THE STORY BEHIND THE SCIENCE

An inside look at a key paper illustrates what it can take to publish new knowledge

Pg. 8

Neuroscience News

SUMMER 2020

THE PICOWER INSTITUTE FOR LEARNING AND MEMORY

DIRECTOR'S **MESSAGE**

Dear Friends,

In this edition we invite you behind the scenes for an inside look at the process of discovery. Through the example of a fascinating paper from the Littleton lab that made important findings about epilepsy and fundamental neuroscience, our goal was to demonstrate and elucidate what it can take to create and publish new knowledge (see page 8).

When we first thought about telling this story, which chronicles a postdoc's hard-earned research success, we had no idea that it would appear within a broader context where all of sudden the ability to pursue such labors of love in the lab seems so precious. But this spring the Covid-19 pandemic required all but a few of us to work remotely rather than at the bench.

Covid-19 brought great difficulty and uncertainty to billions of people around the world. But it has also provided many inspiring examples of creativity, resourcefulness, resilience, generosity and selfless service. On pages 6 and 7 we share stories of how Picower people have risen to the challenge – finding ways to be our most productive from home, launching studies of how the SARS-CoV-2 virus and the body's immune response may affect the brain, donating supplies to hospitals and even caring for Covid-19 patients on the front lines.

Throughout, we have strived to keep our minds on our mission of understanding how the brain works and how dysfunction can give rise to disease. We continue to publish new discoveries, for instance about how memory allows us to understand new experiences, how to reverse symptoms of Fragile X syndrome, and how reactivating an enzyme reduces DNA damage and cognitive decline in aging mice. We are undeterred from persisting with our research and pursuing discovery, whatever the circumstances and whatever it may take.

LI-HUEI TSAI, DIRECTOR
The Picower Institute for Learning and Memory

Neural 'event code' helps us interpret new situations

Though many sensory details may differ each time, our brain can identify segments of experience that are similar to ones you've had before -- like enjoying a three-course meal of a salad, an entree and a dessert. A new study finds the neurons involved. Image by Jose-Luis Olivares/MIT News Office.

Imagine you are having dinner at a new restaurant. You may try dishes you haven't had before, and your surroundings will be completely new to you. However, your brain knows that you have had similar experiences — perusing a menu, ordering appetizers, and splurging on dessert are all things that you have probably done when dining out.

MIT scientists have now identified populations of cells that encode each of these distinctive segments of an overall experience. These chunks of memory, stored in the hippocampus, are activated whenever a similar type of experience takes place, and are distinct from memories of a specific location.

The researchers believe that this kind of "event code," discovered in mice, may help the brain interpret novel situations and learn new information by using the same cells to represent similar experiences.

"When you encounter something new, there are some really new and notable stimuli, but you already know quite a bit about that particular experience, because it's a similar kind of experience to what you have already had before," says Susumu Tonegawa, a professor of biology and neuroscience at the RIKEN-MIT Laboratory of Neural Circuit Genetics at MIT's Picower Institute.

Tonegawa is the senior author of the study in *Nature Neuroscience.* Chen Sun, a former graduate student, is the lead author.

Certain "place cells" in the brain's hippocampus are specialized to store memories of specific locations. In the new study, the MIT team wanted to investigate whether the hippocampus also stores representations of more abstract elements of a memory. That is, instead of firing whenever you enter a particular restaurant, such cells might encode "dessert," no matter where you're eating it.

The researchers measured activity in neurons of the CA1 region of the mouse hippocampus as the mice repeatedly ran a four-lap maze. At the end of every fourth lap, the mice were given a reward. As expected, the researchers found place cells that lit up when the mice reached certain points along the track.

However, the researchers also found sets of cells that were active during one of the four laps, but not the others.

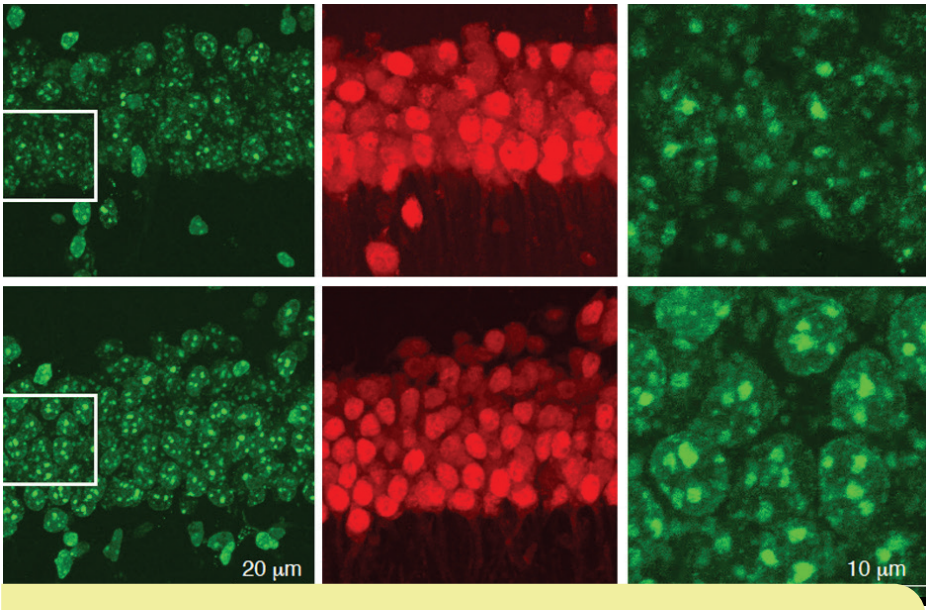
The researchers also trained mice to run a square maze on day 1 and then a circular maze on day 2, in which they also received a reward after every fourth lap. The place cells changed their activity, reflecting the new environment, but the same sets of lap-specific cells were activated during each of the four laps, regardless of the shape of the track. The lap-encoding cells' activity also remained consistent when laps were randomly shortened or lengthened.

The researchers theorize that the hippocampus contains "two mutually and independently manipulatable codes," Sun says. One encodes continuous changes in location, time, and sensory input, while the other organizes an overall experience into smaller chunks that fit into known categories such as appetizer and dessert.

> "We believe that both types of hippocampal codes are useful, and both are important."
> -SUSUMU TONEGAWA

"We believe that both types of hippocampal codes are useful, and both are important," Tonegawa says. "If we want to remember all the details of what happened in a specific experience, moment-to-moment changes that occurred, then the continuous monitoring is effective. But on the other hand, when we have a longer experience, if you put it into chunks, and remember the abstract order of the abstract chunks, that's more effective than monitoring this long process of continuous changes."

Study finds that aging neurons accumulate DNA damage



In this figure, neurons in the bottom row, which are missing the HDAC1 gene, show higher levels of DNA damage (green) than normal neurons.

