McGOVERN INSTITUTE
FOR BRAIN RESEARCH AT MIT

Brain SCAN

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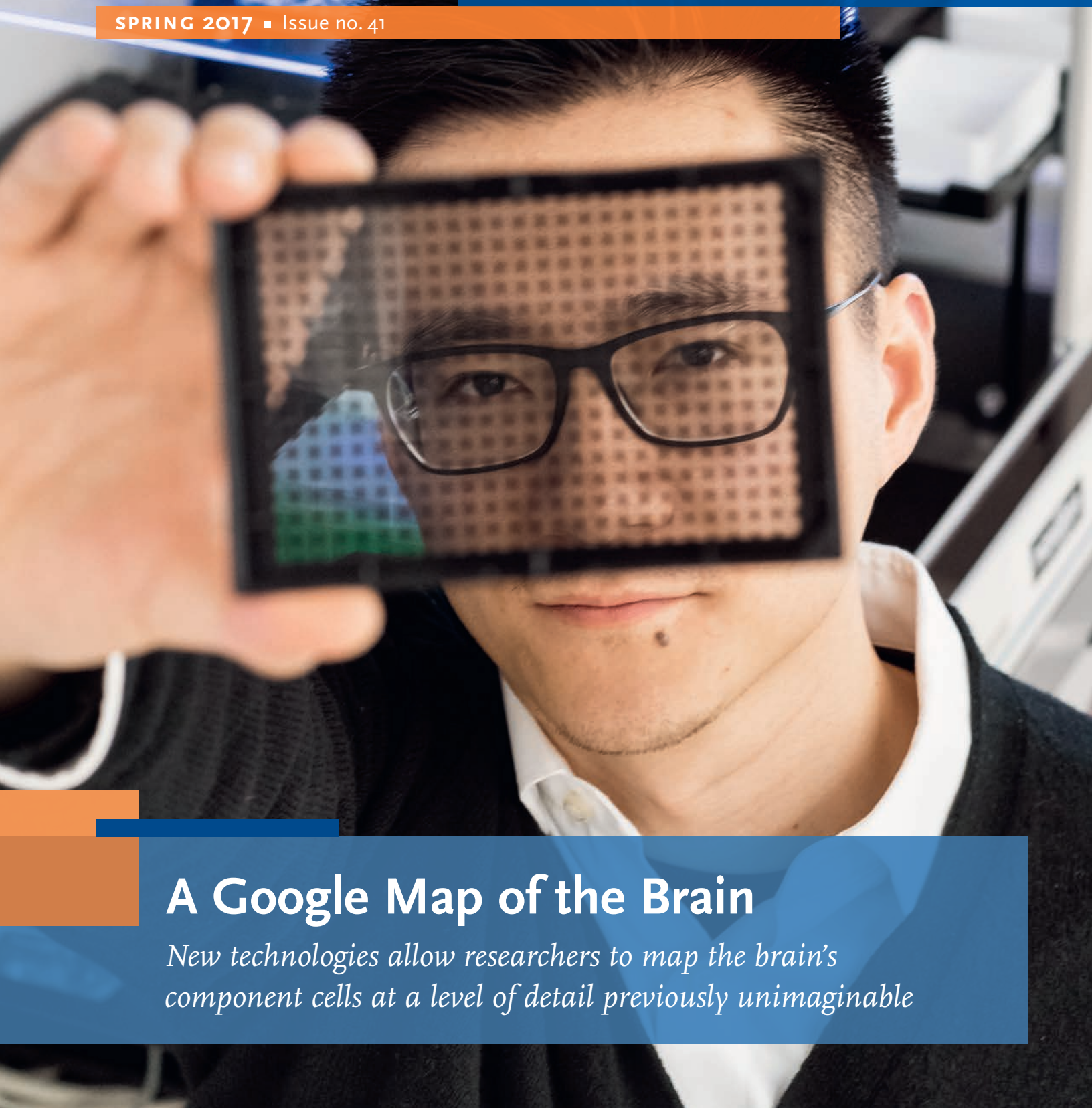

A Google Map of the Brain

New technologies allow researchers to map the brain's component cells at a level of detail previously unimaginable

FROM THE **DIRECTOR**

Our work at the McGovern Institute has benefited greatly from our ties to the Broad Institute, which lies just across the street from our own building. The Broad is a world-leading center for human genome research, with a major focus on the genetics of brain disorders such as schizophrenia, bipolar disorder and autism. It is also a hotbed of technology innovation, and as you can read in this issue, the technologies that we are jointly developing promise to change our view of the brain, allowing us to identify and map its myriad cell types on a scale far greater than previously possible.

