Livestreaming the Brain

To learn how the brain works, Picower Institute labs are advancing technologies and methods to watch it live as it happens.

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Neuroscience News

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THE PICOWER INSTITUTE
FOR LEARNING AND MEMORY

DIRECTOR'S **MESSAGE**

Dear Friends,

Neuroscientists frequently use the metaphor of "mechanisms." We're trying to figure out how the brain works and, in many cases, how we could then make repairs when things break down during disease. Much like mechanics, we examine how parts fit together and how they interact to produce functions. In our case, the parts are molecules and cells, and the functions are learning and memory, consciousness, intelligence, perception, behavior and more.

When your car is in the shop, maybe for engine trouble, sometimes your mechanic will want to take things apart and look at each belt, gear and cylinder. Sometimes the mechanic will want to watch the engine running intact. That's true for neuroscientists, too, but that latter approach–watching how the many molecules and cells of the brain operate together during live behavior–is technically difficult. In this edition, we feature the many technological and methodological innovations Picower scientists have developed to watch the brain work, live as it happens (see p. 9).

We also share several other stories that exemplify the theme of mechanisms in the brain. On the facing page, read about how Earl Miller and colleagues discovered that a critical information processing mechanism–brain waves–follow a pattern that is ubiquitous across the brain's cortex. The study has important implications for an array of disorders. There is also news of my lab's investigation of mechanisms of Alzheimer's disease. One story (p.4) describes a mechanism of disease progression within the brain's microglia immune cells, while another (this page), describes a potential way to treat the cells. On page 5, we discuss how we're continuing to investigate the mechanisms underlying our non-invasive stimulation of brain rhythms to treat Alzheimer's.

When we understand fundamental mechanisms of how the brain works, can design treatments to help it work better.

LI-HUEI TSAI, DIRECTOR

The Picower Institute for Learning and Memory

Nanoparticle-delivered RNA reduces neuroinflammation

A new MIT study shows that a lipid nanoparticle (LNP) could be used for a potential Alzheimer's disease (AD) therapy. In tests in multiple mouse models and with cultured human cells, a newly tailored LNP formulation effectively delivered small interfering RNA (siRNA) to the brain's microglia immune cells to reduce excessive inflammation.

The new results, reported in the journal *Advanced Materials* achieves the reduction in inflammation by directly tamping down expression of the Spi1 gene that encodes a protein called PU.1. More generally, the new study also demonstrates a new way to deliver RNA to microglia, which have been difficult to target so far.

Study co-senior author Li-Huei Tsai, Picower Professor and Director of The Picower Institute and the Aging Brain Initiative, said she hypothesized that LNPs might work as a way to bring siRNA into microglia because the cells, which clear waste in the brain, have a strong proclivity to uptake lipid molecules. She discussed this with Robert Langer, the David Koch Institute Professor widely known for seminal work on nanoparticle drug delivery, and they decided to test reducing PU.1 expression with an LNP-delivered siRNA.

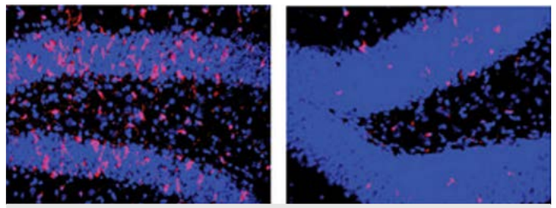
The team optimized an LNP to access microglia. LNPs have four main components and by changing the structures of two of them, and by varying the ratio of lipids to RNA, the researchers were able to come up with seven new formulations to try. Importantly, their testing included trying their formulations on cultured human microglia that they had induced into an inflammatory state. That state, after all, is the one in which the proposed treatment is needed. Among the seven candidates, one the team named "MG-LNP" stood out for its safety and especially high delivery efficiency of a test RNA cargo.

The team next tested their LNP formulations' effectiveness and safety in mice. Among the seven formulations, MG-LNP again proved the most effective at transfecting microglia. Langer says he believes this could potentially someday open new ways of treating certain brain diseases with nanoparticles.

Once they knew MG-LNP could deliver a test cargo to microglia both in human cell cultures and mice, the scientists then tested whether using it to deliver a PU.1-suppressing siRNA could reduce inflammation in microglia. In the cell cultures, a relatively low dose achieved a 42 percent reduction of PU.1 (which is good because microglia need at least some PU.1 to live). Indeed MG-LNP transfection did not cause the cells any harm. It also significantly reduced the transcription of the genes that PU.1 expression increases in microglia, indicating that it can reduce multiple inflammatory markers.

"These findings support the use of MG-LNP-mediated anti-PU.1 siRNA delivery as a potential therapy for neuroinflammatory diseases," the researchers wrote.

A final set of tests evaluated MG-LNP's performance delivering the siRNA in two mouse models of inflammation in the brain. In one, mice were exposed to LPS, a molecule that simulates infection and stimulates a systemic inflammation response. In the other model, mice exhibit severe neurodegeneration and inflammation when an enzyme called CDK5 becomes hyperactivated by a protein called p25. In both models, injection of MG-LNPs carrying the anti-PU.1 siRNA reduced expression of PU.1 and inflammatory markers, much like in the cultured human cells.



Treatment with an siRNA delivered by a lipid nanoparticle (right) reduced expression of the protein PU.1 (red specks) vs. in an untreated control (left).

A universal pattern of **brain wave** frequencies

Throughout the brain's cortex, neurons are arranged in six distinctive layers. A team of MIT neuroscientists has now found that these layers also show distinct patterns of electrical activity consistently over many brain regions and across several animal species, including humans.

In the topmost layers, neuron activity is dominated by rapid oscillations known as gamma waves. In the deeper layers, slower oscillations called alpha and beta waves predominate. The universality of these patterns suggests that these oscillations are likely playing an important role across the brain, the researchers say.

"When you see something that consistent and ubiquitous across cortex, it's playing a very fundamental role in what the cortex does," says Earl Miller, Picower Professor of Neuroscience in The Picower Institute, and one of the senior authors of the new study.

