INSIDE

A Neuron's Neighbors

The field is called neuroscience, but neurons alone can't operate your brain. Non-neural cells indispensably contribute to function. Their roles can make them promising treatment targets in disease. Pg. 8

Neuroscience News

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THE PICOWER INSTITUTE
FOR LEARNING AND MEMORY

DIRECTOR'S **MESSAGE**

Dear Friends,

You don't have to be a sports fan to appreciate that while the stars of any team are the players, they wouldn't be able to do anything without coaches, trainers, equipment managers and a host of other team employees who don't take the field, court or ice.

So it is with the brain. Neurons get the lion's share of the attention, but they depend heavily on less appreciated cells: "glia" such as microglia, astrocytes, and oligodendrocytes, and the epithelial, mural and other cell types of the brain's blood vessels. "Less appreciated" also means "less studied," but several labs in The Picower Institute for Learning and Memory have been intently researching glial and vascular cells for years because they are indispensable to many brain functions and fully implicated in many brain diseases. Our cover story on page 8 features this research and what it means for efforts to improve brain health.

On the pages leading up to that, we cover a wide gamut of other research, including new findings about memory from Matt Wilson's lab and cognition from Mriganka Sur's Lab, and new technologies from the labs of Kwanghun Chung and Wilson that have created amazing new capabilities for fellow scientists. And we feature a new strategy developed in Mark Bear's lab for potentially treating fragile X syndrome, the most common inherited form of autism.

While we are proud to share all the above news, nothing has made our hearts swell with joy more than the story on page 7: The awarding of the National Medal of Science to our colleague Emery N. Brown. The medal is the nation's highest honor for scientists and we are all very excited that he received such a prestigious and well-deserved recognition.

We hope you enjoy all these stories and we thank you for reading and engaging with us in other ways. As friends of the institute, you, too, are part of our team.

LI-HUEI TSAI, DIRECTOR

The Picower Institute for Learning and Memory

How the brain, with sleep, learns meaningful **maps** of spaces

A new study in mice by neuroscientists at The Picower Insitute provides evidence for how the brain forms cohesive cognitive maps of whole spaces and highlights the critical importance of sleep for the process.

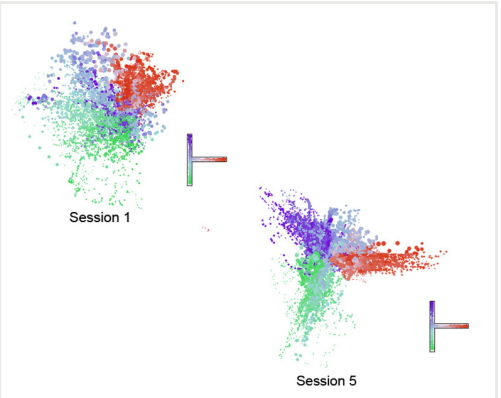
Scientists have known for decades that the brain devotes neurons in a region called the hippocampus to remembering specific locations. So-called "place cells" reliably activate when an animal is at the location the neuron is tuned to remember. But more useful than having markers of specific spaces is having a mental model of how they all relate in a continuous overall geography. Though such "cognitive maps" were formally theorized in 1948, neuroscientists have remained unsure of how the brain constructs them.

The new study in *Cell Reports* finds that the capability may depend upon subtle but meaningful changes over days in the activity of cells that are only weakly attuned to individual locations, but that increase the robustness and refinement of the brain's encoding of the whole space. With sleep, the study's analyses indicate, these "weakly spatial" cells increasingly enrich neural network activity in the hippocampus to link together these places into a cognitive map.

"On day 1, the brain doesn't represent the space very well," said lead author Wei Guo, a research scientist in the lab of senior author Matthew Wilson, Sherman Fairchild Professor in The Picower Institute. "Neurons represent individual locations, but together they don't form a map. But on day 5 they form a map. If you want a map, you need all these neurons to work together in a coordinated ensemble."

To conduct the study, the research team introduced mice to simple mazes of varying shapes and let them explore them freely for about half an hour a day for several days. Meanwhile, Guo and his co-authors monitored hundreds of neurons in the CA1 area of the hippocampus by engineering cells to flash when a buildup of calcium ions made them electrically active. They not only recorded the neurons' flashes when the mice were actively exploring, but also while they were sleeping.

Analysis of the recordings showed that the activity of the place cells developed immediately and remained strong and unchanged over several days of exploration. But Guo extended his analysis to the more subtle and mysterious activity of cells that were not so strongly spatially tuned. Using an emerging technique called "manifold learning" he was able to discern that many of the "weakly spatial" cells gradually correlated their activity with activity patterns among other neurons in the CA1 network. As this was happening, the network encoded a cognitive map of the maze that increasingly resembled the maze.



Neural representations of a cognitive map of a sideways T-shaped maze evolved over five sessions. Each dot is a point in time and each color corresponds to a location in the actual maze (see smaller T's). Over time the cognitive map better resembles the actual maze.

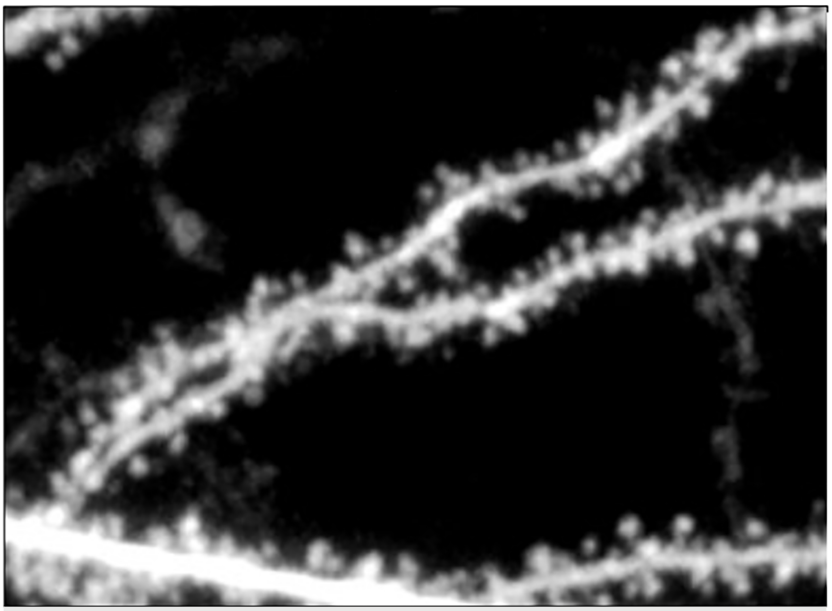
Studies by Wilson's lab and many others have shown that memories are consolidated, refined and processed during sleep and rest. Guo and Wilson's team therefore sought to test whether sleep was necessary for the contribution of weakly spatial cells to latent learning of cognitive maps. To do this they let some mice explore a new maze twice during the same day with a three-hour siesta in between. Some of the mice were allowed to sleep but some were not. The ones that did showed a significant refinement of their mental map, but the ones that weren't allowed to sleep showed no such improvement. Not only did the network encoding of the map improve, but also measures of the tuning of individual cells during showed that sleep helped cells become better attuned both to places and to patterns of network activity.

