeon Rhee, PhD; graduate student Ragini Phansalkar; and research assistants Justin Farry, Anahita Eskandari, Elya Shamskhou and Camille Hironaka.

Researchers at Ball State University and Columbia University also contributed to the work.

The research was supported by the National Institutes of Health, the New York Stem Cell Foundation, the American Heart Association and the Leducq Foundation.

Stanford's departments of Cardiothoracic Surgery and of Biology also supported the work. ISM

Compound identified that may help treat heart failure

By Mandy Erickson

Heart attack survivors may think the worst is behind them. But many later develop heart failure, a progressive disease marked by shortness of breath and swelling in the legs. Symptoms can prevent patients from working, exercising — even picking up grandchildren.

Heart failure occurs after a heart attack when enough of the heart muscle dies, causing the rest of the heart to overwork, which leads to more damage. To protect an overworked, failure-prone heart, cardiologists typically prescribe medications that encourage the heart to take it easy, said Daria Mochly-Rosen, PhD, professor of chemical and systems biology and the George D. Smith Professor in Translational Medicine.

Mochly-Rosen is hoping to tackle heart failure at the molecular level. She and her colleagues developed a compound that in preliminary tests appeared to improve heart function in rats with heart failure caused by a heart attack.

The study was published Jan. 18 in *Nature Communications*. Julio Ferreira, PhD, a professor at the University of Sao Paulo, is the lead author.

One contributor to heart failure following a heart attack is the accumulation of broken or dysfunctional mitochondria, the small organelles in cells that produce energy. The researchers identified a pair of proteins that, when bonded, gum up the normal activity of mitochondria and contribute to heart failure. One of those proteins, protein kinase C beta 2, is found in higher levels in failing human and rodent hearts.

The researchers tapped their chemistry know-how to develop a compound called SAMβA (pronounced "samba"), which can prevent these proteins from bonding, thereby improving mitochondrial function and providing more energy for the heart.

In tests, post-heart-attack rats that developed heart failure and were treated with SAMβA had better cardiac function — measured by how well their left heart ventricles pumped blood with each heart beat — than rats that weren't treated with SAMβA.

"We greatly improved their hearts," Mochly-Rosen said. "If humans are going to be like rats, perhaps we can treat them with a drug that prevents this deterioration."

She added that they also gave healthy rats doses of SAMβA "and it had absolutely no effect," an indication that the compound is nontoxic.

Mochly-Rosen and Ferreira suspect that SAMβA will also be effective in humans. If so, it has the potential to be developed into a drug for human heart attack patients, they believe.

"I'm hopeful SAMβA will be accepted by the industry for drug development because it appears very promising," Mochly-Rosen said. ISM

Longtime Stanford nurse Betty Rose dies at 83

A memorial service for Betty Rose, a nurse who worked at Stanford Hospital for more than 40 years in a variety of leadership roles, will be held at 5 p.m. Jan. 31 at All Saints' Episcopal Church at 555 Waverley St. in Palo Alto.

Rose died Jan. 22 at her home in Palo Alto. She was 83.

In lieu of flowers, donations may be made to the El Observador Foundation (http://elobservador-foundation.org), which Rose and and her husband Hilbert Morales, who survives her, founded in 1983 to support the Bay Area's Hispanic community. The foundation's address is 1042 West Hedding St., Suite 250, San Jose, CA 95126. ISM

Scientists generate myelin-producing cells, track their growth

By Bruce Goldman

For proper brain function, it's crucial that certain neurons be wrapped with myelin, a coating that enhances impulse transmission. Failure can spell outcomes ranging from cerebral palsy to multiple sclerosis.

A better understanding of oligodendrocytes, the brain cells that make myelin, might help correct or prevent these diseases. Yet, while cultured neurons have long been scrutinized and manipulated in efforts to pry loose their secrets, studying human oligodendrocytes has been tough. They're born late in brain development, and they're challenging to generate alongside human neurons and other brain cells in a way that recapitulates the complex interactions occurring among these cell types as they develop.

Now, School of Medicine investigators have proved that a system they developed a few years ago for culturing balls of stem-cell-derived human brain cells, which mimic aspects of real brain circuitry, can generate oligodendrocytes together with neurons and a third type of brain cell called astrocytes.

