McGOVERN INSTITUTE
FOR BRAIN RESEARCH AT MIT

Brain SCAN

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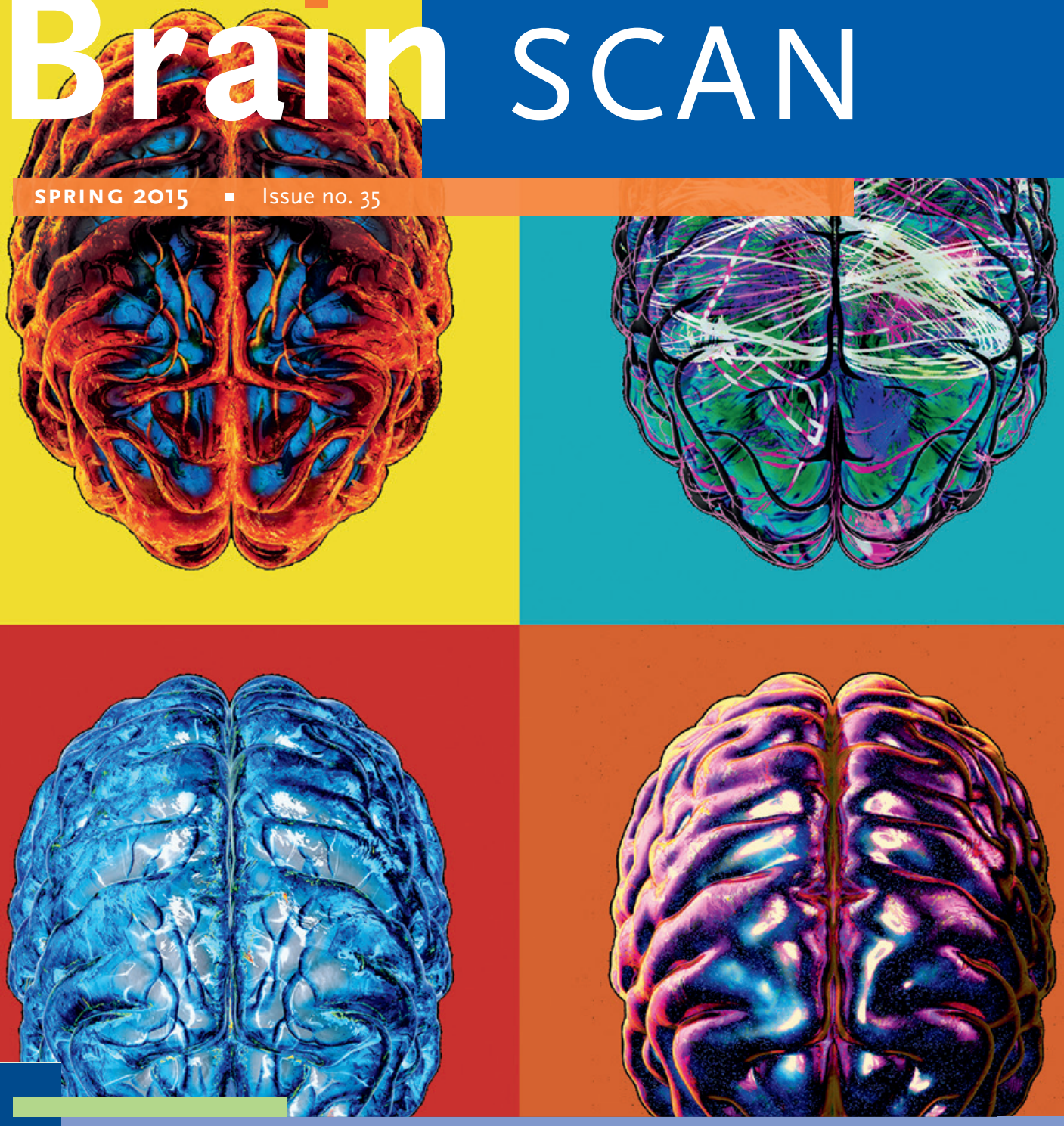


Bold New Microscopies for the Brain

New technologies are changing how scientists study the brain's fine structure

FROM THE **DIRECTOR**

For many people the word 'microscopy' evokes images of dusty laboratories and dry anatomical textbooks. But in fact the past few years have seen extraordinary advances in microscopy, including the development of 'super-resolution' methods that were recognized by the 2014 Nobel Prize.