New molecular strategy for treating **fragile X syndrome**

Building on more than two decades of research, Picower Institute scientists report a new way to treat pathology and symptoms of fragile X syndrome, the most common genetically-caused autism spectrum disorder. The team showed that augmenting a novel type of neurotransmitter signaling reduced hallmarks of fragile X in mouse models of the disorder.

The new approach described in *Cell Reports* works by targeting a specific molecular subunit of the "NMDA" receptors that they discovered plays a key role in how neurons synthesize proteins to regulate their connections, or "synapses," with other neurons in brain circuits. The scientists showed that in fragile X model mice, increasing the receptor's activity caused neurons in the hippocampus region of the brain to increase molecular signaling that suppressed excessive bulk protein synthesis, leading to other key improvements.

"One of the things I find most satisfying about this study is that the pieces of the puzzle fit so nicely into what had come before," said study senior author and Picower Professor Mark Bear. Former postdoc Stephanie Barnes, now a lecturer at the University of Glasgow, is the study's lead author.



Observations of the small protrusions that line the dendrites of neurons, called spines, were critical in understanding the function of NMDA receptors in the new study, as well as a precursor to the research back in 2020.

In 2011, Bear's lab showed that fragile X and another autism disorder, tuberous sclerosis (Tsc), represented two ends of a continuum of a kind of protein synthesis in the same neurons. In fragile X there was too much. In Tsc there was too little. When lab members crossbred fragile X and Tsc mice, in fact, their offspring emerged healthy as if the mutations of each disorder canceled each other out.

Meanwhile, it has long been understood from Bear's work in the 1990s that the flow of calcium ions through NMDA receptors (NMDARs) can trigger a form of synaptic change, or "plasticity" called "long-term depression" (LTD). But in 2020, they found that another mode of signaling by the receptor—one that did not require ion flow—altered protein synthesis in the neuron and caused a physical shrinking of the dendritic "spine" structures housing synapses.

For Bear and Barnes these studies raised the prospect that if they could pinpoint how NMDA receptors affect protein synthesis they might identify a new mechanism that could be manipulated therapeutically to address fragile X.

In the new study, Bear and Barnes's team decided to use the non-ionic effect on spine shrinkage as a readout to dissect how NMDARs signal protein synthesis for synaptic plasticity in hippocampus neurons. They hypothesized that the dichotomy of ionic effects on synaptic function and non-ionic effects on spine structure might derive from the presence of two distinct components of NMDARs: "subunits" called GluN2A and GluN2B. To test that, they used genetic manipulations to knock out each of the subunits. They found that knocking out "2A" or "2B" could eliminate LTD but that only knocking out 2B affected spine size.

How does the 2B subunit signal spine shrinkage? A promising possibility was a part of the subunit called the "carboxyterminal domain," or CTD. So, in a new experiment Bear and Barnes took advantage of a mouse that had been genetically engineered by researchers at the University of Edinburgh so that the 2A and 2B CTDs could be swapped with one another. A telling result was that when the 2B subunit lacked its proper CTD, the effect on spine structure disappeared. The result affirmed that the 2B subunit signals spine shrinkage via its CTD.

Another consequence of replacing the CTD of the 2B subunit was an increase in bulk protein synthesis that resembled findings in fragile X. Conversely, augmenting the non-ionic signaling through the 2B subunit suppressed bulk protein synthesis, reminiscent of Tsc.

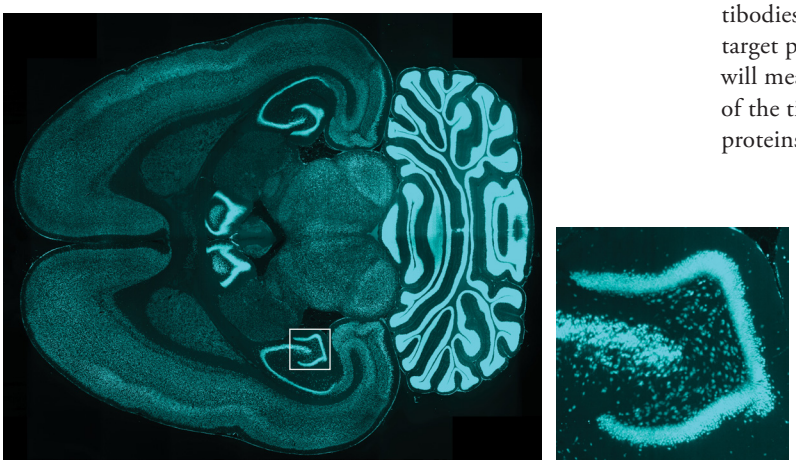
Putting the pieces together, the findings indicated that augmenting signaling through the 2B subunit might, like introducing the mutation causing Tsc, rescue aspects of fragile X.

Indeed when the scientists swapped in the 2B subunit CTD of NMDA receptor in fragile X model mice they found correction of not only the excessive bulk protein synthesis, but also of the altered synaptic plasticity and increased electrical excitability that are hallmarks of the disease. To see if a treatment that targets NMDA receptors might be effective in fragile X, they tried an experimental drug called Glyx-13. This drug binds to the 2B subunit of NMDA receptors to augment signaling. The researchers found that this treatment can also normalize protein synthesis and reduced sound-induced seizures in the fragile X mice.

Bear said he does not know what the prospects are for Glyx-13 as a clinical drug, but he noted that there are some drugs in clinical development that specifically target the 2B subunit of NMDA receptors.