The impact of new technologies often extends beyond the original purpose, a point that is powerfully illustrated by a recent study from Feng Zhang, whose work on CRISPR and gene editing has implications far beyond neuroscience. Now, Zhang and his colleagues at the Broad Institute have described an entirely new application of CRISPR, for the detection of rare DNA sequences from Zika virus or other pathogens in clinical samples. This method, which they call 'SHERLOCK,' holds great promise as a new diagnostic tool, with potential applications ranging from cancer detection to the monitoring of infectious disease. Because it can be delivered as a paper-based test, SHERLOCK may be especially useful in places where access to laboratory facilities is limited—an important factor for monitoring emerging diseases such as Zika virus.

Bob Desimone, Director
Doris and Don Berkey Professor
of Neuroscience

On the cover:
Yinqing Li, a postdoc in Guoping Feng's lab, studies the patterns of gene expression that define individual cell types within the brain.

Photo: Justin Knight

McGovern researchers are developing new ways to study vast numbers of individual brain cells. The results are likely to transform our view of the brain and to provide new insights into many disorders.

At the start of the twentieth century, Santiago Ramón y Cajal's drawings of brain cells under the microscope revealed a remarkable diversity of cell types within the brain. Through sketch after sketch, Cajal showed that the brain was not, as many believed, a web of self-similar material, but rather that it is composed of billions of cells of many different sizes, shapes, and interconnections.

Yet more than a hundred years later, we still do not know how many cell types make up the human brain. Despite decades of study, the challenge remains daunting,

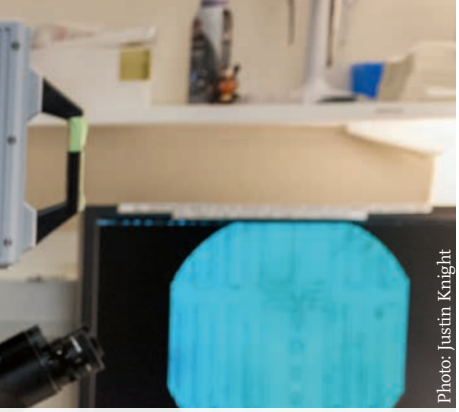

Photo: Justin Knight

A Google Map of the Brain

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as the brain's complexity has overwhelmed attempts to describe it systematically or to catalog its parts.

Now, however, this appears about to change, thanks to an explosion of new technical advances in areas ranging from DNA sequencing to microfluidics to computing and microscopy. For the first time, a parts list for the human brain appears to be within reach.

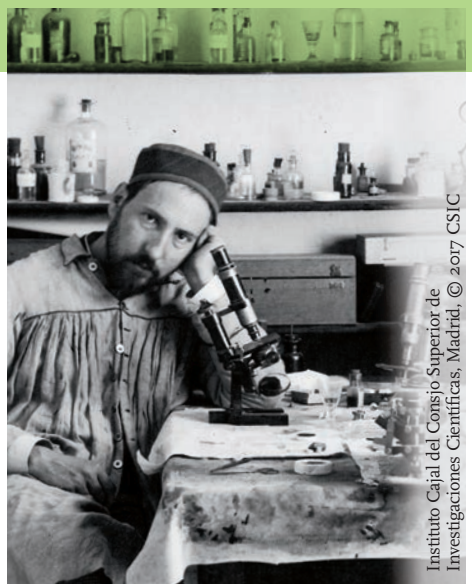
Why is this important? "Until we know all the cell types, we won't fully understand how they are connected together," explains McGovern Investigator Guoping Feng. "We know that the brain's wiring is incredibly complicated, and that the connections are key to understanding how it works, but we don't yet have the full picture. That's what we are aiming for. It's like making a Google map of the brain."