MIT neuroscientists have discovered that an enzyme called HDAC1 is critical for repairing age-related damage to genes involved in memory and other cognitive functions. This enzyme is often diminished in both Alzheimer's patients and normally aging adults.

In a study of mice, the researchers showed that when HDAC1 is lost, a specific type of DNA damage builds up as the mice age. They also showed that they could reverse this damage and improve cognitive function with a drug that activates HDAC1.

The study suggests that restoring HDAC1 could have positive benefits for both Alzheimer's patients and people who suffer from age-related cognitive decline, the researchers say.

"It seems that HDAC1 is really an anti-aging molecule," says Li-Huei Tsai, director of MIT's Picower Institute, founding member of the Aging Brain Initiative and the senior author of the study. "I think this is a very broadly applicable basic biology finding, because nearly all of the human neurodegenerative diseases only happen during aging. I would speculate that activating HDAC1 is beneficial in many conditions."

Picower Institute research scientist Ping-Chieh Pao is the lead author of the study, in *Nature Communications*.

DNA REPAIR AND AGING

There are several members of the HDAC family of enzymes, and their primary function is to modify histones — proteins around which DNA is spooled. These modifications control gene expression by blocking genes in certain stretches of DNA from being copied into RNA.

In 2013, Tsai's lab published two papers that linked HDAC1 to DNA repair in neurons. In the current paper, the researchers explored what happens when HDAC1-mediated repair fails to occur. To do that, they engineered mice in which they could knock out HDAC1 specifically in neurons and another type of brain cells called astrocytes.

For the first several months of the mice's lives, there were no discernable differences in their DNA damage levels or behavior, compared to normal mice. However, as the mice aged, differences became more apparent. DNA damage began to accumulate in the HDAC1-deficient mice, and they also lost some of their ability to modulate synaptic plasticity — changes in the strength of the connections between neurons. The older mice lacking HDAC1 also showed impairments in tests of memory and spatial navigation.

The researchers found that HDAC1 loss led to a specific type of DNA damage called 8-oxo-guanine lesions, which are a signature of oxidative DNA damage. Studies of Alzheimer's patients have also shown high levels of this type of DNA damage, which is often caused by accumulation of harmful metabolic byproducts. The brain's ability to clear these byproducts often diminishes with age.

An enzyme called OGG1 is responsible for repairing this type of oxidative DNA damage, and the researchers found that HDAC1 is needed to activate OGG1. When HDAC1 is missing, OGG1 fails to turn on and DNA damage goes unrepaired. Many of the genes that the researchers found to be most susceptible to this type of damage encode ion channels, which are critical for the function of synapses.

TARGETING NEURODEGENERATION

Several years ago, Tsai and Stephen Haggarty of Harvard Medical School, who is also an author of the new study, screened libraries of small molecules in search of potential drug compounds that activate or inhibit members of the HDAC family. In the new paper, Tsai and Pao used one of these drugs, called exifone, to see if they could reverse the age-related DNA damage they saw in mice lacking HDAC1.

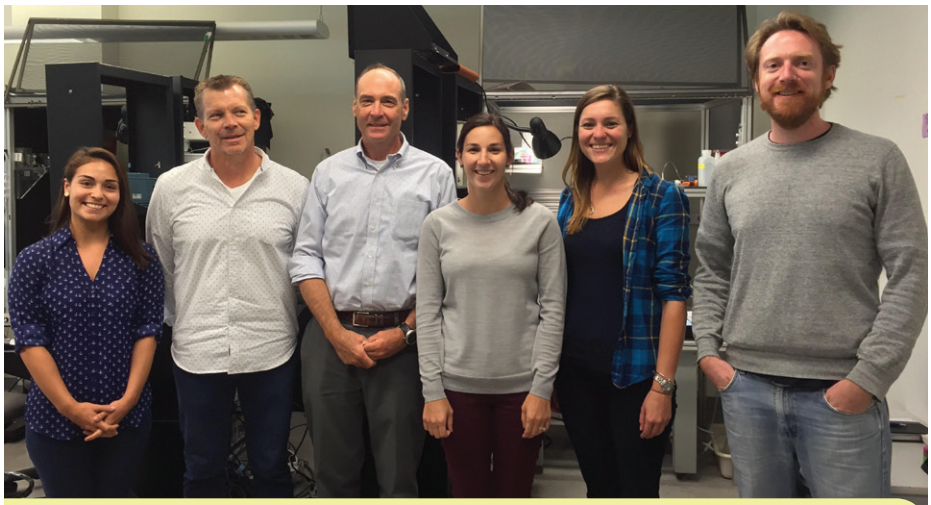
The researchers used exifone to treat two different mouse models of Alzheimer's, as well as healthy older mice. In all cases, they found that the drug reduced the levels of oxidative DNA damage in the brain and improved the mice's cognitive functions, including memory.

Exifone was approved in the 1980s in Europe to treat dementia but was later taken off the market because it caused liver damage in some patients. Tsai says she is optimistic that other, safer HDAC1-activating drugs could be worth pursuing as potential treatments for both age-related cognitive decline and Alzheimer's disease.

"This study really positions HDAC1 as a potential new drug target for age-related phenotypes, as well as neurodegeneration-associated pathology and phenotypes," she says.

Tsai's lab is now exploring whether DNA damage and HDAC1 also play a role in the formation of Tau tangles — misfolded proteins in the brain that are a signature of Alzheimer's and other neurodegenerative diseases.

Scientists find new **Fragile X** treatment strategy



Members of the research team (from right side to the left): Patrick McCamphill, Laura Stoppel, Becca Senter, Mark Bear, Arnold Heynen, and Amanda Coranado. Photo courtesy of FRAXA.

MIT scientists have identified a potential new strategy for treating Fragile X syndrome, the leading heritable cause of intellectual disability and autism.

In mice, the researchers showed that inhibiting an enzyme called GSK3 alpha reversed many of the behavioral and cellular features of Fragile X. The small-molecule compound has been licensed for further development and possible human clinical trials.

The compound may not have the same limitations of another class of Fragile X drugs that failed in prior human clinical trials, says study co-senior author Mark Bear, Picower Professor of Neuroscience in MIT's Picower Institute.

Florence Wagner, director of medicinal chemistry at the Broad Institute's Stanley Center for Psychiatric Research, is also a senior author of the study in *Science Translational Medicine*. The lead authors are MIT postdoc Patrick McCamphill, former MIT graduate student Laura Stoppel, and former MIT postdoc Rebecca Senter.

Fragile X affects about 1 in 2,500 to 4,000 boys and 1 in 7,000 to 8,000 girls, and is caused by a genetic mutation of a protein called FMRP. In addition to intellectual disability, symptoms include epilepsy, attention deficit and hyperactivity, hypersensitivity to noise and light, and autistic behaviors such as hand-flapping.