New methods are urgently needed if we are to understand the brain, whose function depends on the activity of billions of neurons, communicating with each other through trillions of tiny synaptic connections. To trace these connections, we need to see not just the forest but every tree, down to its individual leaves and twigs. Only when we have this 'wiring diagram' can we fully understand how information flows through the brain, and how this flow is disrupted by disease.

Among those pushing the frontiers of microscopy is my colleague Ed Boyden. To cite just one example, in the method known as 'expansion microscopy,' Ed and his colleagues have turned the very idea of microscopy on its head; instead of trying to magnify the image, they devised a way to expand the specimen itself, with some help from baby diapers, as you can read in this issue.

A century ago, in words that still resonate today, the great Spanish neuroanatomist Santiago Ramón y Cajal described the brain as "a world of unexplored continents." But today, I believe that we are entering a new golden age of exploration, and that a map of the brain that Cajal could only have dreamed of is now within our grasp.

Bob Desimone, Director
Doris and Don Berkey Professor
of Neuroscience

On the cover:
Artwork by Crean Quaner
www.neuronize.de

McGovern researchers create unexpected new approaches to microscopy that are changing the way scientists look at the brain.

Ask McGovern Investigator Ed Boyden about his ten-year plan and you'll get an immediate and straight-faced answer: "We would like to understand the brain."

He means it. Boyden intends to map all of the cells in a brain, all of their connections, and even all of the molecules that form those connections and determine their strengths. He also plans to study how information flows through the brain and to use this to generate a working model. "I'd love to be able to load a map of an entire brain into a computer and see if we can simulate the brain," he says.

Photo: Justin Knight

Bold New Microscopies for the Brain

Boyden likens the process to reverse-engineering a computer by opening it up and looking inside. The analogy, though not perfect, provides a sense of the enormity of the task ahead. As complicated as computers are, brains are far more complex, and they are also much harder to visualize, given the need to see features at multiple scales. For example, signals travel from cell to cell through synaptic connections that are measured in nanometers, but the signals are then propagated along nerve fibers that may span several centimeters—a difference of more than a million-fold.

Modern microscopes make it possible to study features at one scale or the other, but not both together. Similarly, there are methods for visualizing electrical activity in single neurons or in whole brains, but there is no way to see both at once. So Boyden is building his own tools, and in the process is pushing the limits of imagination. "Our group is often trying to do the opposite of what other people do," Boyden says.

Boyden's new methods are part of a broader push to understand the brain's connectivity, an objective that gained impetus two years ago with the President's BRAIN Initiative, and with allied efforts such as the NIH-funded Human Connectome Project. Hundreds of researchers have already downloaded Boyden's recently published protocols, including colleagues at the McGovern Institute who are using them to advance their studies of brain function and disease.

Just Add Water

Under the microscope, the brain section prepared by Jill Crittenden looks like a tight bundle of threads. The nerve fibers are from a mouse brain, from a region known to degenerate in humans with Parkinson's disease. The loss of the tiny synaptic connections between these fibers may be the earliest signs of degeneration, so Crittenden, a research scientist who has been studying this disease for several years in the lab of McGovern Investigator Ann Graybiel, wants to be able to see them.