Identifying the cell types is also important for understanding disease. As genetic risk factors for different disorders are identified, researchers need to know where they act within the brain, and which cell types and connections are disrupted as a result. "Once we know that, we can start to think about new therapeutic approaches," says Feng, who is also an institute member of the Broad Institute, where he leads the neurobiology program at the Stanley Center for Psychiatric Disorders Research.

Drop by Drop

In 2012, computational biologist Naomi Habib arrived from the Hebrew University of Jerusalem to join the labs of McGovern Investigator Feng Zhang and his collaborator Aviv Regev at the Broad Institute. Habib's plan was to learn new RNA methods as they were emerging. "I wanted to use these powerful tools to understand this fascinating system that is our brain," she says.

Her rationale was simple, at least in theory. All cells of an organism carry the same DNA instructions, but the instructions are read out differently in each cell type. Stretches of DNA corresponding to individual genes are copied, sometimes thousands of times, into RNA molecules that in turn direct



Instituto Cajal del Consjo Superior de Investigaciones Científicas, Madrid, © 2017 CSIC

Above: Postdoc Naomi Habib uses genomic technologies to study thousands of brain cells in parallel.

Below: A self-portrait from around 1885 by Santiago Ramón y Cajal, whose pioneering studies revealed the diversity of cell types that comprise the brain.

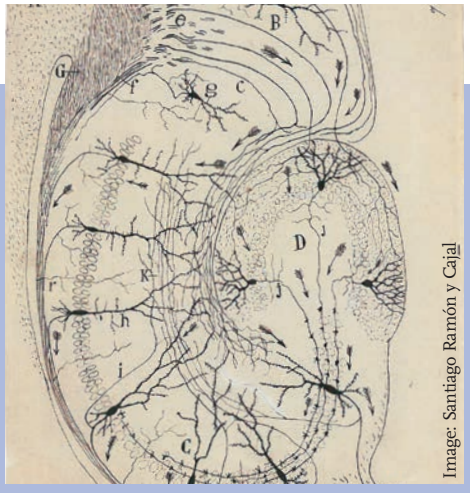


Image: Santiago Ramón y Cajal

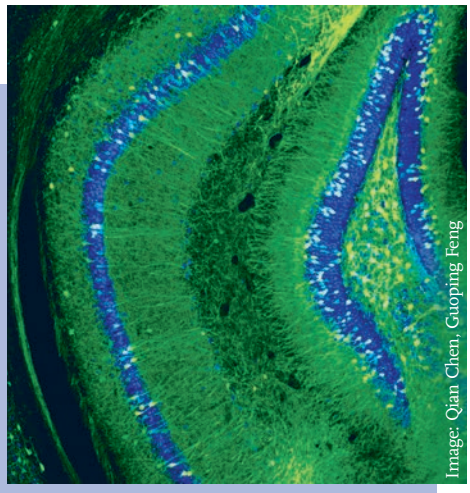


Image: Qian Chen, Guoping Feng

Many of the cell types that Cajal described in structures such as the hippocampus (left) can now be labeled using modern genetic methods (right).

the synthesis of proteins. Differences in which sequences get copied are what give cells their identities: brain cells express RNAs that encode brain proteins, while blood cells express different RNAs, and so on. A given cell can express thousands of genes, providing a molecular "fingerprint" for each cell type.

Analyzing these RNAs can provide a great deal of information about the brain, including potentially the identities of its constituent cell types. But doing this is not easy, because the different cell types are mixed together like salt and pepper within the brain. For many years, studying brain RNA meant grinding up the tissue—an approach that has been compared to studying smoothies to learn about fruit salad.

As methods improved, it became possible to study the tiny quantities of RNA contained within single cells. This opened the door to studying the difference between individual cells, but this required painstaking manipulation of many samples, a slow and laborious process.