Bear's lab, which has been studying Fragile X for about two decades, has previously shown that protein synthesis at synapses, the junctions between neurons, is stimulated by a neurotransmitter receptor called metabotropic glutamate receptor 5 (mGluR5). FMRP normally regulates this protein synthesis. When FMRP is lost, mGluR5-stimulated protein synthesis becomes overactive.

In studies of mice, Bear and others have found that compounds that inhibit the mGluR5 receptor could reverse most of the symptoms of Fragile X. However, none of the mGluR5 inhibitors that have been tested in clinical trials have succeeded.

"We and many other labs have been chipping away at this and trying to understand the key molecular players. There's quite a large number now, and there have been different manipulations in the signaling pathway that can correct Fragile X phenotypes in animals," Bear says. "We like to refer to this as a target-rich environment. If at first you don't succeed therapeutically, you have many other shots on goal."

The GSK3 enzyme comes in two forms, alpha and beta, so Wagner, along with Edward Holson, former director of medicinal chemistry at the Stanley Center, and Edward Scolnick, chief scientist emeritus at the Stanley Center, set out to develop drugs that would inhibit either one or the other.

After a screen of more than 400,000 drug compounds, Wagner identified a handful that inhibited both forms of GSK3. By slightly altering their structures, she then came up with versions that could target selectively the alpha or beta forms.

Bear's lab tested the selective inhibitors in genetically engineered mice that lack the FMRP protein, and found that the inhibitor specific to GSK3 alpha eliminated one of the common Fragile X symptoms — seizures induced by loud tones. Following that, they found that the GSK3 alpha inhibitor also successfully reversed several other symptoms of Fragile X.

These symptoms include overproduction of protein as well as altered synaptic plasticity, impairment of some types of learning and memory, and hyperexcitability of some neurons.

"It checked off all the boxes that we would have expected from inhibiting mGluR5 or the signaling pathway downstream," Bear says. "It's really amazing that if you can correct the excess protein synthesis with a drug compound, a dozen other phenotypes are going to be corrected."

GSK3 is a kinase, which means that it controls other proteins by adding chemical groups called phosphates to them, but its exact role in Fragile X is not yet known. In this study, the researchers found that GSK3 is part of the same signaling pathway controlled by mGluR5, but GSK3 appears to act later in the pathway.

The initial findings in mice suggest that GSK3 alpha inhibitors do not have some of the complications that may have caused the mGluR5 inhibitors to fail in clinical trials, Bear says. For example, one side effect seen in mouse studies of mGluR5 inhibitors is the development of resistance to long-term treatment.

"We don't know whether the mGluR trials failed because of treatment resistance, but it's a viable hypothesis," Bear says. "What we do know is with the GSK3 alpha inhibitor, we do not see that in mice, to the extent that we've looked at it."

Protein like a '**volume dial**' for neural communication

Picower Institute neuroscientists have found that a protein acts like a volume dial for the release of neurotransmitters, the chemicals that neurons release across connections called synapses to stimulate muscles or communicate with other neurons in brain circuits.

Working in flies, the team determined that the protein Synaptotagmin 7 (SYT7), which is also found in humans and other mammals, constrains the number and availability of neurotransmitter-containing blobs, called vesicles, for release at the synapse. When the scientists reduced SYT7, they saw much more neurotransmitter release at synapses. When they increased the protein, neurotransmitter release dropped significantly.

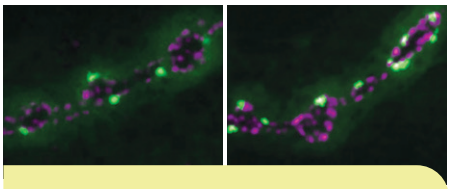
"You can think of this as almost like a radio's volume dial," said Troy Littleton, Menicon Professor of Neuroscience at MIT and senior author of the study in *eLife*. "If a neuron wants to send more signal out all it has to do is basically reduce the levels of SYT7 protein that it is making. It's a very elegant way for neurons to turn up or down the amount of output that they are giving."

The study's co-lead authors are Zhuo Guan, a research scientist, and former graduate student Mónica C. Quiñones-Frías. To study SYT7 the team focused its experiments on synapses in the junction between a neuron and muscle.

They changed the amount of SYT7 the neuron could produce by mutating and breeding flies in which the gene was completely eliminated, only one copy could be expressed, or in which the gene was overexpressed. For each of these fly lines they measured the surprising inverse relationship between SYT7 and synaptic transmission.

Also, using a technique the lab invented to visually flag neurotransmitter release every time it happens, they mapped how active individual synapses at the neuron-muscle junction were over time. In flies engineered to produce less SYT7 they saw more synapses with a high propensity for release.

To understand how SYT7 plays its restrictive role, they studied how neurons recycle vesicles to create a "readily releasable pool" (RRP). Neurons lacking SYT7 had more vesicles in the RRP. Neurons in which SYT7 was overexpressed substantially limited vesicles there.



Synapses glowing light green are engaged in neurotransmitter release. There are many more such synapses in the right panel where SYT7 was knocked out.

Under the microscope they were able to see that SYT7 resides in a network of tubes surrounding, but not within the active zones where neurotransmitters are released.

Understanding more about SYT7 could matter in several ways, Littleton said. Two years ago, researchers showed that the protein is reduced in mice harboring a genetic cause of Alzheimer's disease. And in February another paper showed that patients with bipolar disorder exhibited lower levels of the protein than people who do not have the disorder.

McKnight Scholars Award supports Flavell lab's gut-brain connection studies

A rapidly growing collection of studies suggests that the bacteria resident in the intestine directly influence the brain and contribute to psychiatric disorders, but scientists have yet to pinpoint how that happens. A new McKnight Scholars Award announced May 28 by the McKnight Foundation will help Steven Flavell, a member of The Picower Institute for Learning and Memory at MIT, investigate how the central nervous system senses bacteria in the gut.

"This work will help uncover fundamental signaling mechanisms that might allow the gut microbiome to impact human health and behavior," said Flavell, Lister Brothers Career Development Professor in MIT's Department of Brain and Cognitive Sciences. "This is a research area of great interest right now, but also an area where there is still a need for new mechanistic insights."

Working in the simple but instructive model of the *C. elegans* nematode worm, Flavell's lab recently discovered how an "enteric" neuron called NSM employs specific genes that are also present in humans to deploy bacterial sensors in the worm's alimentary canal. The worms eat bacteria, so NSM's sensors allow the worm to know when it is feeding and adjust its locomotion behavior accordingly (e.g. by slowing down to enjoy the meal).

With the award, Flavell plans to follow up on those findings in three ways. In one set of experiments the lab will seek to identify how the bacteria activate NSM's sensing mechanisms. In another, the scientists will investigate other enteric neurons whose functions aren't known, but whose anatomy suggests may be similarly tapped into the alimentary canal. Finally, the team will examine how the rest of the nervous system feeds back to these enteric sensory neurons. For example, when the worms are sated, the sensory neurons have diminished food responses compared to when they are hungry.