"We now have multiple cell types interacting in one single culture," said Sergiu Pasca, MD, assistant professor of psychiatry and behavioral sciences. "This permits us to look close-up at how the main cellular players in the human brain are talking to each other."

A study describing the work was published online today in *Nature Neuroscience*. Pasca, who directs the human brain organogenesis program at the Wu Tsai Neurosciences Institute at Stanford, is the study's senior author. The lead author is graduate student Rebecca Marton.

From stem cells to oligodendrocytes

In a dish, all three cell types develop from pluripotent stem cells; arrange themselves within three-dimensional brain balls, or spheroids — much as they would in the human forebrain; progressively advance in maturity; and engage in lifelike interactions with one another. From their ringside seats, the Stanford scientists peeked in on oligodendrocytes' movements and watched them wrap their extensions around individual neurons to form the insulating coats of myelin that, in real-life brain tissue, speed up signal transmission.

The researchers were able to determine which genes were active at different stages of oligodendrocyte development in the brain spheroids, and to show that these gene-activation patterns were extremely similar to those of real-life oligodendrocytes at comparable stages of maturation. This enabled them to pinpoint, in these cultured oligodendrocytes, the different times of onset of activation of several genes that, when mutated, cause different congenital myelination disorders — a finding with possible implications for modeling these disorders.

Many of these brain spheroids, which contain as many as 1 million cells and measure as much as one-eighth of an inch in diameter, have survived in culture for at least two years. During that time, another type of brain cell manifested. These cells, called astrocytes, outnumber neurons in the brain and perform many essential tasks, from managing nutrition and energy supplies for neurons to directing the positioning, formation and functionality of synapses, the junctions through which neurons transfer information to one another.

In the human cerebral cortex, most neurons are born by week 26 of gestation. Astrocytes begin to appear around this period and continue to mature for months afterward. Previous culture methods didn't keep cells alive long enough to recapitulate this maturation process.

Human oligodendrocytes take even longer to make their appearance. In higher brain regions such as the cerebral cortex, responsible for advanced cognitive functions such as decision-making, scheduling and foresight, oligodendrocytes begin to form in significant numbers around the time of birth.

In the new study, the researchers modified their previous method of culturing brain spheroids by adding special growth factors and nutrients that promote oligodendrocyte formation, survival and development. By day 100 of culture initiation, oligodendrocytes were present alongside neurons and astrocytes.

Using live-imaging microscopy, Marton was able to see into brain spheroids and record the behavior of oligodendrocytes that had been labeled with a fluorescent marker. She did this extensively between days 65 and 275, monitoring cells' behavior by varying the microscope's depth of field. The researchers watched oligodendrocytes migrating from their points of origin to their neuronal destinations.

What electron microscopy revealed

High-resolution electron microscopy revealed oligodendrocyte extensions sheathing neuronal filaments within three to four months of culture initiation.

When the scientists exposed the brain spheroids to a fat-dissolving emulsifier, "oligodendrocytes were affected the most," Pasca said. "It was as though they were melting."

Generating brain spheroids from patient-derived skin cells allows medical researchers such as Pasca to study neurological and psychiatric diseases on a personalized basis without having to obtain and maintain living brain tissue.

Pasca's group is looking at a number of genetic disorders affecting myelination that arise in fetal development or early childhood. But oligodendrocyte-containing brain spheroids could also prove useful in studying demyelination disorders, such as multiple sclerosis and cerebral palsy — and even some psychiatric conditions not usually thought of as myelin-associated.

"We know that in schizophrenia, myelination, especially in adolescence, is disrupted prior to a patient exhibiting symptoms," Pasca said.

STEVE FISCH

Sergiu Pasca and his team have proved that a system they developed a few years ago for culturing balls of stem cell-derived human brain cells can generate oligodendrocytes together with neurons and a third type of brain cell called astrocytes.

Pasca is a member of Stanford's Maternal & Child Health Research Institute and a faculty fellow of Stanford ChEM-H.

Other study co-authors are postdoctoral scholars Yuki Miura, PhD, Qingyun Li, PhD, and Omer Revah, DVM; former postdoctoral scholar Steven Sloan, PhD; resident physician Rebecca Levy, MD, PhD; and John Huguenard, PhD, professor of neurology and neurological sciences and of neurosurgery.