A breakthrough came in 2015, with the development of automated methods based on microfluidics. One of these, known as dropseq (droplet-based sequencing), was pioneered by Steve McCarroll at Harvard, in collaboration with Regev's lab at Broad. In this method, individual cells are captured in tiny water droplets suspended in oil. Vast numbers of droplets are automatically pumped through tiny channels, where each undergoes its own separate sequencing reactions. By running multiple samples in parallel, the machines can process tens of thousands of cells and billions of sequences, within hours rather than weeks or months. The power of the method became clear when in an experiment on mouse retina, the researchers were able to identify almost every cell type that had ever been described in the retina, effectively recapitulating decades of work in a single experiment.

Dropseq works well for many tissues, but Habib wanted to apply it to the adult brain, which posed a unique challenge. Mature neurons often bear elaborate branches that become intertwined like tree roots in a forest, making it impossible to separate individual cells without damage.

Nuclear Option

So Habib turned to another idea. RNA is made in the nucleus before moving to the cytoplasm, and because nuclei are compact and robust it is easy to recover them intact in large numbers, even from difficult tissues such as brain. The amount of RNA contained in a single nucleus is tiny, and Habib didn't know if it would be enough to be informative, but Zhang and Regev encouraged her to keep going. "You have to be optimistic," she says. "You have to try."

Fortunately, the experiment worked. In a paper with Zhang and Regev, she was able to isolate nuclei from newly formed neurons in the adult mouse hippocampus (a brain structure involved in memory), and by analyzing their RNA profiles individually she could order them in a series according to their age, revealing their developmental history from birth to maturity.

Now, after much further experimentation, Habib and her colleagues have managed to apply the droplet method to nuclei, making it possible for the first time to analyze huge numbers of cells from adult brain—at least ten times more than with previous methods.

This opens up many new avenues, including the study of human postmortem tissue, given that RNA in nuclei can survive for years in frozen samples. Habib is already starting to examine tissue taken at autopsy from patients with Alzheimer's and other neurodegenerative diseases. "The neurons are degenerating, but the other cells around them could also be contributing to the degenerative process," she says. "Now we have these tools, we can look at what happens during the progression of the disease."

Computing Cells

Once the sequencing is completed, the results are analyzed using sophisticated computational methods. When the results emerge, data from individual cells are visualized as colored dots, clustered on a graph according to their statistical similarities. But because the cells were dissociated at the start of the experiment, information about their appearance and origin within the brain is lost.

To find out how these abstract displays correspond to the visible cells of the brain,

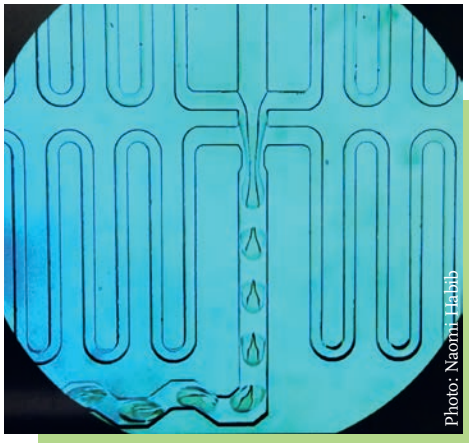


Photo: Naomi Habib

In the droplet sequencing method, a microfluidic device captures individual cells within tiny droplets, allowing thousands of cells to be analyzed in parallel.

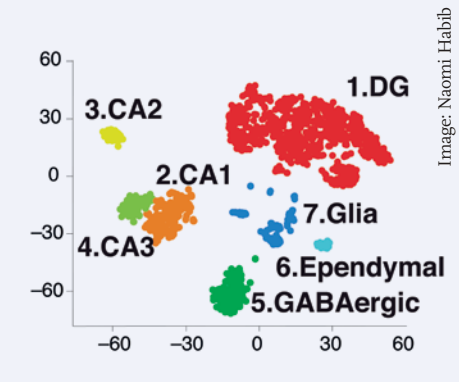


Image: Naomi Habib

Nuclei from mouse brain cells, clustered based on their patterns of gene expression.

Habib teamed up with Yinqing Li, a former graduate student with Zhang who is now a postdoc in the lab of Guoping Feng. Li began with existing maps from the Allen Institute, a public repository with thousands of images showing expression patterns for individual genes within mouse brain. By comparing these maps with the molecular fingerprints from Habib's nuclear RNA sequencing experiments, Li was able to make a map of where in the brain each cell was likely to have come from.