Ultrafast **protein labeling** of millions of densely packed cells in organ-scale tissues

A new technology enables scientists to label proteins across millions of individual cells in fully intact 3D tissues with unprecedented speed, uniformity, and versatility. Using the technology, the team was able to richly label whole rodent brains and other large tissue samples in a single day. In their new study in *Nature Biotechnology,* they also demonstrate that the ability to label proteins with antibodies at the single-cell level across whole brains can reveal insights left hidden by other widely used labeling methods.



A cross-section from a whole rat brain dataset labeled to highlight a protein marker that identifies neurons (inset: a closeup of the dentate gyrus in the hippocampus).

Profiling the proteins that cells are making is a staple of studies in biology, neuroscience and related fields because the proteins a cell is expressing at a given moment can reflect the functions the cell is trying to perform or its response to its circumstances, such as disease or treatment.

"Conventionally, investigating the molecules within cells requires dissociating tissue into single cells or slicing it into thin sections, as light and chemicals required for analysis cannot penetrate deep into tissues. Our lab developed technologies such as CLARITY and SHIELD, which enable investigation of whole organs by rendering them transparent, but we now needed a way to chemically label whole organs to gain useful scientific insights," says study senior author Kwanghun Chung, associate professor in The Picower Institute. "If cells within a tissue are not uniformly processed, they cannot be quantitatively compared. In conventional protein labeling, it can take weeks for these molecules to diffuse into intact organs, making uniform chemical processing of organ-scale tissues virtually impossible and extremely slow."

The new approach, called "CuRVE," represents a major advance by demonstrating a fundamentally new approach to uniformly processing large and dense tissues whole. In the study, the researchers explain how they overcame technical barriers via an implementation of CuRVE called "eFLASH," and provide copious vivid demonstrations of the technology, including how it labeled proteins that didn't show up when using genetic labeling methods.

"This is a significant leap, especially in terms of the actual performance of the technology," said co-lead author Dae Hee Yun, a former MIT graduate student and now a senior application engineer at LifeCanvas Technologies, a startup company Chung founded to disseminate the tools his lab invents. The paper's other lead author is Young-Gyun Park, a former MIT postdoctoral researcher who is now an assistant professor at KAIST in South Korea.

Large, 3D tissue samples are hard to label uniformly because antibodies seep into tissue very slowly, but are quick to bind to their target proteins. Simply soaking an organ in a bath of antibodies will mean that proteins are intensely well labeled on the outer edge of the tissue, but virtually none of the antibodies will find cells and proteins deeper inside.

To improve labeling, the team conceived of a way—the conceptual essence of CuRVE—to resolve the speed mismatch. The strategy was to continuously control the pace of antibody binding while at the same time speeding up antibody permeation throughout the tissue. To figure out how this could work and to optimize the approach, they built and ran a sophisticated computational simulation that enabled them to test different settings and parameters, including different binding rates and tissue densities and compositions.

Then they set out to implement their approach in real tissues. Their starting point was a previous technology, called "SWITCH," in which Chung's lab devised a way of temporarily turning off antibody binding, letting the antibodies permeate the tissue, and then turning binding back on. Yun said the team realized there could be substantial improvements if antibody binding speed could be controlled constantly, but the chemicals used in SWITCH were too harsh for such ongoing treatment. So they screened a library of similar chemicals to find one that could more subtly and continuously throttle antibody binding speed. They found that deoxycholic acid was an ideal candidate. Using that chemical, the team could not only modulate antibody binding by varying the chemical's concentration, but also by varying the labeling bath's PH (or acidity).

Meanwhile, to speed up antibody movement through tissues, the team used another prior technology invented in the Chung Lab: stochastic electrotransport. That technology accelerates the dispersion of antibodies through tissue by applying electric fields.

Implementing this eFLASH system of accelerated dispersion with continuously modifiable binding speed produced the wide variety of labeling successes demonstrated in the paper. In all, the team reported using more than 60 different antibodies to label proteins in cells across whole brains of mice and rats; whole mouse embryos; other whole mouse organs including lung and heart; and blocks of brain tissue from larger animals including humans.

Each of these specimens were labeled within a day, an "ultra-fast" speed for whole, intact organs, the authors said. Moreover, different preparations did not require new optimization steps.

Even after learning a task, humans and animals still **explore** other approaches

Maybe it's a life hack or a liability, or a little of both. A surprising result in a new MIT study may suggest that people and animals alike share an inherent propensity to keep updating their approach to a task even when they have already learned how they should approach it and even if the deviations sometimes lead to unnecessary error.

The behavior of "exploring" when one could just be "exploiting," could make sense for at least two reasons, said Mriganka Sur, senior author of the study in *Current Biology*. Just because a task's rules seem set one moment doesn't mean they'll stay that way in this uncertain world, so altering behavior from the optimal condition every so often could help reveal needed adjustments. Moreover, trying new things when you already know what you like is a way of finding out whether there might be something even better out there than the good thing you've got going on right now.

"If the goal is to maximize reward, you should never deviate once you have found the perfect solution, yet you keep exploring," said Sur, Paul and Lilah Newton Professor in The Picower Institute. "Why? It's like food. We all like certain foods, but we still keep trying different foods because you never know, there might be something you could discover."

Former research technician Tudor Dragoi, now a graduate student at Boston University, and research scientist Hiroki Sugihara led the study in which they and fellow members of the Sur Lab explored how humans and marmosets, a small primate, make predictions about event timing.

Three humans and two marmosets were given a simple task. They'd see an image on a screen for some amount of time—the amount of time varied from one trial to the next within a limited range—and they simply had to hit a button (marmosets poked a tablet while humans clicked a mouse) when the image disappeared. Success was defined as reacting as quickly as possible to the image's disappearance without hitting the button too soon. Marmosets received a juice reward on successful trials.

Though marmosets needed more training time than humans, the subjects all settled into the same reasonable pattern of behavior regarding the task. The longer the image stayed on the screen, the faster their reaction time to its disappearance. This behavior follows the "hazard model" of prediction in which, if the image can only last for so long, the longer it's still there, the more likely it must be to disappear very soon. The subjects learned this and overall, with more experience, their reaction times became faster.