Flavell said the support of the award, $75,000 a year for three years, will be invaluable for pursuing these studies.

"I'm extremely grateful to the McKnight Foundation for supporting our research, and for supporting me as an early-career investigator," he said.

How might **COVID-19** and the immune response affect the brain?

Though the most immediately threatening symptoms of Covid-19 are respiratory, neuroscientists are intently studying the pandemic from the perspective of the central nervous system. To get ahead of the possible long-term neurological problems, multiple Picower Institute labs have been pursuing research to determine how the virus may affect the brain either directly or via the body's heightened immune response.

With deep expertise in neuro-immune interactions, as well as in the neural systems underlying the sense of smell, which is reported to be lost in some Covid-19 patients, institute member Gloria Choi is advancing several collaborative coronavirus studies.

"With these various suspected neurological symptoms, if we can determine the underlying mechanisms by which the immune system affects the nervous system upon the infection with SARS-CoV-2 or related viruses, then the next time the pandemic comes we can be prepared to intervene," said Choi, Samuel A. Goldblith Career Development Assistant Professor of Applied Biology.

Picower Professor Li-Huei Tsai is also planning studies of the neurological impact of Covid-19. Tsai's studies of Alzheimer's disease include investigation of the blood-brain barrier (BBB), which tightly gates what goes into and out of the brain through the circulatory system. Technologies her lab is developing could help assess whether and how coronavirus infection might overrun or evade that safeguard.

"It is critical to know how the coronavirus might affect the brain," Tsai said. "We are eager to bring our technology to bear on that question."

Choi is developing three lines of coronavirus research. Together with Picower colleagues Newton Professor Mriganka Sur and Associate Professor Kwanghun Chung, she hopes to tackle the question of anosmia, the loss of smell. Choi has studied the olfactory system in mice since her graduate and postdoctoral days. Moreover, a key finding of her neuroimmunology research is that because neurons express receptors for some of the signaling molecules, called cytokines, emitted by immune system cells, those interactions can directly affect neural development and activity. Working in mouse models, the team is asking whether such an impact, amid the immune system's heightened response to Covid-19, is occurring in the olfactory system.

Based on her studies with Harvard immunologist Jun Huh that have shown how maternal infection during pregnancy can lead to autism-like symptoms in their offspring, Choi and Huh are concerned about two other aspects of coronavirus infection. One builds on the finding that the risk depended strongly on the composition of the pregnant mother's gut microbiome. Given the wide range of outcomes seen among coronavirus patients, Choi and colleagues are looking into whether microbiome composition could play a role. In the longer term, Choi and Huh also plan to study whether Covid-19 infection among pregnant mothers presents an elevated risk of their offspring developing neurodevelopmental disorders.

How might SARS-CoV-2 access the brain? Tsai's lab may be able to find out using their advanced model of the BBB, which postdoc Joel Blanchard has cultured from human induced pluripotent stem cells. With MIT Institute Professor Robert Langer, the team is integrating the model with iPSC-derived cultures of neurons and other brain cells on a chip (called a "miBrain" chip) to provide a sophisticated and integrated testbed of brain cell and cerebral vascular interaction.

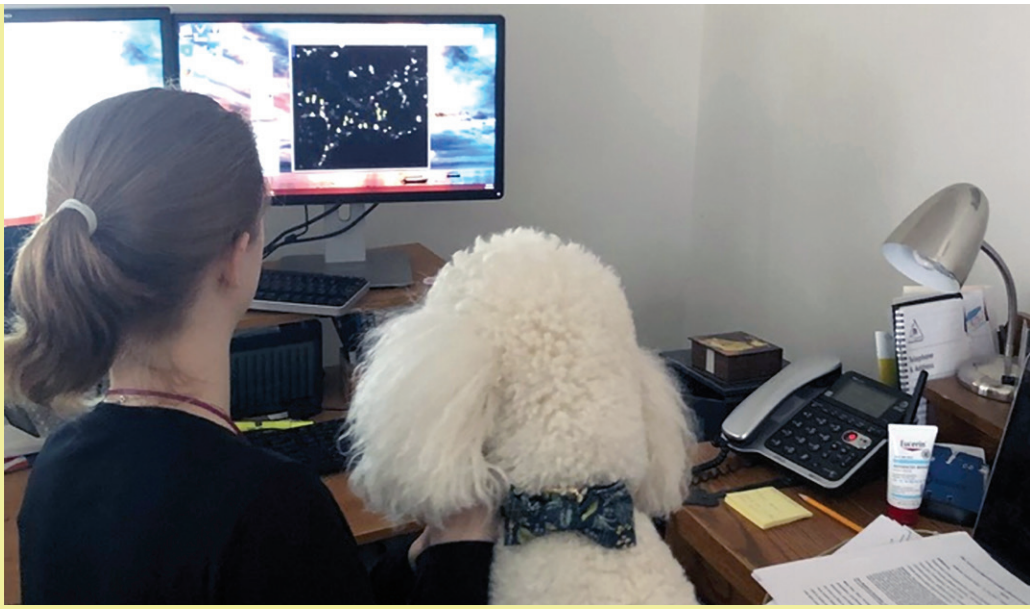
In one planned experiment Tsai's lab could derive miBrain chips from a variety of individuals to see whether the virus is able to permeate the BBB equally or differently in those personalized models. They can also test whether the body's immune system response (a so-called "cytokine storm") increases the BBB's permeability – by using blood serum from Covid-19 patients in the miBrainChip model.

Another way the virus might spread in the nervous system is from neuron to neuron via their connections. With cultures of thousands of neurons, the miBrain chip platform could help them determine whether that's the case and whether specific kinds of neurons are more susceptible.

Finally, there may be genetic differences that increase susceptibility to viral entry to the brain. Using technologies like CRISPR/Cas9, the team can engineer such candidate risk genes into the BBBs to test whether permeability varies. In their Alzheimer's disease research, for example, they study whether variations in a gene called ApoE causes different degrees of amyloid proteins plaque buildup in the BBB model.

Researching from home; donating urgently needed supplies

(Right) On March 20 MIT labs had to ramp staffing down to less than 20 percent of normal to ensure social distancing. But scores of Picower researchers have continued their neuroscience research from home, for instance by analyzing heaps of data, writing research manuscripts, gaining new training and planning out new experiments for the future. Here Senior Research Associate Kendyll Burnell, a member of Professor Elly Nedivi's lab, examines neural imaging data at home with her poodle Soma.



Picower physician-scientist provides front-line respiratory care

Dr. Diane Chan in protective gear at the respiratory illness clinic.

As both a neurologist who sees patients at Massachusetts General Hospital and a Picower Clinical Fellow conducting Alzheimer's disease clinical studies at MIT, Diane Chan already has two demanding jobs. But as eastern New England's need for Coronavirus care surged this spring, she volunteered to take on a third by joining the first wave of non-Internal Medicine doctors to help evaluate patients in MGH's respiratory illness clinics.