It was a good first step, but still not perfect. "What we really need," he says, "is a method that allows us to see every RNA in individual cells. If we are studying a brain disease, we want to know which neurons are involved in the disease process, where they are, what they are connected to, and which special genes might be involved so that we can start thinking about how to design a drug that could alter the disease."

Expanding Horizons

So Li partnered with Asmamaw (Oz) Wassie, a graduate student in the lab of McGovern Investigator Ed Boyden, to tackle the problem. Wassie had previously studied bioengineering as an MIT undergraduate, where he had helped build an electronic "artificial nose" for detecting trace chemicals in air. With support from a prestigious Hertz Fellowship, he joined Boyden's lab, where he is now working on the development of a method known as expansion microscopy.

In this method, a sample of tissue is embedded with a polymer that swells when water is added. The entire sample expands in all directions, allowing scientists to see fine details such as connections between neurons, using an ordinary microscope.

Wassie recently helped develop a way to anchor RNA molecules to the polymer matrix, allowing them to be physically secured during the expansion process. Now, within the expanded samples he can see the individual molecules using a method called fluorescent in situ hybridization (FISH), in which each RNA appears as a glowing dot under the microscope. Currently, he can label only a handful of RNA types at once, but by using special sets of probes, applied sequentially, he thinks it will soon be possible to distinguish thousands of different RNA sequences.

"That will help us to see what each cell looks like, how they are connected to each other, and what RNAs they contain," says Wassie. By combining this information with the RNA expression data generated by Li and Habib, it will be possible to reveal the organization and fine structure of complex brain areas and perhaps to identify new cell types that have not yet been recognized.

Looking Ahead

Li plans to apply these methods to a brain structure known as the thalamic reticular nucleus (TRN) – a sheet of tissue, about ten neurons thick in mice, that sits on top of the thalamus and close to the cortex. The TRN is not well understood, but it is important for controlling sleep, attention and sensory processing, and it has caught the interest of Feng and other neuroscientists because it expresses a disproportionate number of genes implicated in disorders such as autism, attention deficit hyperactivity disorder, and intelligence deficits. Together with Joshua Levin's group at Broad, Li has already used nuclear RNA sequencing to identify the cell types in the TRN, and he has begun to examine them within intact brain using the expansion techniques. "When you map these precise cell types back to the tissue, you can integrate the gene expression information with everything else, like electrophysiology, connectivity, morphology," says Li. "Then we can start to ask what's going wrong in disease."

Meanwhile, Feng is already looking beyond the TRN, and planning how to scale the approach to other structures and eventually to the entire brain. He returns to the metaphor of a Google map. "Microscopic images are like satellite photos," he says. "Now with expansion microscopy we can add another layer of information, like property boundaries and individual buildings. And knowing which RNAs are in each cell will be like seeing who lives in those buildings. I think this will completely change how we view the brain." ■

Photo: Justin Knight

Graduate student Oz Wassie (left) and postdoc Yinqing Li are developing new ways to visualize RNA within individual brain cells.

Photo: McGovern Institute

Graduate Student Runs Boston Marathon in Memory of Bombing Victim

Rachel Romeo is hard to miss. A graduate student in John Gabrieli's lab studying language development in children, Romeo spends most of her time in the Martinos Imaging Center scanning the brains of young kids. These days, however, she can be found hobbling around the McGovern Institute on crutches and sporting a rainbow-colored hairdo—relics of her experience running the 2017 Boston Marathon in memory of a young boy named Martin Richard.

In 2012, Martin and his classmates participated in one of Romeo's studies on reading and language development. One year later, the 8-year-old boy was among three people killed when two bombs exploded near the finish line of the 2013 Boston Marathon.

Photo: Martin Richard Foundation

Romeo ran the 2017 Boston Marathon in memory of Martin Richard, an 8-year-old boy who died during the 2013 marathon bombing, and who had been a research participant in one of Romeo's studies.

"I immediately felt an immense connection to him," Romeo says. "I have kept a piece of paper with his handwritten name on the bulletin board above my desk. It reminds me that we never know when life will end, so we had better be proud of what we've done, said and accomplished every single day."