But as the experiment continued, the team noticed something surprising was also going on. Mathematical modeling of the reaction time data revealed that both the humans and marmosets were letting the results of the immediate previous trial influence what they did on the next trial, even though they had already learned what to do. If the image was only on the screen briefly in one trial, on the next round subjects would decrease reaction time a bit (presumably expecting a shorter image duration again) whereas if the image lingered, they'd increase reaction time (presumably because they figured they'd have a longer wait).

Those results add to ones from a similar study Sur's lab published in 2023 in which they found that even after mice learned the rules of a different cognitive task, they'd arbitrarily deviate from the winning strategy every so often. In that study, like this one, learning the successful strategy didn't prevent subjects from continuing to test alternatives, even if it meant sacrificing reward.

Even with a clear "path" to follow to optimally accomplish a task, people and animals will still sometimes choose to explore other ways to go.

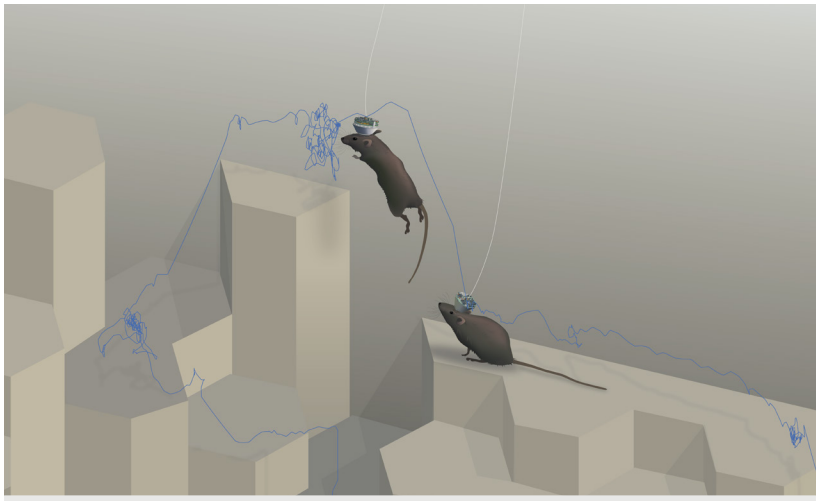
"The persistence of behavioral changes even after task learning may reflect exploration as a strategy for seeking and setting on an optimal internal model of the environment," the scientists wrote in the new study.

The similarity of the human and marmoset behaviors is an important finding as well, Sur said. That's because differences in making predictions about one's environment is posited to be a salient characteristic of autism spectrum disorders. Because marmosets are small, are inherently social, and are more cognitively advanced than mice, work has begun in some labs to establish marmoset autism models, but a key component was establishing that they model autism-related behaviors well. By demonstrating that marmosets model neurotypical human behavior regarding predictions, the study therefore adds weight to the emerging idea that marmosets can indeed provide informative models for autism studies.

Open platform enables new research versatility

Individual technologies for recording and controlling neural activity in mice have each advanced rapidly but the potential of easily mixing and matching them to conduct more sophisticated experiments, all while enabling the most natural behavior possible, has been difficult to realize. To empower a new generation of neuroscience experiments, engineers and scientists at MIT and the Open Ephys cooperative developed a new standardized, open-source hardware and software platform. They described the system, called ONIX, in *Nature Methods*.

ONIX provides labs with a means to acquire data simultaneously from multiple popular implanted technologies (such as electrodes, microscopes and stimulation probes) while also powering and controlling those independent devices via a very thin coaxial cable and unimposing headstage. The system provides a standardized means of acquiring each instrument's data and neatly integrating it all for efficient transmission to desktop software where scientists can then see and work with it. In the study the researchers document ONIX's high data throughput and low latency. They also demonstrate that because the system's headstage and cable are so physically light and resistant to twisting, mice can behave completely naturally and wear the system for days on end. In a large enclosure at MIT with a complex 3D landscape, for instance, mice wearing the system were able to nimbly scamper, climb and leap in experiments comparably to mice wearing no hardware at all.



In this cartoon representing an experiment in the research, a mouse wearing the ONIX system leaps through an open landscape in the lab. The blue line tracks the mouse's position over time.

Jon Newman, a former MIT postdoc and now president of Open Ephys, and MIT postdoc Jie "Jack" Zhang led the work in the lab of co-author Matt Wilson, Sherman Fairchild Professor in The Picower Institute, together with Aarón Cuevas-López at Open Ephys. Wilson, whose lab studies neural processes underlying memory, said the idea behind developing ONIX was to develop a set of standards that would make it easy for any lab to use multiple technologies to acquire rich neural data while animals performed complex behaviors over long time periods.

"Jon's motivation, the principle he used, was that if we need to do experiments that combined things like optogenetics, imaging, tetrode electrophysiology, and neuropixels, could we do it in a way that would not only enable experiments we were doing but also more complex experiments, involving more complex behavior, involving the integration of different recording methodologies that advances the whole community and not just one individual lab?" Wilson said.

As Newman and then Zhang began to develop the technology starting in 2016 with this community-minded, open-source philosophy, Wilson said, it was natural to do so in partnership with Open Ephys, an MIT-born effort, now based in Atlanta, which develops and disseminates open, standardized systems for neuroscience research. Making systems open-source provides researchers with many advantages, explained corresponding author Jakob Voigts, an MIT neuroscience alumnus and co-founder of Open Ephys.

"Anyone can download the plans for the hardware as well as the software that make up the system," Voigts said. "Open source also means that the system works with probes from many manufacturers because the connectors and standards aren't proprietary. Most importantly, the open standards and design allow hardware and software developers to use ONIX as a starting point for completely new tools."

With ONIX, Wilson said, "You can mix and match and combine and then add new technologies without having to re-engineer the whole system."

The researchers conducted several experiments to validate the platform. For instance, they compared the mobility of mice implanted with electrodes but sometimes wearing ONIX (and its 0.3 mm tether cable) vs. sometimes wearing a commonly used and but substantially thicker (1.8 mm) tether cable over an 8-hour neural recording session. The mice proved to be much more mobile while wearing the lighter and thinner ONIX system, showing a broader range of exploration, freer head movement, and much faster running speeds. In a similar experiment in which mice were implanted with tetrodes in the brain's retrosplenial cortex, they even were able to jump while wearing ONIX but did not while wearing the more unweildy system. In another experiment the researchers compared mouse mobility around the enclosure between ONIX-wearing and completely unimplanted mice. The mice explored with equal freedom (as measured by motion tracking cameras) though the ONIX mice didn't run as fast as unimplanted mice.