Imbalances in how these oscillations interact with each other may be involved in brain disorders such as attention deficit hyperactivity disorder.

"Overly synchronous neural activity is known to play a role in epilepsy, and now we suspect that different pathologies of synchrony may contribute to many brain disorders, including disorders of perception, attention, memory, and motor control. In an orchestra, one instrument played out of synchrony with the rest can disrupt the coherence of the entire piece of music," says Robert Desimone, director of MIT's McGovern Institute for Brain Research and a co-senior author.

André Bastos, an assistant professor of psychology at Vanderbilt University, is also a senior author of the paper in Nature Neuroscience. The lead authors are MIT research scientist Diego Mendoza-Halliday and MIT postdoc Alex Major.

In the brain, neurons become synchronized into similar electrical firing patterns, which generate oscillations of electrical activity, or brain waves, of different frequencies. Miller's lab has previously shown that high-frequency gamma rhythms are associated with encoding and retrieving sensory information, while low-frequency beta rhythms act as a control mechanism that determines which information is read out from working memory.

His lab has also found that in certain parts of the prefrontal cortex, different brain layers show distinctive patterns of oscillation: faster oscillation at the surface and slower oscillation in the deep layers. One study, led by Bastos when he was a postdoc in Miller's lab, showed that as animals performed working memory tasks, lower-frequency rhythms generated in deeper layers regulated the higher-frequency gamma rhythms generated in the superficial layers.

In addition to working memory, the brain's cortex also is the seat of thought, planning, and high-level processing of emotion and sensory information. Throughout the regions involved in these functions, neurons are arranged in six layers, and each layer has its own distinctive combination of cell types and connections with other brain areas.

In the new paper, the researchers wanted to explore whether the layered oscillation pattern they had seen in the prefrontal cortex is more widespread, occurring across different parts of the cortex and across species.

Using a combination of data acquired across several participating labs, the researchers were able to analyze 14 different areas of the cortex from four mammalian species including human volunteers.

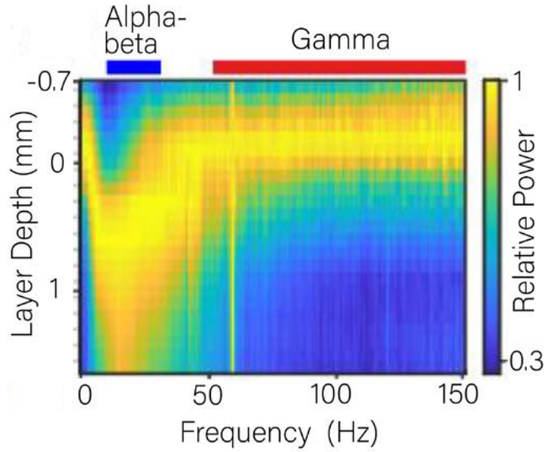
Recording from individual cortical layers has been difficult because each layer is less than a millimeter thick, so it's hard to know which layer an electrode is recording from. For this study, electrical activity was recorded using special electrodes that record from all of the layers at once. That data was then fed into a new computational algorithm the authors designed. This algorithm can determine which layer each signal came from.

Across all species, in each region studied, the researchers found the same layered activity pattern.

The findings support a model that Miller's lab has proposed: that the brain's spatial organization helps it to incorporate new information, carried by high-frequency oscillations, into existing memories and brain processes, which are maintained by low-frequency oscillations. As information passes from layer to layer, input can be incorporated as needed to help the brain perform particular tasks such as baking a new cookie recipe or remembering a phone number.

"The consequence of a laminar separation of these frequencies may be to allow superficial layers to represent external sensory information with faster frequencies, and for deep layers to represent internal cognitive states with slower frequencies," Bastos says.

Under this theory, imbalances between high- and low-frequency oscillations can lead to either attention deficits such as ADHD, when the higher frequencies dominate and too much sensory information gets in, or delusional disorders such as schizophrenia, when the low frequency oscillations are too strong and not enough sensory information gets in.



Across the cortex researchers found the same pattern: Lower frequency alpha/beta rhythms predominated (warmer colors) in deep layers, while higher frequency gamma rhythms ruled in shallow layers.

How a mutation in microglia elevates **Alzheimer's** risk

A rare but potent genetic mutation that alters a protein in the brain's immune cells, known as microglia, can give people as much as a three-fold greater risk of developing Alzheimer's disease. A new study by researchers in The Picower Institute details how the mutation undermines microglia function, explaining how it seems to generate that higher risk.

"This TREM2 R47H/+ mutation is a pretty important risk factor for Alzheimer's disease," said study lead author Jay Penney, an assistant professor at the University of Prince Edward Island who performed the research as a postdoc in the MIT lab of Picower Professor Li-Huei Tsai. "This study adds clear evidence that microglia dysfunction contributes to Alzheimer's disease risk."

The study in the journal *GLIA* shows that human microglia with the R47H/+ mutation in the TREM2 protein exhibit several deficits related to Alzheimer's pathology. Mutant microglia are prone to inflammation yet are worse at responding to neuron injury and less able to clear harmful debris including the Alzheimer's hallmark protein amyloid beta. And when the scientists transferred TREM2 mutant human microglia into the brains of mice, the mice suffered a significant decline in the number of synapses, or connections between their neurons, which can impair the circuits that enable brain functions such as memory.

Early studies of how the TREM2 R47H/+ mutation contributes to Alzheimer's suggested that the mutation simply robbed the protein of its function, but the new evidence paints a deeper and more nuanced picture. While the microglia do exhibit reduced debris clearance and injury response, they become overactive in other ways, such as their overzealous inflammation and synapse pruning.

Rather than rely on mouse models of TREM2 R47H/+ mutation, the researchers focused their work on human microglia cell cultures. To do this they used a stem cell line derived from skin cells donated by a healthy 75-year-old woman. In some of the stem cells they used CRISPR gene editing to insert the R47H/+ mutation and then cultured both edited and unedited stem cells to become microglia. This strategy gave them a supply of mutated microglia and healthy microglia, to act as experimental controls, that were otherwise genetically identical.