The boy's family later established the Martin W. Richard Charitable Foundation Inc., a charity that invests in education, athletics and community-building. Romeo decided to raise money for the foundation by running the 2017 Boston Marathon with Team MR8, the official running team of the Martin Richard Foundation. Running a marathon is difficult even for elite runners, but for Romeo, a self-described "non-athlete," it would be the challenge of a lifetime.

Staying the Course

In December 2016, Romeo bought a new pair of running shoes and began training for the marathon. While working full-time on her PhD thesis in the Gabrieli lab, she gradually increased her mileage by running long stretches of the marathon route on the weekends. In a fundraising twist, she dyed her hair a different color every month—electric blue, hot pink, emerald green—based on requests from her supporters. Despite injuries, snowstorms and other setbacks, Romeo reached her training goals and, by April, when she lined up for the Boston Marathon with bright rainbow-colored hair, she had only one goal left: to finish the race.

Six miles into the historic 26.2-mile race, however, Romeo slipped on a water bottle and fractured her foot. She had a choice: continue or seek medical assistance. Despite the pain, she kept running.

"Every step hurt," Romeo says, "but I just looked at the picture of Martin I was carrying in my hand and I knew that I couldn't give up."

Romeo finished the race, raising more than $11,000 for the Martin Richard Foundation in the process. Although she will be on crutches for several weeks while her foot recovers, Romeo continues to pursue her research with the same tenacity that she showed on the marathon course, dividing her time between her PhD dissertation and studying for her license in speech language pathology. She also plans to return to running as soon as possible. "I do think I've caught the marathon bug," she says. "I hope to run another in the not too distant future." ■

Article and photos printed with permission from the Richard family.

Photo: Rachel Romeo

Romeo with the Richard family at the finish line of the 2017 Boston Marathon.

RESEARCH NEWS

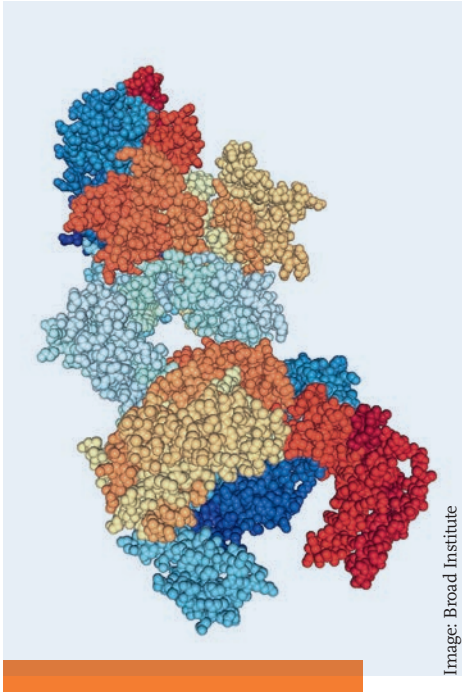


Image: Broad Institute

A CRISPR-associated enzyme called Cas13a is at the heart of a new diagnostic method called SHERLOCK.

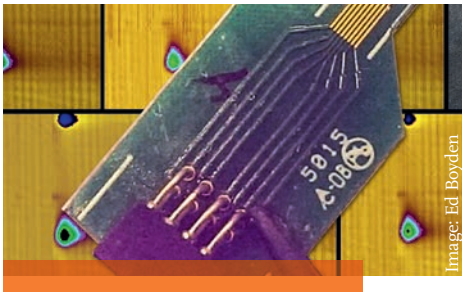


Image: Ed Boyden

A new device for measuring the neurotransmitter dopamine within the living brain.

Feng Zhang, along with collaborators at the Broad Institute, the McGovern Institute, the Institute for Medical Engineering and Science at MIT, and the Wyss Institute for Biologically Inspired Engineering at Harvard, has adapted a CRISPR protein that targets RNA (rather than DNA), for use as a rapid, inexpensive and sensitive diagnostic tool for the detection of Zika virus and other pathogens. The new method, called SHERLOCK, can be delivered as a paper-based test and could have wide-ranging implications for public health, including the management of disease outbreaks in rural settings that lack infrastructure for laboratory-based testing.