In further experiments, Voigts's team at HHMI Janelia Research Campus used ONIX to record for 55 hours because the system kept its cable tangle-free over that long-duration activity.

Finally, the researchers showed that ONIX could transmit recordings not only from implanted electrodes and tetrodes but also from miniscopes and neuropixels. They also showed how Open Ephys's data acquisition software Bonsai enabled the brain activity recordings to be synchronized with behavior tracking cameras to correlate neural activity and behavior.

Emery N. Brown earns National Medal of Science

Emery N. Brown, Edward Hood Taplin Professor of Medical Engineering and Computational Neuroscience in The Picower Institute for Learning and Memory, has won the National Medal of Science, the nation's highest recognition for scientists and engineers, the Biden White House announced Jan 3.

Emery N. Brown in his MIT office

"This is an enormous pleasure to be recognized by the President with this high honor," said Brown, who shared this year's honor with three MIT colleagues and 23 colleagues around the country in total. Senior White House science officials at the time including Arati Prabhakar, Director of the Office of Science and Technology Policy and Assistant to the President for Science & Technology, bestowed the medals on the winners.

A neuroscientist, a statistician and an anesthesiologist at Massachusetts General Hospital, Brown has for decades led innovations and discoveries in the analysis of large and complex data sets. This includes studying how neurons represent signals and stimuli in their ensemble spiking activity. His advances of statistical methods and signal processing algorithms have, for example, aided studies of learning and memory, brain-computer interfaces, human circadian rhythms, and other questions in systems neuroscience.

Brown's official citation, as read to an audience at the Jan. 3 ceremony was: "The National Medal of Science has been awarded to Emery Neal Brown for his revolutionary contributions to neuroscience and anesthesiology. Emery Brown's neuroscientific approach to understanding anesthesia's exact impact on the brain has been transformational for relieving patient suffering and has provided a new foundation for how we think about the very thing that makes us human, our consciousness."

Over the last 20 years, Brown has maintained a special focus on applying his signal processing research to studying the neurophysiology of how anesthesia acts in the brain to create altered states of arousal such as sedation and unconsciousness. His goal is to improve anesthesiology patient care and also to use his understanding of how anesthesia works to gain a deeper understanding of brain function and to improve brain health.

Brown's studies have led to numerous discoveries about how different anesthetic drugs, acting on their specific molecular targets, affect neural networks to produce signature patterns of oscillations, or brain waves, that can be readily monitored during surgery as a readout of the patient's state of unconsciousness. He has shown how the readings characteristically vary with the patient's age and medical condition.

The discoveries have direct implications for improving patient care. As Brown himself does in the operating room, anesthesiologists who monitor EEG readings during surgery can obtain a more direct and precise measurement of a patient's state of arousal and adjust anesthetic dosing accordingly. This practice can reduce the amount of drugs that are administered, thereby reducing post-operative side effects, while maintaining exactly the desired level of unconsciousness and control of nociception (the body's subconscious processing of pain).

In a study in 2023, Brown's lab demonstrated a closed-loop system that uses EEG signals to precisely adjust anesthetic dose to maintain target consciousness levels in animals. The lab is continuing to work on a system that can be used clinically.

At MIT and MGH Brown also recently founded the Brain Arousal State Control Innovation Center, a research program to unify studies of anesthesiology with studies in other areas of clinical neuroscience such as psychiatry, neurology and sleep medicine. Investigations underway via the effort include studies of how anesthesia can be used to treat depression, whether certain anesthetics can be modified to produce more physiologically sound sleep aids, and whether approaches used to accelerate emergence from anesthesia can be adapted to facilitate coma recovery.

Brown receives the National Medal of Science from Arati Prabhakar, former director of the White House Office of Science and Technology Policy. *Image from ceremony webcast.*

In addition to the National Medal of Science, Brown has also been honored with the Gruber Prize in Neuroscience, the Society for Neuroscience's Swartz Prize in Theoretical and Computational Neuroscience, and the Pierre Galletti Prize of the American Institute for Medical and Biological Engineering. Brown is also an elected member of the National Academies of Science, Engineering and Medicine.

A Neuron's Neighbors

The field is called neuroscience, but neurons alone can't operate your brain. Research is revealing how non-neural cells indispensably contribute to function, and why their roles can make them promising treatment targets in disease.

Microglia, stained red, fill the field of view in this image created as part of research published by the Tsai lab in 2023. *Image courtesy Mat Victor.*

Estimates vary, but if you took away all the cells in the human brain that aren't neurons, you'd be left with half the brain. So what are all those neural neighbors doing there?

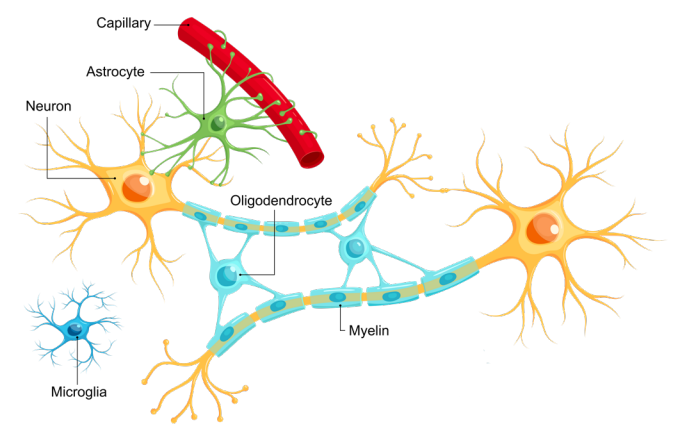
The vasculature circulates blood, of course, but for the rest of the cells the earliest guess in the 1800s was that they just held neurons together, earning them the collective name "glia," the Greek word for glue. Neuroscientists have come a long way from that starting point. Today some of the most exciting discoveries in The Picower Institute and the broader field are being made in various glial cells and the cells of the brain's vasculature, whose complexity and consequence are both gaining recognition. These studies are making two things increasingly clear: non-neuronal cells are intimately involved in enabling sophisticated functions including learning and memory, and they are critical co-conspirators in disease.