To aid patients she was trained to identify upper and lower respiratory infections, to evaluate whether someone has the virus, and to determine whether those patients need to go to the emergency department, or can go home. She also trained on how to carefully doff and don personal protective equipment. Chan's residency included a full year of internal medicine, so with this new training and the constant presence of her internal medicine colleagues, she said she felt well prepared and protected for this urgent new work.

"I'm grateful that I have skills to contribute during this time when the hospital needs our help and patients need our help," Chan said. "I'm really grateful to my internal medicine colleagues for training us to be able to do this in the respiratory clinic."

Of course she had already been helping people. Twice a week at MGH, but more recently via videoconferencing, she sees neurology patients with conditions such as dementia. Even as they grappled with the unexpected need to use a new technology for remote care, she says, many patients were still happy to remain in touch with their doctor.

At MIT her work in the lab of Picower Professor Li-Huei Tsai consists of running a program of studies testing whether light and sound stimulation at the 40 Hz frequency of gamma brain rhythms can improve memory and cognition in patients with mild Alzheimer's disease and reduce the condition's progression. Patients participate in the randomized, controlled and blinded study from home but because the pandemic has delayed in-person visits, key evaluations are being delayed and so the study has been extended. Although she acknowledges feeling stress, for instance about the possibility of bringing the virus home to her family, what rises above for Chan is again a feeling of gratitude.

"I feel grateful to the people in our study," she says. "They have been working with us for a very long time. People were looking forward to their appointments that have had to be canceled. We are at the time when people in the control group would be switched to active stimulation. I'm very grateful to the whole group, everybody, that they would delay this time point and continue doing the stimulation at home until we reopen."

Chan has seen respiratory patients from as far away as New Hampshire and Rhode Island. Most are referred by their primary care physician after a virtual meeting where they describe symptoms worrying enough to merit the in-person care that MGH still provides. At the clinics, doctors like Chan listen to their lungs, check blood oxygen levels, take chest X-rays, and administer the nasalpharyngeal swabs needed for virus testing.

The most critical decision is whether patients require hospitalization right away. Some did, such as a man in his 30s who had already tested positive for the virus but when he came to the clinic he struggled to speak in full sentences and his blood oxygen level was down.

But some patients who come in for care turn out to be more nervous than sick. The skill set of counseling patients, attending to their state of mind as well as their physical health, is a big part of neurology.

"I think that I'm using some of those reassurance skills for some of these patients," Chan says.

Even in normal times, a lot of patients need Chan's reassurance. Now she is extending her care to many more that she never would have expected to see.

Recognizing the urgent need hospitals would face in the coming months, members of several Picower Institute labs (including Bear, Chung, Heiman, Miller, Nedivi and Tsai) gathered supplies including gloves, masks, and RNA test kits to donate in mid March. Here Diane Chan, a scientist in the Tsai lab and a neurologist at Massachusetts General Hospital, examines many of the donations in the Picower lobby before they were loaded into a van sent by the hospital

THE STORY BEHIND THE SCIENCE:

HOW DISCOVERY DEVELOPS

An inside look at a key paper on **epilepsy and basic neuroscience** illustrates what it can take to publish new knowledge

Scientific discoveries can sometimes seem like products on a store shelf. Packaged neatly in the wrapping of a journal article or maybe a news story, there remain few hints of what they really took to produce – the struggle and surprises, the ingenuity and serendipity, the toil and triumph. Perhaps it's no wonder that many members of the public (7 in 10, by one National Science Foundation measure) feel bewildered about how scientists know what they know or do what they do.

To deconstruct and perhaps demystify discovery, let's unwrap the inside story of a paper published by the lab of Troy Littleton, Menicon Professor of Neuroscience in the Departments of Biology and Brain and Cognitive Sciences. The study reported important findings both about a possible mechanism of seizures in epilepsy, which affects 60 million people worldwide, and also the underappreciated relationship between neurons and the brain cells called glia that help them function. Through four years of work led by former postdoc Shirley Weiss, Littleton's team thoroughly unraveled the complex breakdown that makes fruit flies with a genetic mutation prone to seizures and showed multiple ways to intervene, including with human medicines. Published in April 2019 in *eLife*, a far-reaching "open access" journal that is free for all to read, the paper has since been viewed thousands of times.

Figuring out the puzzle of exactly how the mutation made glial cells fail to prevent seizures was a source of particular excitement for Weiss. Littleton adds that the discovery could open up new strategies for developing drugs to address epilepsy in humans, which the flies model well.

Shirley Weiss holds daughter Amit during a lab outing several years ago with Professor Troy Littleton (far right) and other lab members.

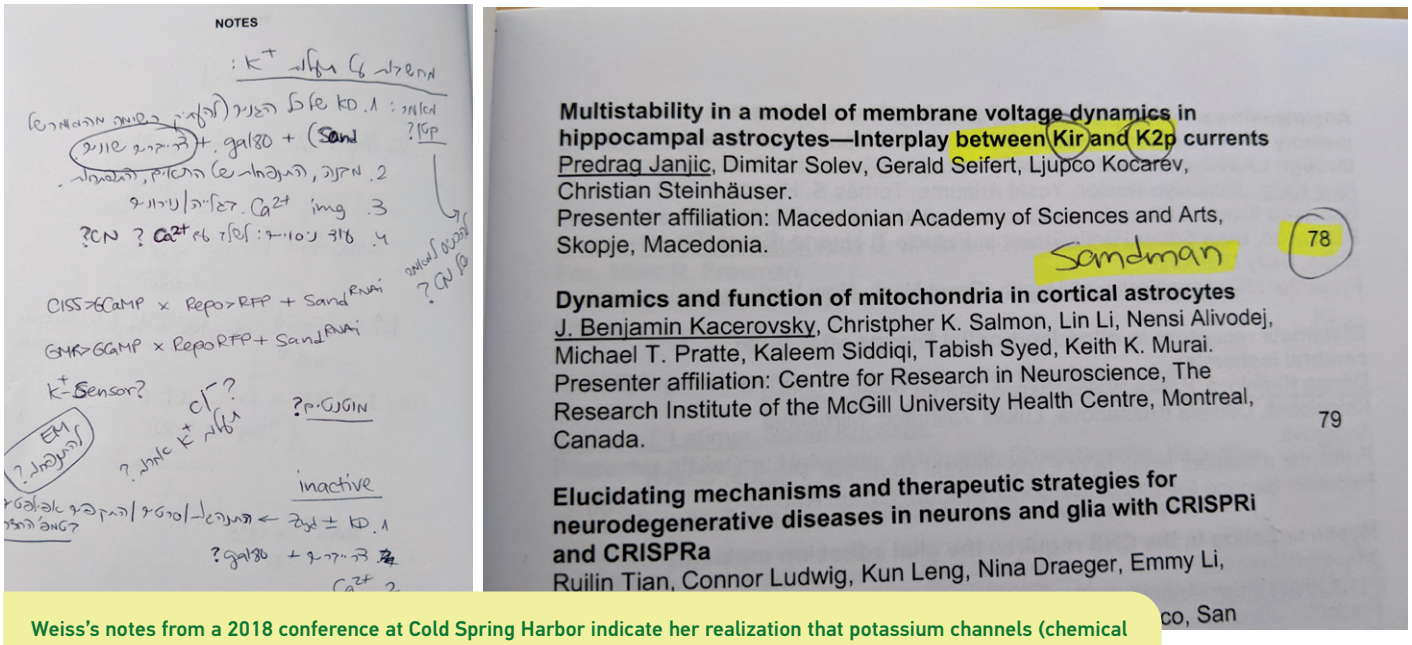
"One of the long-term motivations for the field in general, not just our lab, is there might be pharmacological access to glial cells that might have less side effects than would happen if you target neurons directly," he said.