The team then looked to see how harboring the mutation affected each cell line's expression of its genes. They measured more than 1,000 differences but an especially noticeable finding was that microglia with the mutation increased their expression of genes associated with inflammation and immune responses. Then, when they exposed microglia to chemicals that simulate infection, the mutant microglia demonstrated a significantly more pronounced response than normal microglia, suggesting that the mutation makes microglia much more inflammation-prone.

The team exposed the cells to three kinds of the debris microglia typically clear away in the brain: myelin, synaptic proteins and amyloid beta. The mutant microglia cleared less than the healthy ones.

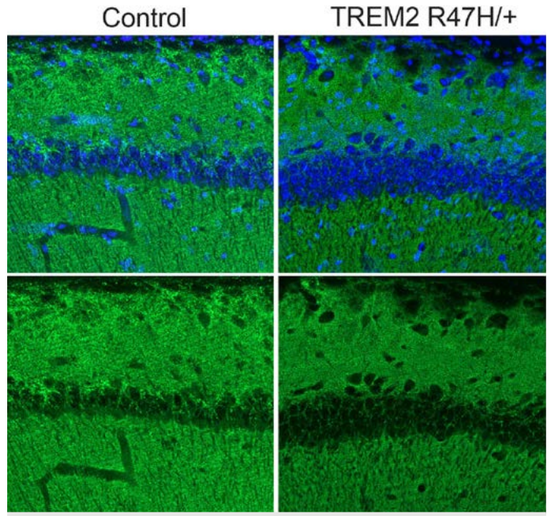
Another job of microglia is to respond when cells, such as neurons, are injured. The team co-cultured microglia and neurons and then zapped the neurons with a laser. For the next 90 minutes after the injury, the team tracked the movement of surrounding microglia. Compared to normal microglia, those with the mutation proved less likely to head toward the injured cell.

Finally, to test how the mutant microglia act in a living brain, the scientists transplanted mutant or healthy control microglia into mice in a memory-focused region of the brain called the hippocampus. The scientists then stained that region to highlight various proteins of interest. Proteins associated with synapses were greatly reduced in mice where the mutated microglia were implanted.

The researchers were able to formulate new ideas about what drives at least some of the mutant microglial misbehavior. They noticed a decline in the expression of a "purinergic" receptor protein involving sensing neuronal injury, perhaps explaining why mutant microglia struggled with that task. They also noted that mice with the mutation overexpressed "complement" proteins used to tag synapses for removal. That might explain why mutant microglia were overzealous about clearing away synapses in the mice, Penney said, though increased inflammation might also cause that by harming neurons overall.

As the molecular mechanisms underlying microglial dysfunction become clearer, Penney said, drug developers will gain critical insights into ways to target the higher disease risk associated with the TREM2 R47H/+ mutation.

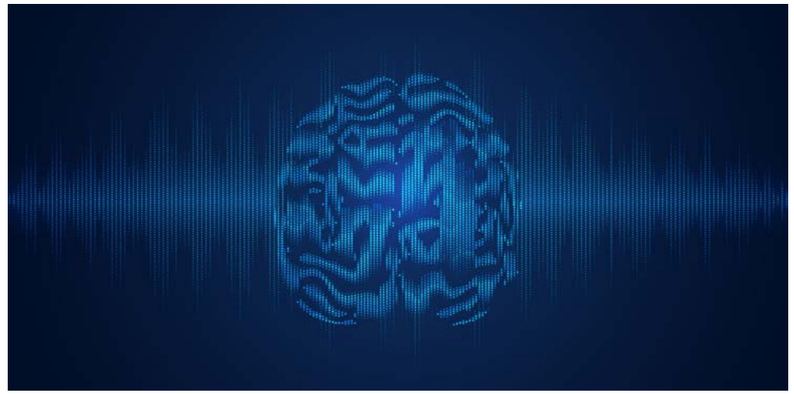
"Our findings highlight multiple effects of the TREM2 R47H/+ mutation likely to underlie its association with Alzheimer's disease risk and suggest new nodes that could be exploited for therapeutic intervention," the authors conclude.



Green staining in hippocampus tissue indicates levels of a protein associated with synapses. The staining is brighter in a mouse that received healthy human microglia (control) compared to in a mouse that received mutant microglia.

Early evidence: **gamma rhythm** stimulation can treat disorders

A surprising MIT study published in *Nature* at the end of 2016 helped to spur interest in the possibility that light flickering at the frequency of a particular gamma-band brain rhythm could produce meaningful therapeutic effects for people with Alzheimer's disease. In a new review paper in the *Journal of Internal Medicine*, the lab that led those studies takes stock of what a growing number of scientists worldwide have been finding out since then in dozens of clinical and lab benchtop studies.



Brain rhythms (also called brain "waves" or "oscillations") arise from the synchronized, network activity of brain cells and circuits as they coordinate to enable brain functions such as perception or cognition. Lower-range gamma frequency rhythms, those around 40 cycles a second, or Hz, are particularly important for memory processes, and MIT's research has shown that they are also associated with specific changes at the cellular and molecular level. The 2016 study and many others since then have produced evidence initially in animals and more recently in humans that various non-invasive means of enhancing the power and synchrony of 40Hz gamma rhythms helps to reduce Alzheimer's pathology and its consequences.

"What started in 2016 with optogenetic and visual stimulation in mice has expanded to a multitude of stimulation paradigms, a wide range of human clinical studies with promising results, and is narrowing in on the mechanisms underlying this phenomenon," wrote the authors including Li-Huei Tsai, Picower Professor in The Picower Institute.

Though the number of studies and methods has increased and the data has typically suggested beneficial clinical effects, the article's authors led by former postdoc Cristina Blanco-Duque also caution that the clinical evidence remains preliminary and that animal studies intended to discern how the approach works have been instructive but not definitive.