New technologies have enabled a recent acceleration of new findings, said Newton Professor Mriganka Sur. His leadership in the field earned him the invitation to be a panelist in a discussion of glia and brain function at last year's Society for Neuroscience Annual Meeting. In a companion paper of the event in the *Journal of Neuroscience*, the panelists wrote, "A broader role of glia in the central nervous system has begun to emerge, pointing out these cells' regulatory role in complex, higher-order brain functions and behaviors."

As Sur has published discoveries about how glial cells called astrocytes contribute to learning and memory, John and Dorothy Wilson Associate Professor Myriam Heiman has revealed mechanisms of how blood vessels become compromised in the neurodegenerative disorders Huntington's disease, frontotemporal dementia, and ALS. And Picower Professor Li-Huei Tsai has published numerous studies over the last decade identifying ways in which the catastrophe of Alzheimer's disease manifests not only in the vasculature, but also the three major glial cell types: microglia, astrocytes, and oligodendrocytes.

While it might seem like bleak news that diseases known for killing neurons also involve glia and the vasculature, Heiman says that actually presents an opportunity. For instance, many diseases show similar dysfunction in the cerebrovasculature that may be easier to target than problems lurking in neurons.

"Across all of these diseases, Alzheimer's, Parkinson's, ALS, and Huntington's, vascular dysfunction has been noted, so could there be a therapeutic that would be easily administered in the blood because it targets blood vessels?" Heiman said. "Such a therapeutic could be effective across disorders and thus is very appealing."



A cartoon of the major cell types and tissues in a neuron's neighborhood. Blood vessels such as capillaries are made of up to 11 different cell types.

Meet the neighbors

To better understand how glial cells and the vasculature both enable healthy brain function and participate in disease dysfunction, let's meet the major types.

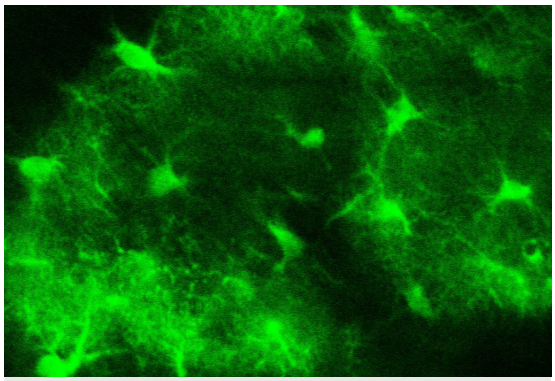
- **Astrocytes:** Sur cites two factoids that illustrate how crucial astrocytes are—They may be more numerous than neurons in the human brain and each one may directly contact as many as 100,000 "synapses"—the circuit connections neurons forge with each other. Astrocytes can manage levels of the neurotransmitter chemicals that facilitate communication across synapses. They also grasp blood vessels to help regulate blood flow and what gets exchanged with the blood, and they can provide neurons with chemical energy.

- **Microglia:** The brain's immune cells sense damage and disease and manage inflammation responses. They also gobble up waste and debris. During development, this job includes destroying synapses that are no longer necessary. Such "synaptic pruning" is an essential function for helping brain circuits mature.
- **Oligodendrocytes:** Neurons have that long extension (called an "axon") that reaches out to forge synapses with other cells. Oligodendrocytes specialize in wrapping axons in a fatty insulation called myelin that enables faster transmission of electrical signals. This vital process for making neural circuits work well is notably compromised in diseases such as Alzheimer's.
- **Vascular cells:** In 2022, Heiman co-led one of the first cellular "atlases" of the brain's vasculature. Her team cataloged 11 different subtypes of cells that make vessels function not as passive conduits of blood flow, but as dynamic and responsive tissues that stringently filter what molecular materials can move through the "blood-brain barrier."

Glia in learning and memory

In all, glia and the vasculature feed, maintain, protect, enhance, edit and help manage the functions of neurons. Several recent studies by Picower Institute researchers have revealed examples of how that enables brain function.

In 2023 Sur's lab studied the role of astrocytes in learning movement skills. They trained mice to push a lever after hearing a tone. Then in some mice the scientists disrupted their astrocytes' ability to soak up the neurotransmitter glutamate. This intervention caused the mice's motions to become erratic. In other mice they hyperactivated the astrocytes' ability to signal neurons with calcium. This not only undermined the smoothness of the mice's motions, but also impeded their ability to learn the task and their reaction time after hearing the cue tone. Under the microscope, in both cases, the researchers could see why behavior got worse. For lack of proper astrocyte function, the coordination among groups of neurons responsible for controlling the movements became compromised.



Astrocytes gleam green in this microscope image captured in the Sur Lab. *Image courtesy Jiho Park.*

A "preprint" (i.e. not yet peer reviewed) study from the lab posted late last year provided another example of how astrocyte regulation of neurotransmitters affects learning. Sur lab members used CRISPR gene editing to disrupt how astrocytes maintain the supply of the inhibitory neurotransmitter GABA in neurons of the visual cortex. Doing so impaired the ability of the mice to properly represent (in patterns of neural activity) what they were seeing.

In another study in 2022, Sur's lab investigated how surprise helps the brain learn. For instance, if a move you make on the chessboard has disastrous consequences, you really remember that. The surprise signal is carried by the neuromodulatory chemical norepinephrine. In the study, mice were trained in a motor task with an uncertain cue. The mice were therefore sometimes surprised to learn, via an irritating puff of air, that they responded wrong. Somehow after neurons in the prefrontal cortex (responsible for reasoning about what to do) received a transient norepinephrine signal, the mice adjusted their behavior to reflect the correction on the next go-round.

"But there is a conundrum, which is that the responses that we recorded after a false alarm, they kind of die out," Sur said. "There is not enough activity that we could see overtly persisting several seconds later until the next trial. Yet in the next trial, when the stimulus comes on, the neuronal responses are reorganized."

That's where astrocytes come in. In a new preprint, Sur's lab provides evidence that the norepinephrine acts not on neurons but on astrocytes. It triggers a sustained increase in their calcium levels. That promotes them to release chemicals that are usually associated with energy metabolism to signal neurons to alter their activity. "So that's our story, that the norepinephrine system actually acts via astrocytes, which then influence neurons," Sur said. "Therefore, astrocytes are not only not handmaidens, they are actually crucial for this very important behavior of reinforcement learning."