The study was funded by the National Institutes of Health, the National Science Foundation, the MQ Foundation, the Robertson New York Stem Cell Foundation, the Wu Tsai Neurosciences Institute, the Kwan Research Fund, the California Institute for Regenerative Medicine, Stanford Bio-X and the Office of the Dean of the Stanford School of Medicine.

Pasca and Marton are co-authors of a patent that Stanford's Office of Technology Licensing has filed with the federal government.

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

Gun

continued from page 1

45 percent of the bill through Medicaid and, to a lesser extent, Medicare and other public insurance programs, the researchers found.

With most gunshot-wound patients surviving their initial hospital stay, the analysis, which was published online Jan. 23 in *PLOS ONE,* sheds new light on the chronic nature of firearm injuries and the resulting medical costs.

However, the estimates offer only a piece of a larger puzzle, as the numbers do not include the costs of non-inpatient services, such as medications, rehabilitation, long-term care or home health care, said Sarabeth Spitzer, a fourth-year medical student at Stanford who is the study's lead author.

"So often gun injuries are talked about in terms of mortality, as one-time events for medical care," Spitzer said. "What tends to be forgotten are the long-term effects these injuries have on the people who survive and the monetary costs to the health care system."

> "We end up as a society paying a huge amount for these injuries."

Thomas Weiser, MD, associate professor of surgery, is the study's senior author.

Risk of readmission

Examining six years of data from the Healthcare Cost and Utilization Project's Nationwide Readmissions Database, Spitzer and her colleagues found that 93 percent of the patients initially admitted to the hospital with firearm injuries survived.

Of those 155,574 patients, the study showed that 15.6 percent were readmitted one or more times within six months, amounting to more than 33,000 hospital readmissions during the study period. More than half occurred within 30 days of the original discharge.

Risk of readmission was highest for patients who were older, suffered more severe injuries, stayed in the hospital longer during their initial admission or required surgery. Patients covered by Medicare had the highest risk of readmission, followed by patients covered by Medicaid.

Self-paying patients were the least likely to be rehospitalized for a firearm injury. Spitzer said this could be because, without insurance coverage, these patients often face full hospital charges that they can't afford: For example, the average readmission charge for self-paying patients was $49,087. Most live in ZIP codes where the population has incomes lower than average, according to the study.

Sarabeth Spitzer

"We do not necessarily think their injuries are less severe," Spitzer said. "It could be that they don't seek care in circumstances where other patients, who are covered by insurance, would take advantage of that health insurance."

Hospitalization, readmission costs

Overall, government insurance paid the most for hospitalizations related to firearm injuries during the six-year period, according to the study. Of a total $5.47 billion in costs, Medicaid contributed $2.1 billion and Medicare provided $389 million. Private insurance and self-paying patients each accounted for about $1.1 billion, while the remainder was covered by other payers.

On average, the total cost of inpatient hospital care for a patient with a firearm injury was $32,700, the study found, and readmission accounted for 9.5 percent of that bill.

Spitzer said she hopes the numbers help fill in a larger picture of the effects of gun violence. She is founder and co-director of the nonprofit Scrubs Addressing the Firearm Epidemic, or SAFE, which promotes research, education and evidence-based policy as a means to reduce firearm injuries.

"We end up as a society paying a huge amount for these injuries," Spitzer said. "These numbers draw attention to the fact there are consequences we all face when people are injured by guns."

Other Stanford co-authors are David Spain, MD, professor of surgery; surgery resident Charlotte Rajasingh, MD; research scientist Lakshika Tennakoon; and medical student Daniel Vail.

The Stanford Medical Scholars Research Program supported this work.

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

Medical school communications office receives six writing awards

By Susan Ipaktchian

Writers in the School of Medicine's Office of Communication and Public Affairs received six awards for their work in the Association of American Medical Colleges' annual competition.