Ann Graybiel has collaborated with MIT bioengineers Michael Cima and Robert Langer to develop a new device for measuring the neurotransmitter dopamine, which is involved in brain responses to reward and which is disrupted in Parkinson's disease and many other disorders. The method involves an array of tiny carbon electrodes, which can measure the distribution of dopamine with great precision. The researchers have used the device to track dopamine levels in the rat striatum, a structure that in humans is an important target for Parkinson's therapy.

Another approach to measuring dopamine has been developed by **Ed Boyden** in collaboration with MIT chemist Michael Strano. Strano previously showed that single-walled carbon nanotubes (SWCNTs) can be converted into fluorescent sensors by wrapping them in DNA or other polymers. Working with Boyden, Strano has now developed a SWCNT-based sensor that serves as a sensitive indicator of dopamine. Although not yet tested in intact brain, the new sensor makes it possible to image dopamine release from cultured neurons with very high spatial and temporal resolution.

Bob Horvitz, along with postdoc Shuo Luo, examined the genetic mechanisms by which nerve cells can arise from muscle. Most neurons are formed during development from the ectoderm, the outer layer of the embryo, but in some species, including nematode worms, neurons can also arise from muscle. Understanding how this happens will allow researchers to ask whether similar mechanisms exist in mammals, and may also enable new ways to generate neural stem cells artificially for regenerative medicine.

Glorial Choi collaborated with Polina Anikeeva, a materials scientist at MIT, to test a new design of flexible polymer probes for recording and manipulating brain activity. The multifunctional probes, developed in Anikeeva's lab, include channels for delivery of viral vectors or drugs; optical waveguides for optogenetic stimulation; and electrode channels for recording electrical activity. ■

AWARDS & HONORS

Mehrdad Jazayeri has received a 2017 McKnight Scholar Award, described by the chair of the selection committee as "one of the most prestigious early-career honors that a young neuroscientist can receive."

Ed Boyden is among eleven MIT faculty members elected to the American Academy of Arts and Sciences this year. Earl Miller of the Picower Institute for Learning and Memory and Mary Potter, professor emerita of psychology in the Department of Brain and Cognitive Sciences, were also elected. Boyden is the seventh McGovern Investigator to be elected to the Academy. ■

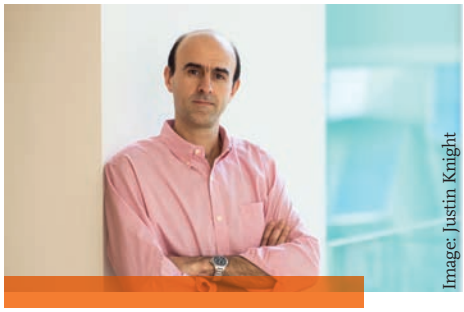


Image: Justin Knight

Merhdad Jazayeri was named a 2017 McKnight Scholar.

EVENTS

Photo: Justin Knight

2017 Scolnick Prize winner Catherine Dulac with Ed Scolnick (left) and Bob Desimone.

Catherine Dulac Delivers Scolnick Lecture

Catherine Dulac of Harvard University received the 2017 Scolnick Prize at a ceremony on March 13. In her prize lecture she described the role of mammalian pheromone receptors and the pathways through which they control brain activity and behavior.

Photo: Justin Knight

Symposium organizer Gloria Choi (center) with Lore McGovern, Bob Desimone and speakers from the 2017 McGovern Symposium.

McGovern Institute Annual Symposium

The topic of this year's annual McGovern symposium was neuromodulators, the ever-expanding list of signaling molecules that shape how neurons respond to neurotransmitters. The eight speakers, who were from the US, Israel and China, discussed how neuromodulators control almost every aspect of behavior, including breathing, feeding, sleep, social and reproductive behavior.

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 Lore Harp McGovern and the late Patrick J. 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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Editors: Charles Jennings, Julie Pryor
Writer: Elizabeth Dougherty
Director of Development: Kara Flyg
Design: Sametz Blackstone Associates, Boston

MCGOVERN INSTITUTE
FOR BRAIN RESEARCH AT MIT

Massachusetts Institute of Technology
77 Massachusetts Avenue 46-3160
Cambridge, MA 02139