What about other cell types and mechanisms of memory? For years, Tsai has shown that in the rush to encode a memory, neurons will snap open both strands of their DNA to enable rapid expression of the genes responsible. In 2021, though, her lab found a surprising result when they dug deeper into how mice remember stressful events (like getting a little shock on the foot). When they looked at all cells –not just neurons—they found that many glia also employed double-strand breaks for rapid response, particularly to express genes associated with glucocorticoid hormones, which signal stress. This suggests that the glia played a key role in incorporating the mouse's sense of stress in encoding the memory.

As noted above, microglia help refine neural circuits. Oligodendrocytes do, too. In a study in 2020, William and Linda R. Young Professor Elly Nedivi and colleagues at Harvard showed that one of the ways that visual cortex neurons change to incorporate new visual experiences is by changing their myelin. The study showed how oligodendrocytes contribute to that dynamic.

Roles in disease

The inevitable corollary of glia having important roles in healthy brain function is that when things go wrong with them, brain function suffers, too. Tsai's lab has illustrated this via an investigation of why the

(Continued on next page)

APOE4 variation of the APOE gene constitutes the biggest genetic risk factor for Alzheimer's.

In five papers spanning 2018-2022, her lab engineered cultures of neurons, astrocytes, microglia, oligodendrocytes and the vasculature in which cells bearing APOE3 could be compared with cells whose only difference was harboring APOE4. In each case the researchers showed how the variation undermined their function. APOE4 microglia, for instance, left excess fat molecules, or lipids, in the environment around neurons. The lipids bound to potassium channels on the neurons, degrading their electrical activity. Astrocytes with APOE4 became overwhelmed by a buildup of cholesterol and triglycerides that affected many crucial functions. Oligodendrocytes with APOE4 failed to properly transport cholesterol to myelinate axons. And in a complex multicellular culture the lab developed of the vasculature and the blood brain barrier (BBB), they found that when pericyte cells have APOE4, they churn out too much APOE protein, which causes amyloid to clump together in blood vessels undermining BBB performance.

Tsai's lab has identified other problems with microglia and oligodendrocytes amid Alzheimer's disease. A landmark paper in 2015 analyzed gene expression patterns amid the disease, finding that many genes associated with Alzheimer's were most active in microglia and that they promoted an inflammatory response. A follow up study in 2023 showed that microglia take on as many as 12 distinct states in Alzheimer's. As the disease progresses, more microglia assume inflammatory states and fewer remain in states that promote normal brain function.

The latter study employed the emerging technology of single cell RNA sequencing (scRNAseq) to examine differences in gene expression, cell by cell, in Alzheimer's vs. comparable non-Alzheimer's brain samples. In 2019, when Tsai and colleagues used the method to map gene expression in the Alzheimer's brain, they produced new insights into why myelination suffers during the disease, especially among women.

Associate Professor Myriam Heiman and postdoc Francisco Garcia look over images of cerebrovasculature in the lab. *Image by Whit Wales*

In some good news, another scRNAseq study in 2024 showed that when astrocytes express genes associated with antioxidant activity and metabolism of the nutrient choline, that helps protect the brain from Alzheimer's.

When Heiman and colleagues used scRNAseq to create their atlas of cell types in the cerebrovasculature in 2022, it wasn't just for cellular cartography. Heiman studies neurodegenerative diseases including Huntington's disease and for years she and her lab had been curious about why patients exhibit cerebrovascular abnormalities long before they show other disease symptoms. So Heiman's team not only studied healthy brains, but also ones with Huntington's. In doing so, her team showed a deficit of expression in key BBB proteins and abnormal innate immune activation in the endothelial cells that line blood vessels.

In a separate study last year, Heiman's collaboration looked at brains with ALS and a kind of frontotemporal dementia. There, too, they found direct evidence that in these neurodegenerative disorders, expression of proteins needed to maintain the BBB is impaired.

And notably, in a recent preprint, Sur's lab combined many of the techniques described above to show that the BBB suffers in the neurodevelopmental disorder Rett syndrome because of a specific molecular defect in vascular endothelial cells.

Non-neuronal therapies

The identification of specific genetic or molecular problems in non-neuronal cells gives Heiman new hope for developing therapies. It means that scientists are not confined to intervening in neurons.

"Improving the health of the cerebrovasculature has promise for maintaining brain healthspan both in normal aging and in disease," Heiman said. "Are there ways that, for example, one could target gene therapy to the cerebrovasculature of the brain to promote its health? Could that help delay onset of disease symptoms across neurodegenerative diseases? Those are questions we and others in the field are actively pursuing right now."

In each of her APOE4 studies, Tsai has demonstrated specific compounds or drugs that can rescue the deficiencies she's detected in glial or vascular cells. She's testing the finding that choline, a common nutrient, aids APOE4 astrocytes in a clinical trial. Moreover, Tsai has developed several experimental drugs in her lab that, in different ways, target molecular mechanisms of microglial inflammation. For instance, last year she worked with MIT engineer Bob Langer to develop a lipid nanoparticle that delivers an RNA to microglia, suppressing the effects of an inflammation promoting protein she first identified in her 2015 study.

Also last year Tsai discovered that astrocytes and the vasculature play a key role in her research showing that stimulating the 40Hz gamma frequency rhythm in the brain can promote clearance of some of the amyloid protein that plagues Alzheimer's brains. The clearance mechanism is the brain's recently discovered "glymphatic" system, which is essentially a plumbing system of cerebrospinal fluid that runs alongside brain blood vessels. Tsai's lab found that 40Hz activity induced by light and sound at that frequency stimulates certain neurons to release a peptide. The peptide makes the blood vessels pulsate, increasing the flow of fluid in the glymphatic system. It also causes astrocytes to open up more channels to let those fluids flow across the brain tissue, washing away amyloid.

Meanwhile, Sur and Heiman, in collaboration with Picower Institute Associate Professor Gloria Choi, have begun a project in which they are asking whether manipulating glial cells and the vasculature could help deliver a molecule with potential to help treat autism.

No one disputes the centrality of neurons in neuroscience, but studies of glia and cerebrovascular cells show that being the center of the neighborhood depends a great deal on the support of the neighbors.