The authors list and summarize results from 16 published clinical studies. These employ gamma frequency sensory stimulation (e.g. exposure to light, sound, tactile vibration, or a combination), trans cranial alternating current stimulation (tACS), in which a brain region is stimulated via scalp electrodes, or transcranial magnetic stimulation (TMS), in which electric currents are induced in a brain region using magnetic fields.

With many variances among them, the clinical studies taken together offer a blend of uneven but encouraging evidence, the authors write. Across clinical studies involving patients with Alzheimer's disease, sensory stimulation has proven safe and well tolerated. Multiple sensory stimulation studies have measured increases in gamma power and brain network connectivity. They have also reported improvements in memory and/or cognition as well as sleep. Some have yielded apparent physiological benefits such as reduction of brain atrophy, in one case, and changes in immune system activity in another. So far, sensory studies have not shown reductions in Alzheimer's hallmark proteins, amyloid or tau.

Clinical studies stimulating 40Hz rhythms using tACS, ranging in sample size from only one to as many as 60, are the most numerous so far and many have shown similar benefits. Most report benefits to cognition, executive function and/or memory (depending sometimes on the brain region stimulated) and some have assessed that benefits endure even after treatment concludes. Some have shown effects on measures of tau and amyloid, blood flow, neuromodulatory chemical activity, or immune activity. Finally a 40Hz stimulation clinical study using TMS in 37 patients found improvements in cognition, prevention of brain atrophy and increased brain connectivity.

In parallel, dozens more studies have shown significant benefits in mice including reductions in amyloid and tau, preservation of brain tissue and improvements in memory.

Animal studies also have offered researchers a window into the cellular and molecular mechanisms by which gamma stimulation might have these effects. Before MIT's original studies in 2016 and 2019, researchers had not attributed molecular changes in brain cells to changes in brain rhythms, but those and other studies have now shown that they affect not only the molecular state of neurons, but also the brain's microglia immune cells, astrocyte cells that play key roles in regulating circulation, and the brain's vasculature system. A hypothesis of Tsai's lab right now is that sensory gamma stimulation might promote the clearance of amyloid and tau via increased circulatory activity of brain fluids.

More definitive clinical studies are needed, the authors note. Indeed, there are now 15 new clinical studies of gamma stimulation underway. Among these is a phase 3 clinical trial by the company Cognito Therapeutics, which has licensed MIT's technology. That study plans to enroll hundreds of participants.

Meanwhile, some recent or new clinical and preclinical studies have begun looking at whether gamma stimulation may be applicable to neurological disorders other than Alzheimer's, including stroke or Down syndrome.

Workshop forges new skills, new connections

Starting on New Year's Day, when many people were still clinging to holiday revelry, scores of students and faculty members from about a dozen partner universities instead flipped open their laptops for MIT's Quantitative Methods Workshop, a jam-packed, week-long introduction to how computational and mathematical techniques can be applied to neuroscience and biology research. But don't think of QMW as a "crash course." Instead the program's purpose is to help elevate each participant's scientific outlook both through the skills and concepts it imparts, and the community it creates.

"It broadens their horizons, it shows them significant applications they've never thought of and introduces them to people whom as researchers they will come to know and perhaps collaborate with one day," said Susan L. Epstein, a Hunter College computer science professor and education coordinator of MIT's Center for Brains, Minds, and Machines, which hosts the program with the Departments of Biology and Brain and Cognitive Sciences and The Picower Institute for Learning and Memory. "It is a model of interdisciplinary scholarship."

This year 83 undergraduates and faculty members from institutions that primarily serve groups underrepresented in STEM fields took part in the QMW, said organizer Mandana Sassanfar, senior lecturer and Director of Diversity and Outreach across the four hosting MIT entities. Since the workshop launched in 2010, it has engaged more than 1,000 participants of whom more than 170 have gone on to participate in MIT Summer Research Programs (such as MSRP-BIO), and 39 have come to MIT for graduate school.

Individual goals, shared experience

Undergraduates and faculty in various STEM disciplines often come to QMW to gain an understanding of, or expand their expertise in, computational and mathematical data analysis. Computer science and statistics-minded participants come to learn more about how such techniques can be applied in life sciences fields. In hands-on labs using the computer programming language Python to process, analyze, and visualize data, in lectures, and in less formal settings such as tours and lunches with MIT faculty, participants work and learn together, and inform each other's perspectives.

And regardless of their field of study, participants make connections with each other and with the MIT students and faculty who teach and speak over the course of the week.

Biology Assistant Professor Brady Weissbourd converses with QMW student participants during a lunch break. *Photo by Mandana Sassanfar.*

Hunter College computer science sophomore Vlad Vostrikov said that while he has already worked with machine learning and other programming concepts, he was interested to "branch out" by seeing how they are used to analyze scientific datasets. He also valued the chance to learn the experiences of the graduate students who teach QMW's intensive hands-on labs.

Jariatu Kargbo, a biology and chemistry sophomore at University of Maryland Baltimore County, said when she first learned of the QMW she wasn't sure it was for her. It seemed very computation focused. But after an advisor encouraged her attend to expand her research skills, she also realized it would be a good opportunity to make connections at MIT in advance of perhaps applying for MSRP this summer.

"I thought this would be a great way to meet up with faculty and see what the environment is like here because I've never been to MIT before," Kargbo said. "It's always good to meet other people in your field and grow your network."

QMW is not just for students. It's also for their professors, who said they can gain valuable professional education for their research and teaching.

Fayuan Wen, an assistant professor of biology at Howard University, is no stranger to computational biology, having performed big data genetic analyses of sickle cell disease (SCD). But she's mostly worked with the R programming language and QMW's focus is on Python. As she looks ahead to projects in which she wants analyze genomic data to help predict disease outcomes in SCD and HIV, she said a QMW session delivered by biology graduate student Hannah Jacobs was perfectly on point.

"This workshop has the skills I want to have," Wen said.