TOO MUCH EXCITEMENT

First, a little biological background. Neurons are electrical. Their participation in the brain circuits that guide behavior, emotion, reasoning and memory depends on how they build up or dissipate electrical charge by taking in or ejecting ions of calcium, potassium and sodium. If they remain too electrically charged up because of an imbalance of these ions, they can become hyperactive and, in groups, produce seizures. In this study, it turned out that a certain kind of glial cells were responsible for regulating the balance of potassium ions around their neuronal neighbors to help govern their electrical charge and activity. The mutation, Weiss discovered, caused the glia to leave too much potassium outside the neurons, making it harder for the neurons to get rid of the potassium they had built up inside when they were electrically active. Without the ability to get their potassium out, the neurons stayed too excited, producing seizures.

The lab first discovered the mutation, which the Louisianan Littleton named "zydeco," in 2005 when a team led by Zhuo Guan used genetic screening techniques Littleton learned when he was a postdoc in the 1990s at the University

(Cover image) A green glow indicates elevated activity of the protein calcineurin in cortex glia cells.



Weiss's notes from a 2018 conference at Cold Spring Harbor indicate her realization that potassium channels (chemical symbol K) and specifically the protein "Sandman" might be what becomes too internalized in the Zydeco mutant.

of Wisconsin. The team's broader goal was to learn about how neurons communicate with each other, so they looked for flies with mutations that either shut the process down, leading to a readily observable symptom of paralysis, or amped it way up, leading to seizures. Zydeco fell into that second category, making fruit flies seize dramatically when stressed by heat or by getting jostled around.

"It was so striking, it was hard to ignore," Littleton said. "Whatever this gene was, it was doing something very important in the brain."

When Weiss joined the Littleton lab after earning her PhD at Hebrew University in Jerusalem in 2013, the lab had just published a new paper about zydeco. It was a long-awaited follow-up. Zydeco disrupted a gene on the fly's X chromosome which at the time was poorly understood. Led by former graduate student Jan Melom, the lab finally was able to clone the gene that was mutated in Zydeco and showed that it specifically affected "cortex glial" cells and that it caused them to retain too much calcium. But what remained completely unclear was how this made the neurons that those glia contact so susceptible to seizures.

Though Weiss had a research specialty in studying calcium in brain cells, at first she worked on a few other ideas. But because Melom had left the lab, Weiss soon picked up the zydeco baton. In so doing, she was taking on what would become an especially extensive effort involving scores of experiments and a vast array of techniques, some of which she would have to learn along the way.

NO HYPOTHESIS NEEDED OR HEEDED

Based on the 2013 findings, Littleton had formulated a working hypothesis about what might be going on to cause the seizures. He figured the excess of calcium in the cortex glia probably caused them to emit too much of some kind of signal to the neurons, in turn causing them to remain too active.

That turned out to be wrong, Littleton acknowledges with a smile.

"What Shirley did was to disprove my very strong impression of what was actually happening," Littleton said. "Sometimes that's very difficult to do. Once you have an idea of how you think the biology is working, that can reinforce the sorts of experiments you do and affects how you think about the project. It was very exciting in the end that Shirley was able to get past my pre-conceived notions and figure out what was really happening."

The team didn't fall into that trap because the experimental approach they chose didn't depend on what they thought. Weiss's key initial inquiries were based on a wide-open, free-ranging manipulation of the zydeco flies' genes. Her strategy was to "knock down" or interfere with the cell's ability to make use of 847 different genes covering a wide variety of potentially relevant glial cell functions. If knocking down any particular gene stopped the seizures, that would give them a huge clue about how the seizures happen. And whatever worked, if anything, would work regardless of anyone's guesses up front.

"The great thing about using forward genetics is you don't have to have a very strong hypothesis," Weiss said. "You can let the genetics lead the way. I tried to be hypothesis free and to be as unbiased as I could be."

The knockdown screening yielded about 50 genes where interference totally or partially alleviated the seizures. One in particular squared well with what Melom had observed about a specific cellular process (scientists call it a "pathway") that related to handling calcium.

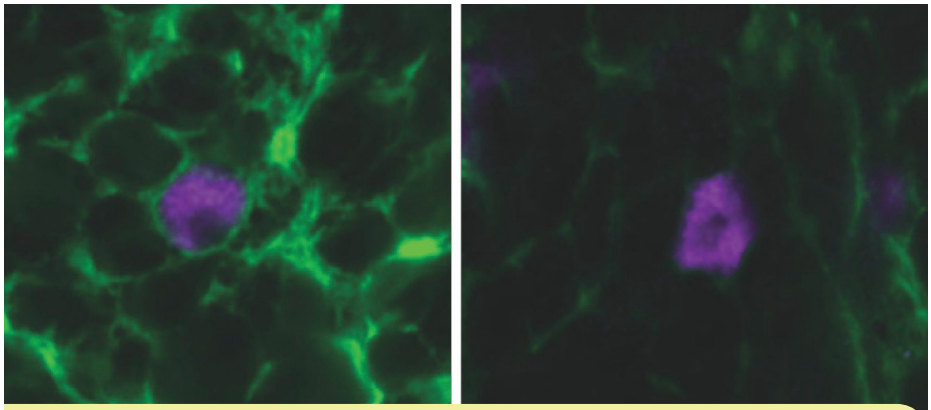
Around that time, though, life outside the lab intervened. In February 2015 Weiss and her husband Kfir Sharabi, also a postdoc, and their then four-and-a-half year old daughter, Amit, welcomed their second daughter, Ma'ayan, to the world. With two young kids and the rest of her family in Israel, Weiss came back from maternity leave and got back to investigating the most promising hits of the knockdown screen.

A CALCIUM CONUNDRUM

The particular hit related to calcium that caught Weiss's eye was a gene was called CanB2. Zydeco flies with that gene knocked down experienced no more seizure troubles at all. Moreover, she found that it was specifically helpful to knock it down in cortex glia and that knocking it down in healthy flies didn't do any discernable harm.