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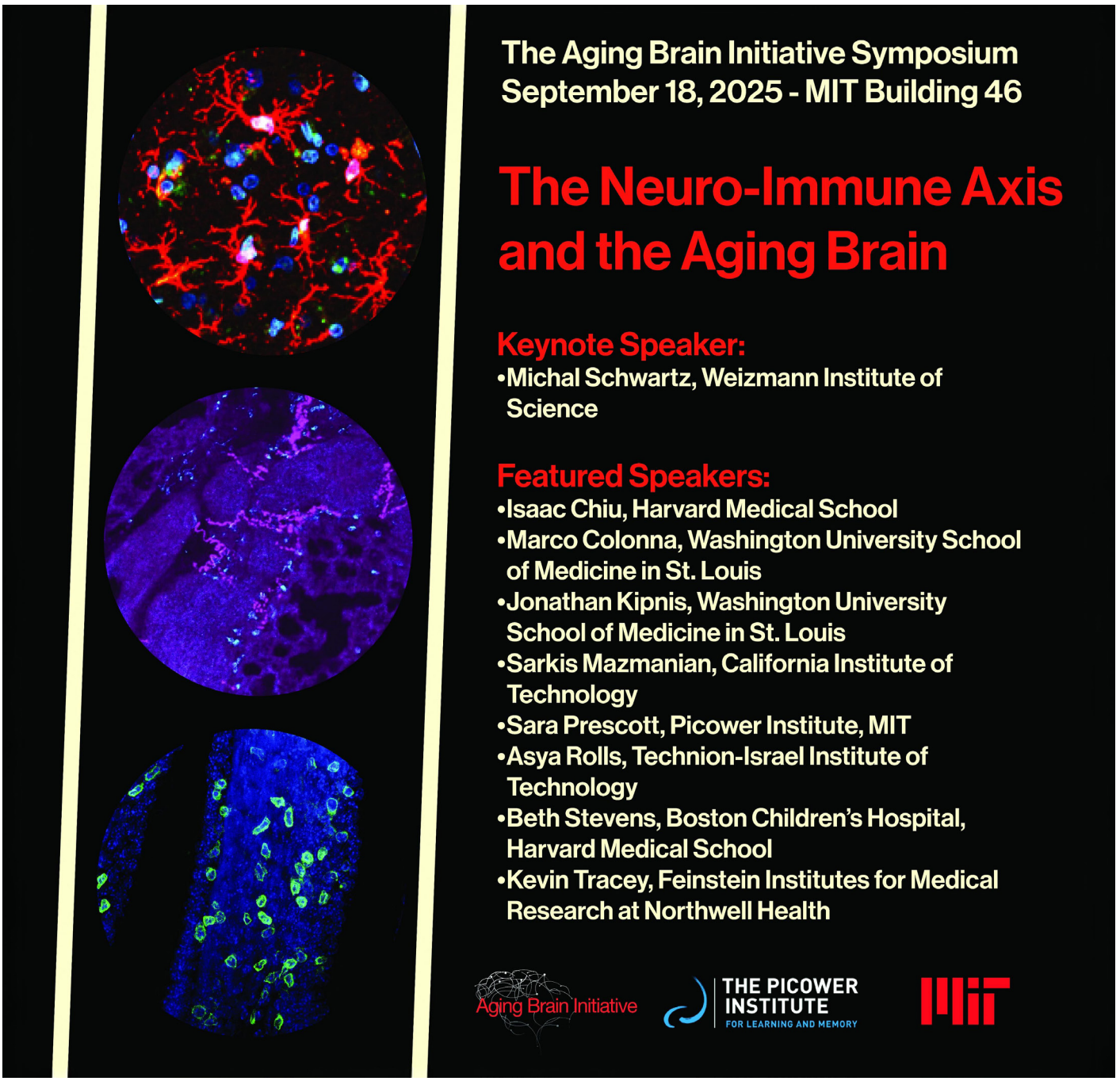

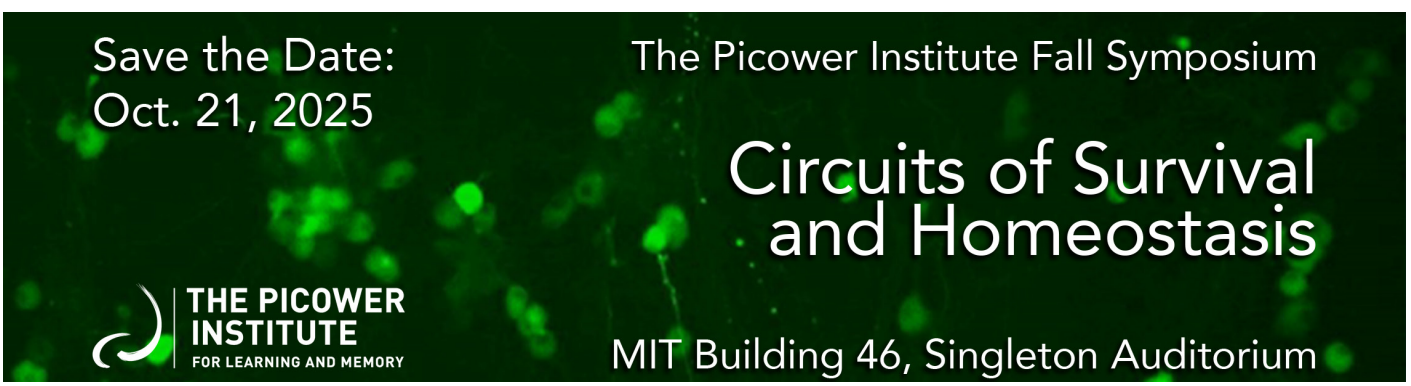

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BOTTOM ROW: Earl Miller, Picower Professor of Neuroscience, Department of Brain and Cognitive Sciences; **Elly Nedivi**, William R. (1964) & Linda R. Young Professor of Neuroscience, Departments of Brain and Cognitive Sciences and Biology; **Sara Prescott,** Assistant Professor of Biology; **Mriganka Sur**, Paul E. Newton Professor of Neuroscience, Director of The Simons Center for the Social Brain, Department of Brain and Cognitive Sciences; **Susumu Tonegawa**, Picower Professor of Biology and Neuroscience, Departments of Brain and Cognitive Sciences and Biology, HHMI Investigator, 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.