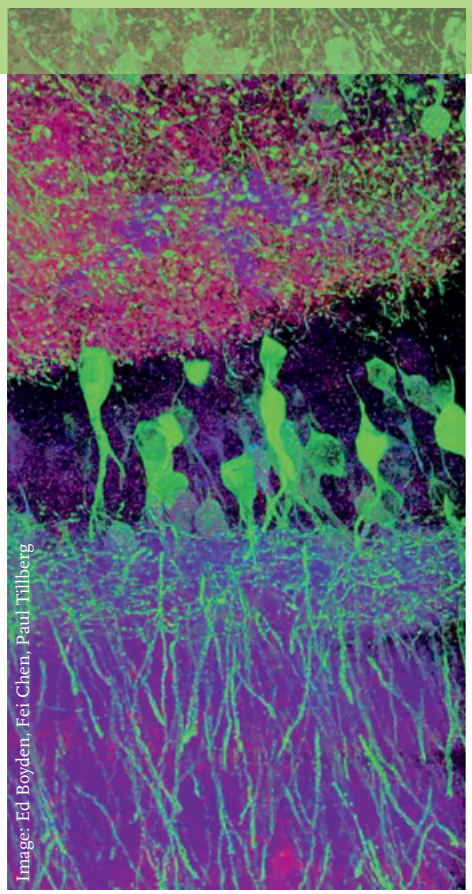


Image: Ed Boyden, Fei Chen, Paul Tillberg

Fine structures within the mouse brain are revealed by expansion microscopy, a new technique pioneered by Ed Boyden and colleagues.

But she can't. They are far too small—smaller than a wavelength of light, meaning they are beyond the limit for optical microscopy. To bring these structures into view, one of Boyden's technologies, called expansion microscopy (ExM), simply makes the specimen bigger, allowing it to be viewed on a conventional laboratory microscope.

The idea is at once obvious and fantastical. "Expansion microscopy is the kind of thing scientists daydream about," says Paul Tillberg, a graduate student in Boyden's lab. "You either shrink the scientist or expand the specimen."

Leaving Crittenden's sample in place, Tillberg adds water. Minutes later, the tissue has expanded and become transparent, a ghostly and larger version of its former self.

Crittenden takes another look through the scope. "It's like someone has loosened up all the fibers. I can see each one independently, and see them interconnecting," she says. "ExM will add a lot of power to the tools we've developed for visualizing the connections we think are degenerating."

It took Tillberg and his fellow graduate student Fei Chen several months of brainstorming to find a plausible way to make ExM a reality. They had found inspiration in the work of MIT physicist Toyoichi Tanaka, who in the 1970s had studied smart gels, polymers that rapidly expand in response to a change in environment. One familiar example is the absorbent material in baby diapers, and Boyden's team turned to this substance for the expansion technique.

The process they devised involves several steps. The tissue is first labeled using fluorescent antibodies that bind to molecules of interest, and then it is impregnated with the gel-forming material. Once the gel has set, the fluorescent markers are anchored to the gel, and the original tissue sample is digested, allowing the gel to stretch evenly in all directions.

When water is added, the gel expands and the fluorescent markers spread out like a picture on a balloon. Remarkably, the 3D shapes of even the finest structures are faithfully preserved during the expansion, making it possible to see them using a conventional microscope. By labeling molecules with different colors, the researchers can even distinguish pre-synaptic from post-synaptic structures. Boyden plans eventually to use hundreds, possibly thousands, of colors, and to increase the expansion factor to 10 times original size, equivalent to a 1000-fold increase in volume.

ExM is not the only way to see fine structures such as synapses; they can also be visualized by electron microcopy, or by recently-developed 'super-resolution' optical methods that garnered a 2014 Nobel Prize. These techniques, however, require expensive equipment, and the images are very time-consuming to produce.

"With ExM, because the sample is physically bigger, you can scan it very quickly using just a regular microscope," says Boyden.