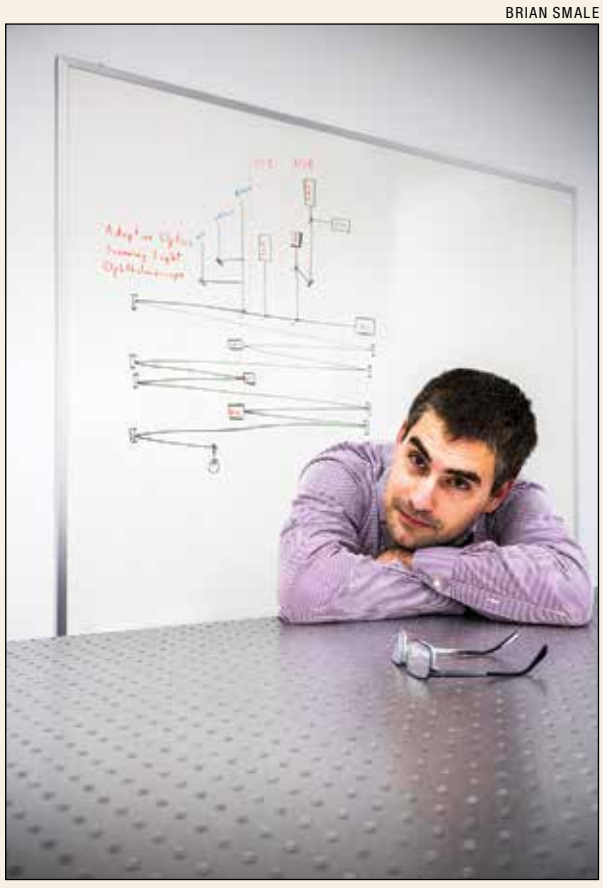
In all, the office received four of the five gold awards in the national contest's writing categories, along with a silver and a bronze award. The awards were for work published in 2017-18.

Science writer Krista Conger received the gold award in the basic-science writing category for "Eye spy," a feature that was published in the summer 2017 issue of *Stanford Medicine* magazine. It describes the journey of Uruguayan-native Alfredo Dubra: As a young boy, Dubra suffered from strabismus, or crossed eyes; today, he is a renowned physicist who is spending his life trying to find better ways of examining the retina. "A perfect piece!" the judges wrote. "Well-written, with clear descriptions, a natural flow and wonderful narrative (excellent story and quote to illustrate how people get into a career because of a personal experience)."

Science writer Bruce Goldman received the silver award in the same category for "Brain balls," a feature in the winter 2018 issue about researcher Sergiu Pasca's efforts to develop brain organoids that can be grown and studied in the lab. Judges called it a "superb feature" with "evocative explanations of the science. A very enjoyable long read."

"Magical moment," which recounted the events surrounding the first U.S. adult heart transplant, earned a gold award in the general staff writing category. Science writer Tracie White brought to life the historic surgery that took place 50 years earlier at Stanford Hospital. The story was published in the winter 2018 issue of the magazine. Judges for the contest called the story "perfect," and said it was "an astoundingly beautiful and informative piece of writing that takes the reader back in time to an historical medical milestone."

BRIAN SMALE



An award-winning story described how Alfredo Dubra went from a child with strabismus, or crossed eyes, to a physicist working to find better ways of examining the retina.

In the solicited articles category, novelist Joyce Maynard received the gold award for an essay she wrote about life while her husband was being treated for cancer and how the experience changed her. The essay, "In the fog of loss," was published in *Stanford Medicine*'s summer 2017 issue. Judges commented, "This was beautiful. A great service — giving space to an excellent writer to tell a medical story in a clear, emotional, personal way."

The magazine is edited by Rosanne Spector.

Conger also received a gold award in the news release category for her Jan. 31, 2018, release about a potential vaccine therapy for cancer developed by researcher Ronald Levy and his colleagues. The story drew huge amounts of coverage from the news media, resulting in inquiries from cancer patients throughout the world who hoped they could take part in a planned clinical trial of the experimental therapy. To date, the news release has garnered more than 1.5 million views on the school's website. Judges called it "a model news release for the current age."

Goldman received the bronze award for his Oct. 27, 2017, news release about researcher Michael Eisenberg's findings linking the frequency of marijuana use to the frequency of sexual intercourse. Judges said the release was nicely written and that they could easily see why the findings drew such widespread news coverage.

The news releases are edited by John Sanford.