Moreover, Wen said she is looking to start a machine learning class in the Howard biology department and was inspired by some of the teaching materials she encountered at QMW, including online curriculum modules developed by Taylor Baum, a graduate student in EECS and Picower Institute labs, and Paloma Sánchez-Jáuregui, a coordinator who works with Sassanfar.

Tiziana Ligorio, a Hunter computer science doctoral lecturer who together with Epstein will teach a deep machine learning class again this spring at the City University of New York campus, felt similarly. Rather than require a bunch of prerequisites that might drive students away from the class, Ligorio was looking to QMW's intense but introductory curriculum as a resource for designing a more inclusive way of getting students ready for the class.

Instructive interactions

Each day runs 9 to 5, including morning and afternoon lectures and hands-on sessions. Class topics ranged from statistical data analysis and machine learning to brain-computer interfaces, brain imaging, signal processing of neural activity data and cryogenic electron microscopy.

"This workshop could not happen without dedicated instructors—grad students, postdocs, and faculty—who volunteer to give lectures, design and teach hands-on computer labs and meet with students during the very first week of January," Saassanfar said.

(Continued on next page)

The sessions surround student lunches with MIT faculty members. For example, at midday Tuesday Biology Assistant Professor Brady Weissbourd, an investigator in The Picower Institute, sat down with seven students in one of Building 46's curved sofas to field questions about his neuroscience research in jellyfish and how he uses computational techniques as part of that work. He also described what it's like to be a professor and other topics that came to the students' minds.

Then the participants all crossed Vassar Street to Building 26's room 152 where they formed different but similarly sized groups for the hands-on lab "Machine Learning Applications to Studying the Brain," taught by Baum. She guided the class through Python exercises she developed illustrating "supervised" and "unsupervised" forms of machine learning, including how such methods can be used to discern what a person is seeing based on magnetic readings of brain activity.

Enduring connections

As new QMW attendees soaked in the experience for the first time, Luis Miguel de Jesús Astacio could recall how attending QMW as an undergraduate back in 2014 helped to launch his career as a physics faculty member at the University of Puerto Rico Rio Piedras Campus. After QMW he returned to MIT that summer as a student in the lab of neuroscientist and Picower Professor Susumu Tonegawa, and then in 2016 in the lab of physicist and Francis Friedman Professor Mehran Kardar. What's endured for the decade has been his connection to Sassanfar. So while he was once a student at QMW, this year he was back with a cohort of undergraduates as a faculty member.

Michael Aldarondo-Jeffries, director of Academic Advancement Programs at the University of Central Florida seconded the value of the networking that takes place at QMW. He has brought students for a decade, including four this year. What he's observed is that as students come together in settings like QMW or UCF's McNair program, which helps to prepare students for graduate school, they become inspired about a potential future as researchers.

Graduate student Taylor Baum (standing, black stripes) leads a class on principles of machine learning for analyzing neuroscience data. *Photo by David Orenstein.*

"The thing that stands out is just the community that's formed," he said. "For many of the students, it's the first time that they're in a group that understands what they're moving toward. They don't have to explain why they're excited to read papers on a Friday night."

Or why they are excited to spend a week including New Year's Day at MIT learning how to apply quantitative methods to life sciences data.

Emery N. Brown joins American Philosophical Society

On November 18, Emery N. Brown, Edward Hood Taplin Professor of Medical Engineering and Computational Neuroscience, signed the Roll Book of the nation's oldest learned society.

The ceremonial moment at the fall meeting of the American Philosophical Society in Philadelphia marked Brown's first participation as a member in the society, which has a founding mission of "promoting useful knowledge" through research, fellowships, and public outreach. Brown earned election earlier in 2023.

An anesthesiologist at Massachusetts General Hospital, Brown is also a neuroscientist and a statistician. Throughout his career as a researcher and clinician he has uniquely blended all three interests. In his early work he made numerous contributions to statistical analysis of neuroscience data. His neuroscience statistical analyses have had wide applications in studies of learning and memory, brain-computer interfaces, and systems neuroscience.

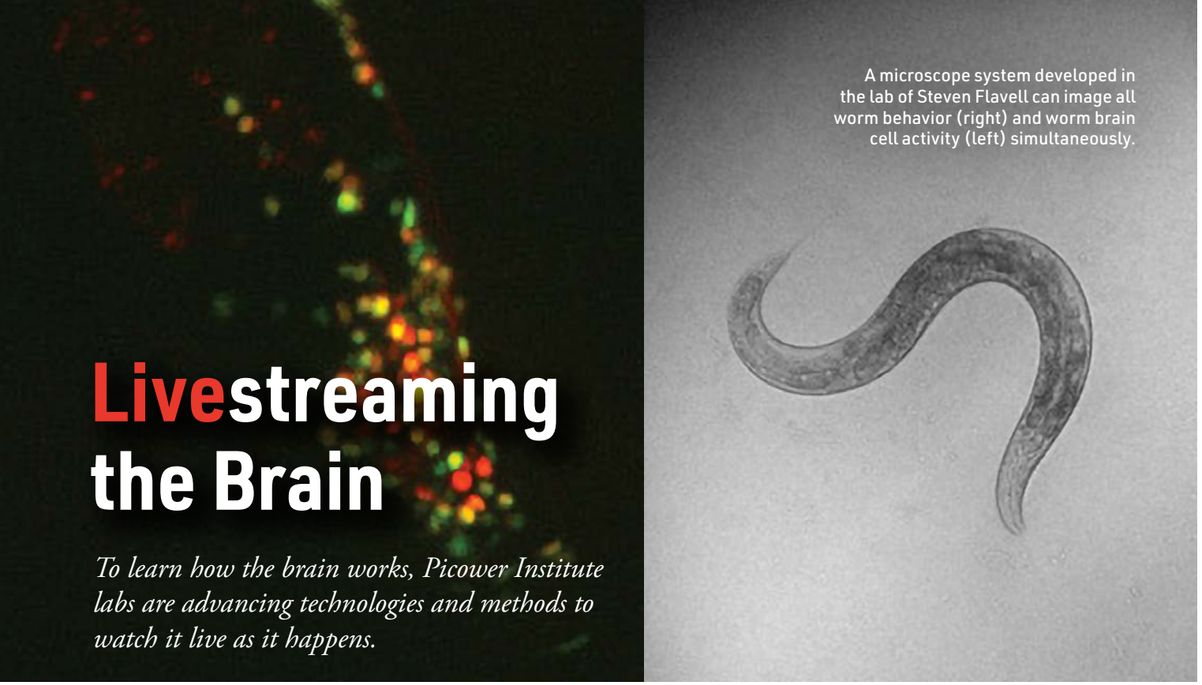
For nearly 20 years, Brown's lab has also applied his statistics and neuroscience expertise directly to anesthesiology by advancing fundamental studies of how each major anesthetic drug affects brain circuits to induce and maintain simultaneous but reversible states of unconsciousness, amnesia, immobility, and analgesia. At the systems neuroscience level, Brown and collaborators have shown how doses of each of these drugs produces oscillation signatures in EEG measurements that directly convey brain state. In his most recent paper he demonstrated how a closed-loop automated system can employ such brain state measurements to constantly optimize doses of propofol to maintain a desired level of unconsciousness. In the clinic this approach can reduce post-operative complications, such as cognitive dysfunction.