Boyden is already talking to other leading researchers in the field, including Kwanghun Chung at MIT and

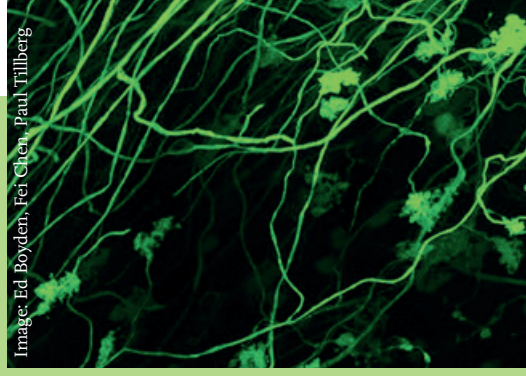


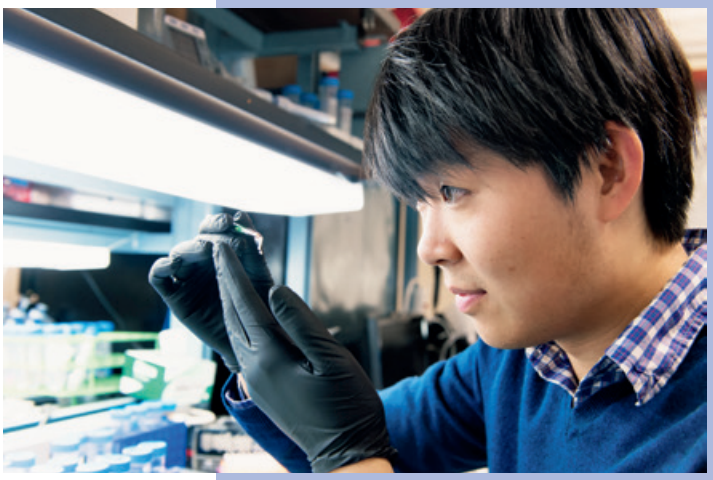
Image: Ed Boyden, Fei Chen, Paul Tillberg

Neurons in the mouse cerebellum visualized through expansion microscopy.

George Church at Harvard, about ways to further enhance the ExM method. Within the McGovern Institute, among those who expect to benefit from these advances is Guoping Feng, who is developing mouse models of autism, schizophrenia and other disorders by introducing some of the same genetic changes seen in humans with these disorders. Many of the genes associated with autism and schizophrenia play a role in the formation of synapses, but even with the mouse models at his disposal, Feng isn't sure what goes wrong with them because they are so hard to see. "If we can make parts of the brain bigger, we might be able to see how the assembly of this synaptic machinery changes in different disorders," he says.

3D Movies Without Special Glasses

Another challenge facing Feng and many other researchers is that many brain functions, and many brain diseases, are not confined to one area, but are widely distributed across the brain. Trying to understand these processes by looking through a small



Photos: Justin Knight

Left, graduate student Fei Chen examines a specimen in Ed Boyden's lab. Right, postdoc Jill Crittenden in the lab with Ann Graybiel.

microscopic window has been compared to watching a soccer game by observing just a single square foot of the playing field.