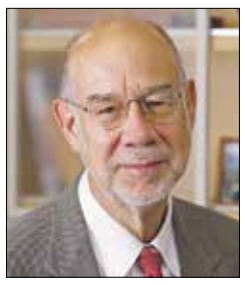
The awards are given by the AAMC's Group on Institutional Advancement, which includes communications, development and alumni relations staff at academic medical centers. This year's awards will be presented April 11 in Orlando, Florida, at the group's annual meeting. ISM

OF NOTE

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

Megan Albertelli

Ruben Alvero



Harry Greenberg

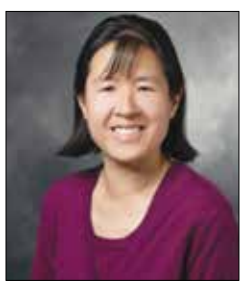
Ed Kopetsky

Nicholas Leeper

Robert Ohgami

Nidhi Rohatgi

Maria Grazia Roncarolo



Lisa Shieh

David Svec

MEGAN ALBERTELLI, DVM, PhD, was promoted to associate professor of comparative medicine, effective Nov. 1. Her research focuses on genetic variation in breast and prostate cancers, and the development and refinement of mouse models of cancer and celiac disease. She also investigates ways to improve the welfare of animals in laboratory settings.

RUBEN ALVERO, MD, was appointed professor of obstetrics and gynecology, effective Dec. 1. His clinical focuses include infertility, polycystic ovary disease, endometriosis and reproductive surgery. His research aims to improve the understanding and treatment of unexplained infertility.

HARRY GREENBERG, MD, the Joseph D. Grant Professor in the School of Medicine and professor of medicine and of microbiology and immunology, is the recipient of the 2019 Distinguished Achievement Award in Basic Science from the American Gastroenterological Association. The award honors a senior investigator whose work has significantly advanced the science or practice of gastroenterology, or both, and includes a $5,000 honorarium.

ED KOPETSKY, chief information officer for Stanford Children's Health, was awarded the 2018 John E. Gall Jr. CIO of the Year award by the College of Healthcare Information Management Executives and the Healthcare Information and Management Systems Society. Under Kopetsky's leadership, Stanford Children's Health has undergone large-scale advances in enterprise systems and customer service.

NICHOLAS LEEPER, MD, associate professor of surgery and of medicine, has been awarded an Emerging Investigator Award by the National Institutes of Health. The seven-year, $6 million grant will support his research on clonal expansion, resistance to efferocytosis (the removal of dead or damaged cells) and innate immunity in atherosclerosis.

ROBERT OHGAMI, MD, PhD, was promoted to associate professor of pathology, effective Dec. 1. His research focuses on classifying and understanding hematopoietic diseases such as Castleman disease, acute myeloid leukemia and T- and B-cell disorders using molecular technologies, digital imaging approaches and computational methodologies.

NIDHI ROHATGI, MD, clinical associate professor of medicine, has been named one of the 10 top hospitalists of 2018 by *ACP Hospitalist*, a publication of the American College of Physicians. The editorial board selected her from nominees across the nation for her dedication to patient care. She was honored for her work improving transitions in care, preventing medical complications in surgical patients and preventing hospital-acquired delirium.

MARIA GRAZIA RONCAROLO, MD, the George D. Smith Professor in Stem Cell and Regenerative Medicine and professor of pediatrics and of medicine, received the 2019 Translational Pioneer Award from *Cell & Gene Therapy Insights*. The award recognizes the contributions of her translational research studies, which have led to greater understanding of the mechanisms underlying immune tolerance in stem cell transplantation, and have been fundamental to the development of novel stem cell and gene therapies for patients with genetic and acquired diseases of the hematopoietic and immune systems.

Manpreet Singh

Joy Wu

LISA SHIEH, MD, PhD, clinical professor of medicine, and **DAVID SVEC**, MD, MBA, clinical assistant professor of medicine, received the Malinda Mitchell Award from Stanford Health Care for their work to decrease inappropriate use of intermediate intensive care unit level care. The award, which recognizes excellence in quality and service, is named for the former president and chief executive officer of Stanford Health Care.

MANPREET SINGH, MD, was promoted to associate professor of psychiatry and behavioral sciences, effective Nov. 1. She directs the Stanford Pediatric Mood Disorders Program. Her work focuses on characterizing the origins and pathways for developing mood disorders, and conducting translational research to protect and preserve brain function after symptoms begin. In addition, she was selected to receive the 2019 American Psychiatry Association's Blanche F. Ittleson Award, which includes a $2,000 honorarium and recognizes a psychiatrist whose work has had significant impact on child and adolescent psychiatry.

JOY WU, MD, PhD, assistant professor of medicine, has joined the Endocrine Society's leadership team as a member of its council. In her role representing basic science, she will work toward the society's mission to promote optimal health by advancing endocrine science, education and care. ISM