In a new research endeavor at MIT and MGH, the Brain Arousal State Control Innovation Center, Brown is working to establish more research programs that will integrate anesthesiology and neuroscience.

Brown said he was motivated by the society's history and mission.

"I am highly honored to be inducted to the American Philosophical Society," he said. "This august society was founded in 1743 by Benjamin Franklin. Its objective of fostering the growth of useful knowledge is even more relevant today."

Emery N. Brown signs the Roll Book of the American Philosophical Society for the first time as a member. Photo by Virginia Andradas.



Livestreaming the Brain

To learn how the brain works, Picower Institute labs are advancing technologies and methods to watch it live as it happens.

A microscope system developed in the lab of Steven Flavell can image all worm behavior (right) and worm brain cell activity (left) simultaneously.

For many Picower Institute neuroscientists, asking some of their most important research questions requires pushing the limits of live imaging technology. It takes vision to see the brain in action.

Back in 2016 as Associate Professor Steve Flavell imagined how his new lab would study how whole nervous systems produce sustained but flexible behaviors, he envisaged a microscope capable of tracking every behavior and the activity of every neuron in the simple brain of his model organism, the *C. elegans* worm. It took almost a decade of ingenuity, building on the previous inventions of other neuroscientists, but now Flavell's lab has begun making striking discoveries.

"For a long time it was a dream of neuroscientists to be able to make all of these measurements to see everything," Flavell said. "Because how can you relate neural activity and behaviors if you can't record them all at the same time?"

Similarly inspired, neuroscientists across The Picower Institute are advancing live imaging techniques to reveal fundamental mechanisms of memory, how animals learn to intelligently navigate their environments, and how they decide to act on perceptual cues. Meanwhile, others are developing different live imaging techniques to discover how the brain forms and remodels the circuits that enable these essential functions.

The appeal of watching the brain work seems intuitive, but little of what's happening can be seen directly. Neural communication activity (i.e. when neurons electrically "spike" to transmit signals) is electrochemical, so it's invisible. Electrodes can directly tap these signals (a practice called "electrophysiology"), but for some studies, it's not feasible to precisely poke many wires into the brain of a living, moving animal. Moreover, electrodes can't reveal which exact neurons they are eavesdropping on.

While the brain's anatomy is visible, it requires great cleverness to see it in ways that answer many crucial neuroscience questions. Sometimes it's enough to put a stained slice of tissue under a microscope to capture a fixed, single moment in time. But Picower scientists seek to observe how these structures change daily in animals as they develop or see and learn new things.

Meanwhile, live imaging techniques suited for human volunteers, such as MRI, can't resolve the scale of molecules, cells and circuits that many Picower Institute researchers must see to understand fundamental mechanisms of brain function.

The methods to use depend on what a scientist wants to know. Some Picower labs don't emphasize live imaging, but those that do have surged to the vanguard.

Tracking activity and behavior

Flavell's dream to image all behaviors and the whole brain activity of live, freely moving animals required two revolutions. One was a way to make the brain's electrical activity visible. That arose when neuroscientists engineered neurons with a protein that glows when calcium ions build up (a correlate of spiking). The other was the advent of artificial intelligence powerful enough to automatically track worm movement and behavior and also to spot the glowing cells in images.

When Flavell arrived at MIT, like-minded scientists at Princeton and Harvard were starting to develop prototype microscopes to image behavior and simultaneous neural activity. The first postdoc Flavell hired, Ni Ji, came from the Harvard lab. Ji and Flavell had their first version of a system up and running within a year, but even so, imaging defined cells across the entire brain of the worm along with its full repertoire of behaviors remained beyond any lab's reach. For a study examining how worms integrate sensory information to decide on the optimal feeding behaviors, for instance, Ji imaged a small selection of the hundreds of neurons in the worm's brain.

Lab members iteratively improved the system, including its ability to follow the worm's movements to keep it perfectly centered. But the breakthrough needed for whole-brain imaging emerged when two graduate students who arrived in 2018, Adam Atanas and Jungsoo Kim, were home during the pandemic. They wrote software that ensured that no matter how much the worm wriggled and twisted, thereby shifting, warping and sometimes partially obscuring the neurons within them, the computers could still keep track of the cells to register their flashes of activity.

Last year saw big payoffs from those efforts. The lab published two studies in *Cell* that employed whole-brain imaging. In one they unveiled a predictive model of how most of the worm's neurons encode its behaviors. In the other they mapped out how the worm's whole nervous system responds to the neuromodulatory chemical serotonin, which in humans is the most frequent target of psychiatric drugs.