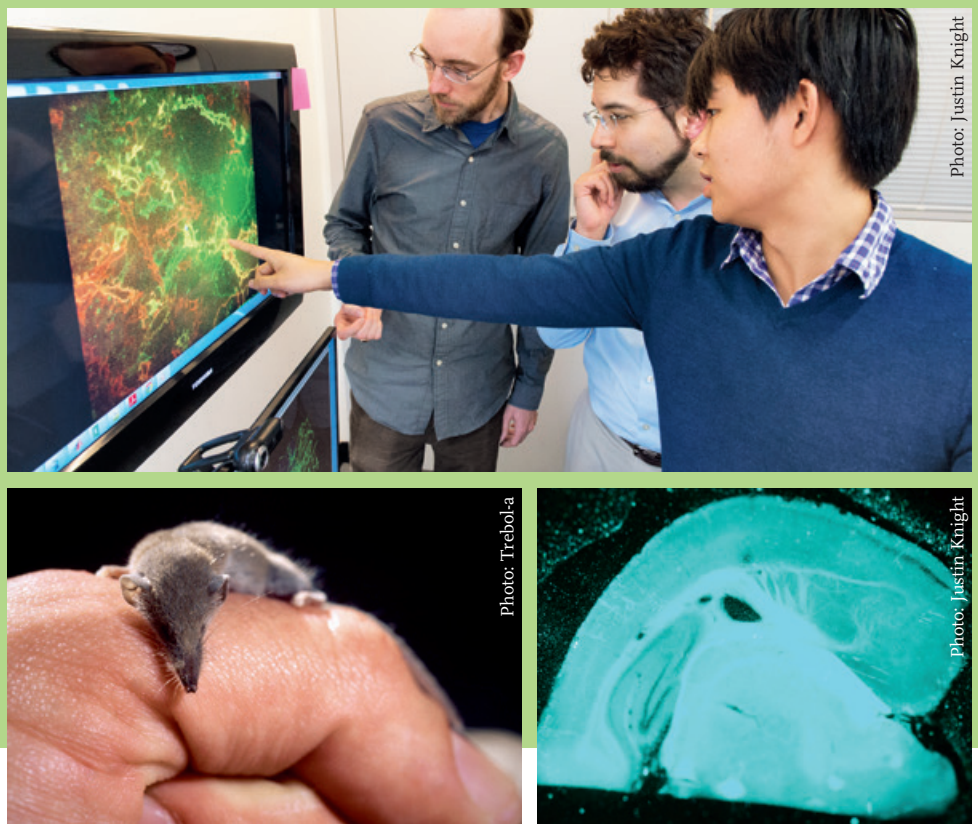
No current technology can capture millisecond-by-millisecond electrical events across the entire living brain, so Boyden and collaborators in Vienna, Austria, decided to develop one. They turned to a method called light field microscopy (LFM) as a way to capture 3D movies of an animal's thoughts as they flash through the entire nervous system.

The idea is mind-boggling to imagine, but the hardware is quite simple. The instrument records images in depth the same way humans do, using multiple 'eyes' to send slightly offset 2D images to a computer that can reconstruct a 3D image of the world. (The idea had been developed in the 1990s by Boyden's MIT colleague Ted Adelson, and a similar method was used to create Google Street View.) Boyden and his collaborators started with a microscope of standard design, attached a video camera, and inserted between them a six-by-six array of miniature lenses, designed in Austria, that projects a grid of offset images into the camera and the computer.

The rest is math. "We take the multiple, superimposed flat images projected through the lens array and combine them into a volume," says Young-Gyu Yoon, a graduate student in the Boyden lab who designed and wrote the software.

Another graduate student, Nikita Pak, used the new method to measure neural activity in *C. elegans*, a tiny worm whose entire nervous system consists of just 302 neurons. By using a worm that had been genetically engineered so that its neurons light up when they become electrically active, Pak was able to make 3D movies of the activity in the entire nervous system. "The setup is just so simple," he says. "Every time I use it, I think it's cool."

The team then tested their method on a larger brain, that of the larval zebra fish. They presented the larvae with a noxious odor, and found that it triggered activity in around 5000 neurons, over a period of about three minutes. Even with this relatively simple example, activity is distributed widely throughout the brain, and would be difficult to detect with previous techniques. Boyden is now working towards recording activity over much longer timespans, and he also envisions scaling it up to image the much more complex brains of mammals.



Top, graduate students Paul Tillberg (left) and Fei Chen (right) with Ed Boyden. Bottom left, the smallest known mammal, the Etruscan shrew, whose tiny brain has many advantages for neuroscience research. Right, expanded tissue from the mouse brain.

He hopes to start with the smallest known mammal, the Etruscan shrew. This animal resembles a mouse, but it is ten times smaller, no bigger than a thimble. Its brain is also much smaller, with only a few million neurons, compared to 100 million in a mouse.

Whole brain imaging in this tiny creature could provide an unprecedented view of mammalian brain activity, including its disruption in disease states. Feng cites sensory overload in autism as an example. "If we can see how sensory activity spreads though the brain, we can start to understand how overload starts and how it spills over to other brain areas," he says.