So what does CanB2 do? In general the gene, along with two others, make a protein called calcineurin. No one had ever characterized what calcineurin does in glia. If Weiss could become the first to figure that out, she could

CONTINUES ON PAGE 10



Side-by-side images show glowing indications of Sandman on cortex glia membranes at normal levels (left) and well below normal levels in the Zydeco mutant (right).

then explain why knocking it down could fix whatever problems the zydeco mutation causes.

By manipulating all three calcineurin genes, Weiss was able to confirm that calcineurin activity was indeed crucial for zydeco seizures. She engineered cortex glia so that a glowing green protein would start to be expressed when calcineurin was active. She could see the protein light up under the microscope. This told her there was a lot more calcineurin activity in zydeco mutant brains than in normal fly brains. Apparently, the excess calcium in the cortex glia correlated with increased calcineurin activity.

There are human medicines that ratchet back calcineurin activity. They are typically used to suppress the immune system after a transplant. Weiss wanted to see whether they could reduce seizures in the zydeco flies. When she fed them the drugs the seizures did subside, providing a clear demonstration that intervening in this glial pathway could hold promise for drug development.

Any such effort, to be truly well targeted, would require more than just an association between calcineurin and seizures. Weiss was determined to discover the mechanism that linked the two.

Figuring out what that mechanism was and how it led to seizures, would turn out to be the heart of the discovery and the most challenging phase of her four year endeavor.

A POTASSIUM EPIPHANY

Weiss needed to find out what process this excess calcineurin activity might be putting into overdrive. She went back to genetics. She performed a screen to knock down direct targets of calcineurin. It didn't appear to yield anything helpful. She did another screen of pathways where calcineurin was implicated. In that case, a process called "endocytosis" came up and sure enough, Weiss found that by inhibiting the process in the cortex glia she could again stop seizures in the zydeco flies.

Endocytosis is how cells ingest material from their surroundings, including regulating the content of their cell surface membrane proteins. The process can therefore affect the proteins they employ on the membrane to interact with the environment outside the cell. Excess endocytosis could mean that the way by which cortex glia interact with their environment is altered in zydeco, perhaps affecting the neurons they support. But how might that matter in this case?

Weiss struggled with this question for months. She received feedback and advice in meetings with Littleton and in lab meetings where the members discuss, challenge and refine ideas. The discussions were helpful, but it turns out that the key breakthrough came from Weiss attending a conference in Cold Spring Harbor, N.Y. in July, 2018.

Among Weiss's genetic screen results of calcineurin targets was a gene called "SAND" that makes a protein in flies called "sandman" (the human version is called TRESK). Sandman, when deployed to the cell membrane, forms a channel (picture a portal though the cell's surface) that allows a cell to bring in potassium ions from outside. At first this result didn't strike Weiss as all that notable, but at the conference, potassium channels kept coming up as a topic in talks. An idea started to percolate as she took notes. Then at the conference posters she started talking with a scientist who said that problems with potassium channels in glial cells have been linked to epilepsy. Potassium channels apparently merited another look.

"I already had the result," Weiss said. "I just didn't connect the two dots."

One of her screens indeed showed that knocking down SAND in healthy flies caused seizures just like the ones seen in zydeco mutants. Further genetic manipulations confirmed that SAND knockdown and zydeco affected the same pathway in cortex glia cells.

By September 2018, a new hypothesis was emerging: Elevated calcium in cortex glia triggered excess calcineurin activity, which spurred increased endocytosis that hindered sandman's intake of potassium. This came at a good time as Weiss was nearing the point where she had to start thinking of wrapping up her postdoctoral appointment at MIT. The hypothesis, and the evidence she'd built up, seemed enough to submit a paper to a journal.

Always mindful that a paper was the goal, Weiss had been writing as she went and developing the key figures. When she had a draft done, Littleton then set to polishing it and giving her feedback.

eLife wasn't the first journal they submitted the paper to, but the editors received it enthusiastically. All three of the scientists who reviewed the manuscript for *eLife*, however, said the same thing: If endocytosis was pulling sandman back from the membrane of the cortex glia, thereby disrupting its ability to take in potassium ions, they wanted to see it happening. Weiss and Littleton not only agreed with that critique, they had even anticipated it.

"You sort of know your own holes in the story," Littleton said. "This is what we were planning to do next anyway."

Since sending in the paper, Weiss had already produced those smoking gun images, showing that in zydeco cortex glia, sandman was much less abundant on the membranes than in the non-mutant flies. This cemented the argument, neatly wrapped up in the paper, that neurons become more susceptible to seizing when zydeco cortex glia, saddled with too much calcium and resulting calcineurin activity, overdo the endocytosis of sandman potassium channels, leaving too much potassium outside of neurons, causing increased excitability and the onset of seizures.

Since publication, the paper has garnered some mentions in the scientific press. It has also earned a new National Institutes of Health grant for Littleton's lab, where they are following in Melom's and Weiss' footsteps to study how calcium levels in glia affect the flux of membrane proteins, not just in disease, but as a matter of course in healthy cells. And for Weiss, the paper impressed funders in Israel, providing her with the money to support her new position where she continues studying glia, calcium and seizures.

It was a hard-earned success. Though their end product is knowledge, scientists spend the vast majority of their time with the unknown. Between the lines of most every paper are years of effort in which scientists persistently asked open questions with open minds so that the evidence could lead them to a discovery they could share with the world.