Deeper insight

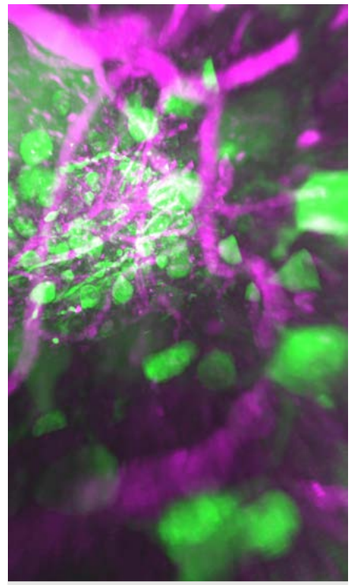
For studies of cognitive abilities such as how mice learn to act on perceptual cues, Newton Professor Mriganka Sur is also a calcium imaging "power user." But mice present different challenges than worms. Mouse brains have millions of neurons and are thicker and opaque. Calcium imaging requires external light stimulation, which has trouble penetrating the tissue. So-called "2-photon" microscopes can image calcium flashes a little way below the brain's surface, but Sur's lab wanted to visualize activity through the brain's entire cortex, which is where mice (and humans) perform sophisticated information processing. About eight years ago Sur therefore teamed up with former postdoc Murat Yildirim and Mechanical Engineering Professor Peter So to refine the nascent concept of "3-photon" microscopy (first developed at Cornell). In 2019 they published the first study to image live neural activity all the way through the cortex's six layers, a depth of more than a millimeter.

Sur's lab has also pioneered calcium imaging of targeted neuronal populations that connect disparate regions, for example the prefrontal cortex and the superior colliculus. Alongside, they have imaged the activity of single axons that connect brain regions to decipher the communications such pathways enable. And they have developed tools to simultaneously image multiple cell types, such as neurons and astrocytes, to understand how they influence each other within cortical networks.

Sur has also tackled calcium imaging's slowness. Calcium flashes last hundreds of milliseconds but a single spike is comparatively instantaneous. When spikes occur rapidly, calcium imaging fails to resolve each cleanly. To improve inference of spiking patterns from calcium signals, Sur has co-authored two studies, including one last year, presenting increasingly efficient algorithms.

A 'new frontier of imaging'

In parallel, scientists are developing a quicker technology to visualize neural electrical activity, called Genetically Encoded Voltage Indicators, or GEVIs. Among them are new Picower Institute Assistant Professor Linlin Fan, and Y. Eva Tan Professor of Neurotechnology Ed Boyden, an affiliate member of The Picower Institute. GEVIs are becoming so quick and sensitive that their glow can indicate a neuron's voltage even if it's less than the peak represented by a spike. GEVIs therefore offer the potential to view subtle neural activity across wide areas of an animal's brain with enough resolution to show activity in individual cells and enough quickness and sensitivity to rival electrophysiology.



A "three-photon" microscope developed in the lab of Mriganka Sur can image neural activity (green) and neural axon structure (pink) all the way through the mouse cortex.

Though it's still emerging, that promise intrigues Sherman Fairchild Professor Matt Wilson, whose decades of electrophysiology innovations have advanced studies of how rodents learn and remember how to navigate their physical environments. The work requires tracking activity among hundreds of neurons in multiple brain regions. Recently Wilson and postdoc Jie "Jack" Zhang began collaborating with Boyden and his team to develop a system that optimizes a GEVI, a new microscope design, and a new kind of camera.

"This is pursuing the technology that will allow us to use the same principles that we've embraced—direct recording of electrophysiological activity—and move that into the new frontier of imaging," Wilson said. "You want to be able to ask and address questions and not be limited by the technology."

Their first testbeds are larval zebrafish, which are transparent and have simpler brains than mice but more complex ones than *C. elegans*. The goal is to image brainwide with enough resolution and speed to capture meaningful neuroscience measurements. A key limitation to overcome, Wilson said, is extracting as much signal as possible. When GEVIs are used in mice, the light they emit will be dampened by the brain's opacity. That's where the novel camera comes in. It's designed so that each pixel in the sensor can be individually controlled. The collaboration's strategy is to control neighboring pairs of pixels so that in the same spot one pixel will image quickly (to capture fast neural dynamics) while the other stays on longer (to gather more total light).

The work is progressing well, Wilson said, with two papers on their systems currently under review by journals.

Although in a spatially focused way, rather than brain-wide, Fan is already applying GEVIs in mice and doing so in combination with another technology, optogenetics, which enables neural activity to be controlled with light. As a graduate student at Harvard and then a postdoc at Stanford she led papers in *Cell* in which she pioneered the combination of these methods to produce new insights into how circuits in the cortex processes the sense of feel and how neural activity in the hippocampus changes circuit connections to encode spatial memories.

These unique feats required more than just getting the GEVIs to work properly. With two paths of light going into the brain (one to stimulate the GEVIs and one for optogenetic control), and another coming out from the GEVIs, Fan's team had to design microscopes that could avoid interference. Using a sophisticated arrangement of advanced optical components, she precisely sculpted the incoming light sources to target different parts of cells in very close proximity.

With a long-term goal of studying how the momentary neural activity upon encountering something new ultimately produces an enduring neural encoding of that memory, Fan is eager to push the limits of "all optical physiology" to enable investigations of larger populations of neurons in wider areas of the brain. Memories are encoded in circuits and their formation, storage and recall can depend on multiple brain regions.

"We are actively paving the way, by showing these are useful and functional *in vivo*," Fan said. "We are one of the few groups really using these tools to discover new knowledge."

To help advance the field's techniques and their use, Fan is co-organizing a three-day conference in Paris in June: "Sculpted Light in the Brain."