Visions of Convergence

While Boyden's microscopy technologies are providing his colleagues with new ways to study brain disorders, Boyden himself hopes to use them to understand the brain as a whole. He plans to use ExM to map connections and identify which molecules are where; 3D whole-brain imaging to trace brain activity as it unfolds in real time; and optogenetics techniques to stimulate the brain and directly record the resulting activity. By combining all three tools together, he hopes to pin stimuli and activity to the molecules and connections on the map and then use that to build a computational model that simulates brain activity.

The plan is grandiose, and the tools aren't all ready yet, but to make the scheme plausible in the proposed timeframe, Boyden is adhering to a few principles. His methods are fast, capturing information-dense images rapidly rather than scanning over days, and inclusive, imaging whole brains rather than chunks that need to be assembled. They are also accessible, so researchers don't need to spend large sums to acquire specialized equipment or expertise in-house.

The challenges ahead might appear insurmountable at times, but Boyden is undeterred. He moves forward, his mind open to even the most far-fetched ideas, because they just might work. ■

INSTITUTE NEWS

McGovern Institute Welcomes Mark Harnett

Our newest faculty member, Mark Harnett, joined the McGovern Institute in April of this year. Harnett, who is also an assistant professor in MIT's Department of Brain and Cognitive Sciences, studies the biophysical mechanisms that enable neurons and neural circuits to perform computations and control behavior.

His lab focuses on the role of dendrites, the elaborate tree-like structures through which neurons receive the vast majority of their synaptic inputs. The thousands of inputs a single cell receives can interact in complex ways that depend on their spatial arrangement and biophysical properties. Harnett addresses the hypothesis that the brain's computational power arises from these fundamental integrative operations within dendrites. He focuses in particular on sensory processing and spatial navigation, with the goal of understanding the mechanistic basis of these brain functions.

Harnett received his BA in Biology from Reed College and his PhD in Neuroscience from the University of Texas at Austin.


Photo: Justin Knight

Prior to joining MIT, he was a postdoctoral researcher at the Howard Hughes Medical Institute's Janelia Research Campus in Ashburn, VA, where he worked with Jeff Magee.

You can read more about Mark Harnett's research on our website. ■


Photo: Justin Knight

Charles Gilbert of The Rockefeller University delivers the 2015 Scolnick Prize Lecture.

Charles Gilbert Delivers Scolnick Lecture

The 2015 Scolnick Prize was awarded to Charles Gilbert of The Rockefeller University. In his prize lecture, Gilbert discussed his work on the function of the visual cortex, which he has studied over a period of four decades. His work has revealed the importance of lateral connections, which enable cortical cells to respond not only to stimuli in their primary receptive fields, but also to information elsewhere in the visual scene.

These connections are critical for visual perception, since they allow the brain to link different components of the image into a coherent whole. They are also important for understanding how the brain responds to injury, and how it rewires itself in response to experience.

The video of the lecture can be viewed on the McGovern website. ■

AWARDS AND HONORS

Nancy Kanwisher has been selected by the postdocs of the Brain and Cognitive Sciences Community at MIT as the winner of the 2015 Outstanding Postdoc Mentor Award. The award was established "to recognize excellence in mentoring and to raise awareness of the essential role that mentors play in the career development of postdocs."


Photo: Susana Correia

Nancy Kanwisher pictured with members of her lab.

Feng Zhang has received one of six new awards from the Paul G. Allen Family Foundation. Zhang was awarded $1 million to develop a genomic engineering system to produce and study human neural cell types relevant to neurological disorders. ■

RESEARCH NEWS


Photo: Justin Knight

McGovern Investigator Feng Zhang.

Feng Zhang and colleagues published a review discussing the potential therapeutic applications of genome-editing technology, including the challenges that must be overcome in order to develop successful treatments for human genetic diseases.

Ed Boyden, working with collaborators at Georgia Institute of Technology, described a miniature chip for patch clamping, a technique that allows neuroscientists to record electrical signals from a single neuron with great precision. The new design is expected to make patch clamping easier and more affordable, allowing the technique to be applied to many neurons simultaneously.

A collaborative study involving the labs of **Rebecca Saxe**, **John Gabrieli** and **Nancy Kanwisher** used a combination of MRI-based methods to demonstrate a surprisingly precise and fine-grained relationship between anatomical connections and functional activity in the human brain. ■

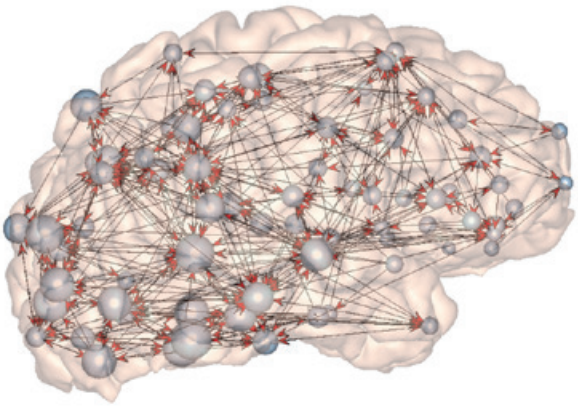


Image: David Osher and Zeynep Saygin

IN THE MEDIA

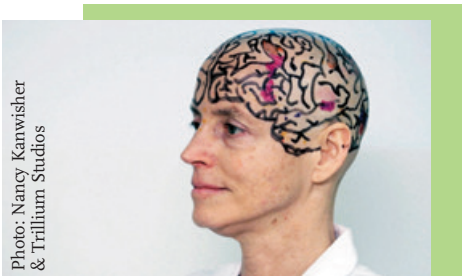


Photo: Nancy Kanwisher & Trillium Studios

Traditional methods of brain imaging help scientists understand how the brain functions. But **Nancy Kanwisher** has taken extreme measures to reveal where certain regions of the brain are located. In a 90-second video posted on her "Nancy's Brain Talks" website, Kanwisher shaves her head and directs graduate student Rosa Lafer-Sousa to draw functionally specific regions of the brain directly onto her scalp. The video was quickly picked up by various online news outlets, and has garnered more than 150,000 hits on YouTube. Kanwisher hopes the video will draw attention to her website, which contains short talks on the different scientific methods neuroscientists can use to study the human mind and brain.

In a separate study conducted with colleagues at Wellesley College, Lafer-Sousa explored the science of color perception behind the viral internet sensation known as #thedress. Her study was picked up by various news sites including the *Washington Post, New York Times,* and *Los Angeles Times.*

The Washington Post ran a story on **John Gabrieli**'s study linking brain anatomy, academic achievement and family income. "The thing that really stands out is how powerful the economic influences are on something as fundamental as brain structure," Gabrieli said. "It's just very striking." ■

Is this dress white and gold or blue and black?

EVENTS

Photos: Justin Knight

Josh Tenenbaum of MIT (right) was among the speakers at the 2015 McGovern Institute symposium.

McGovern Annual Symposium

Theories of motor control have advanced the idea that the brain uses internal models to generate reliable motor commands and predict the sensory consequences of those commands. In this year's symposium, ten speakers explored the recent advances in the study of internal models in perception, cognition and action, and discussed the extent to which they reveal the common computational principles across neural circuits and behaviors.

Selected talks from the symposium may be viewed on our website.

The McGovern Institute for Brain Research at MIT is led by a team of world-renowned neuroscientists committed to meeting two great challenges of modern science: understanding how the brain works and discovering new ways to prevent or treat brain disorders. The McGovern Institute was established in 2000 by Patrick J. McGovern and Lore Harp McGovern, with the goal of improving human welfare, communication and understanding through their support for neuroscience research. The director is Robert Desimone, who is the Doris and Don Berkey Professor of Neuroscience at MIT and former head of intramural research at the National Institute of Mental Health.

Further information is available at: http://mcgovern.mit.edu

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