Structure & Physiology

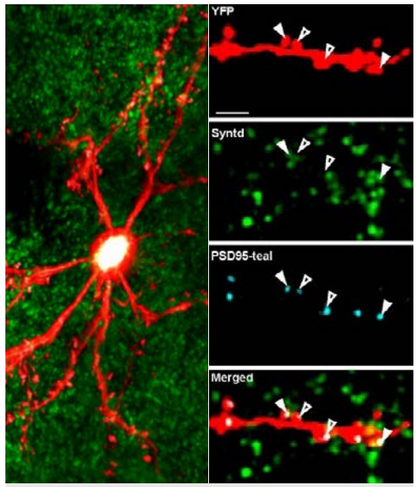
Sculpting light to see and control activity is one thing. Using live imaging to reveal how the brain sculpts itself is another. That's what has motivated other Picower Institute professors to make their own imaging innovations.

Like Flavell, William R. and Linda Young Professor Elly Nedivi knew she'd need new imaging technologies right from the establishment of her lab in 1998. She wanted to study the molecular basis of "plasticity," the way the brain builds and edits circuit connections between neurons, called synapses, to enable learning and memory. Nedivi didn't want to rely on making statistical inferences from observations in dissected brain slices—she wanted to directly watch it happen. At the time, one couldn't buy a two-photon microscope to image in mice, but she engaged the help of So, setting off a collaboration that continues today.

With that first scope Nedivi was able to see entire dendritic arbors (the vineline branches that extend from the neuron body) of individual cortical neurons and track them over time, seeing they were not as hardwired as everyone thought. The changes were subtle but one could track them by watching daily in the same animal. Inhibitory neurons constantly remodeled connections with their excitatory partners.

Inhibitory synaptic connections had never been visualized live before because they don't form a distinguishable shape like the excitatory synapses that reside on small spine protrusions that are physically apparent. This led Nedivi to a new challenge: How can the two different kinds of synapses—excitatory and inhibitory— be tracked as they come and go, or shrink and grow? Doing that would require developing synaptic fluorescent markers and two-photon microscopy for simultaneous tracking of multiple colors, something she and So developed together.

First they developed a two-color system. Then, when Nedivi and So developed a three-color version, it meant she could image both excitatory and inhibitory synapses at the same time. That enabled her to discover that even when excitatory synapses were mostly stable in the adult brain, inhibitory ones would often come and go dynamically to modulate the degree of excitatory activity.



Elly Nedivi's lab can image neurons labeled with three colors: Red highlights the overall cell. In the accompanying detail, green marks incoming connections from other neurons, while teal highlights the neuron's side of the connections.

"We were only able to figure that out because we saw them right next to each other at the same time," said Nedivi, who notes that many other labs have now adopted multi-color imaging.

Last year, the three-color system extended to also visualize specific inputs to synapses. This enabled her lab's unprecedented analysis in *Nature Neuroscience* of how inputs from a brain region called the thalamus delivered sensory information to neurons in layer 2/3 of the visual cortex.

Now Nedivi, So, and graduate student Kendyll Burnell are developing a four-color system. As before, adding a color takes a lot of tinkering both with the microscope optics and with the proteins, or "fluorophores," that glow to mark a key molecule. In this round, for instance, one of the new fluorophores turned out to be so bright that they had to ratchet down how much they stimulated it to avoid drowning out the others. Once four colors are fully working they hope to use it to examine how the thalamus connects to mature vs. immature synapses and to track differences in the synapses' molecular compositions.

Menicon Professor Troy Littleton has developed new live imaging methods for his studies of how neurons in drosophila fruit flies develop their circuit communications infrastructure. In a 2018 study in *eLife*, his lab debuted "optical quantal imaging" in which they engineered synapses to flash whenever the neurotransmitter glutamate crossed from the sending cell's side of the synapse to the receiving cell's side. The technique yielded the insight that along the same neuron's connection to a muscle a few synapses become very active while most remain comparatively weak. Then, using "intravital imaging" they were able to anesthetize, image, and then revive larval fruit flies to measure the day-by-day development of these different synapses. Last year, Littleton's lab used intravital imaging again in a study showing that without the protein perlecan, neural axons can literally unravel, disrupting synapse formation.

And the Sur Lab's three-photon microscope has provided deeper looks at structures thanks to another technology embedded in the scope called "Third Harmonic Generation." THG detects differences in how materials bend light. It can therefore resolve the membranes of cells and blood vessels. In 2020 the lab used THG to examine how the functions of distinct brain regions correlated with differences in their structure. And in 2022 they used the scopes to image advanced 3D cell cultures modeling early brain development in Rett syndrome. They showed that newborn neurons in the cultures struggled to migrate to their proper places, lending insight into how disease symptoms develop.

Driven to discover, Picower Institute labs are advancing live brain imaging.

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September 24, 2024: Lectures

Michelle Monje, MD, PhD
HHMI, Stanford University

Erin Schuman, PhD
Max Planck Institute for Brain Research

September 25, 2024: Workshops

Hosted by The Picower Institute for Learning and Memory, MIT

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BOTTOM ROW: **Earl Miller**, Picower Professor of Neuroscience, Department of Brain and Cognitive Sciences; **Elly Nedivi**, William R. (1964) & Linda R. Young Professor of Neuroscience, The Picower Institute for Learning and Memory, Departments of Brain and Cognitive Sciences and Biology; **Sara Prescott,** Assistant Professor of Biology; **Mriganka Sur**, Paul E. Newton Professor of Neuroscience, Director of The Simons Center for the Social Brain; **Susumu Tonegawa**, Picower Professor of Biology and Neuroscience, Departments of Brain and Cognitive Sciences and Biology, Investigator, Howard Hughes Medical Institute, Investigator and Director of the RIKEN-MIT Center for Neural Circuit Genetics; **Li-Huei Tsai**, Picower Professor of Neuroscience, Department of Brain and Cognitive Sciences, Director, The Picower Institute for Learning and Memory; **Brady Weissbourd,** Assistant Professor of Biology; **Matthew Wilson**, Sherman Fairchild Professor in Neurobiology, Departments of Brain and Cognitive Sciences and Biology, Associate Director, The Picower Institute for Learning and Memory.