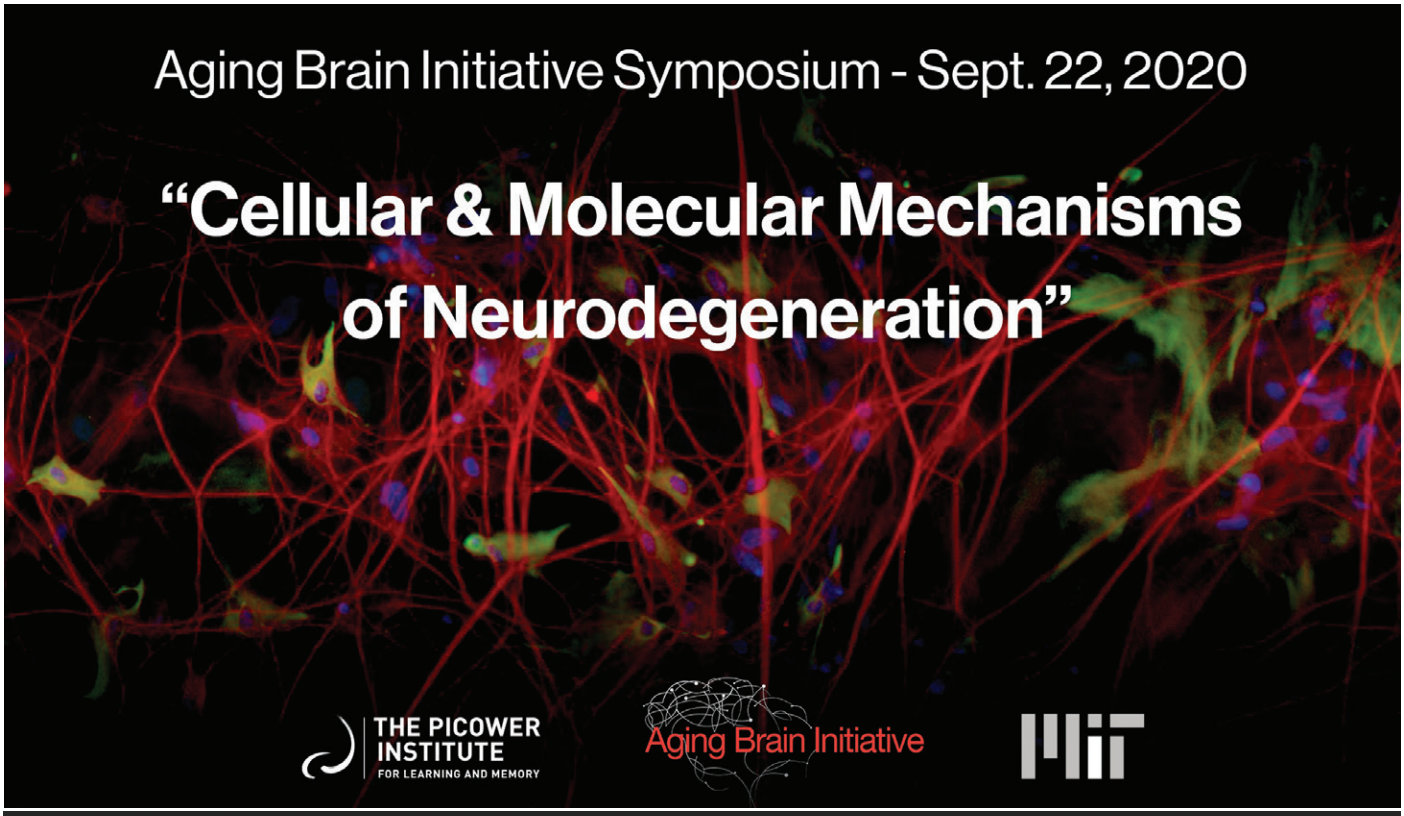
Upcoming **EVENTS**

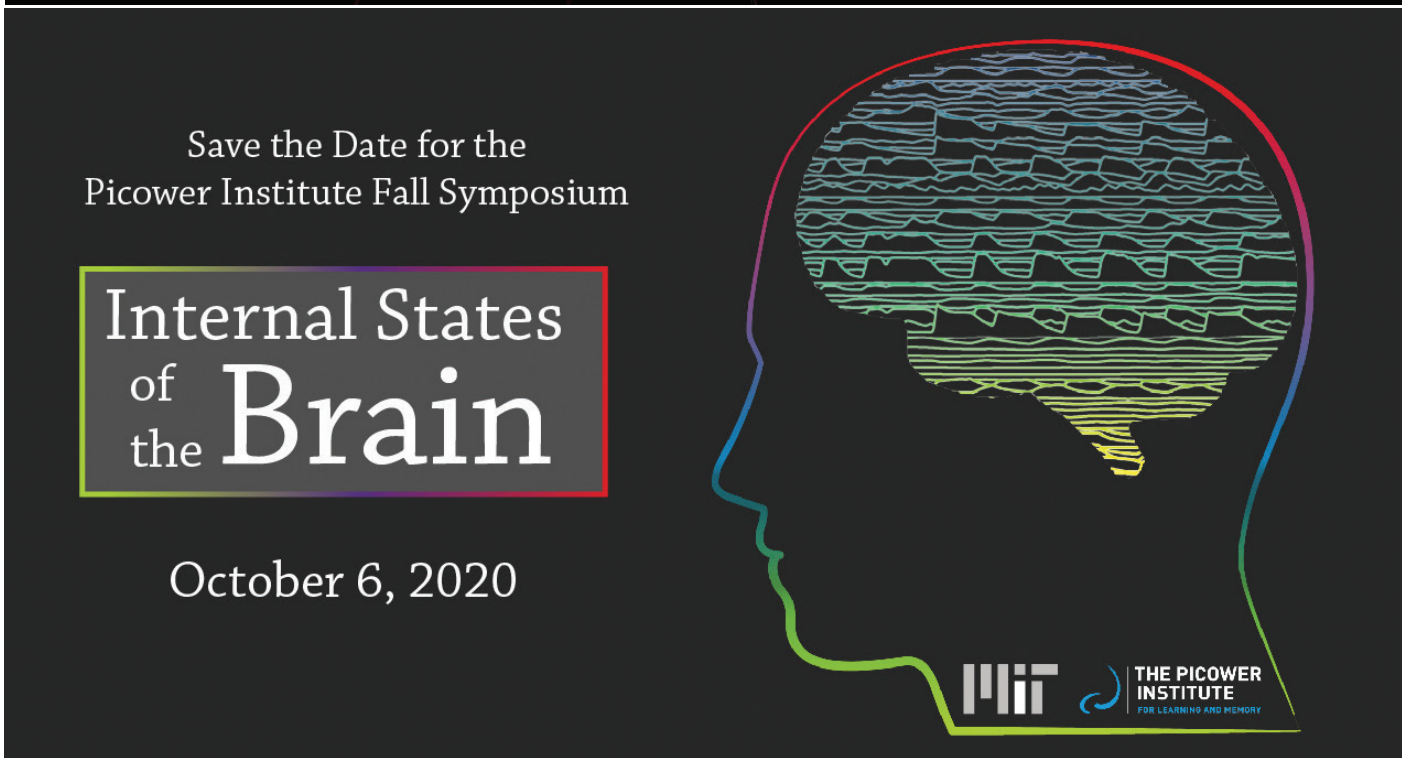
For the *latest* information on all our lectures, symposia and other events, please visit: **picower.mit.edu/events**

Upcoming Seminars and Colloquia

Due to the Covid-19 Pandemic our expectation is to hold these events via videoconference. Please check the web address listed above for the latest information about these events and the rest of our fall schedule.

Thank you for your understanding.





THE PICOWER INSTITUTE
FOR LEARNING AND MEMORY

Massachusetts Institute of Technology

Massachusetts Institute of Technology
77 Massachusetts Avenue Building 46 Room 1303
Cambridge, MA 02139-4307
picower.mit.edu

Neuroscience News Summer 2020

OUR VISION

The Picower Institute is a community of scientists dedicated to understanding the mechanisms that drive learning and memory and related functions such as cognition, emotion, perception, and consciousness. Institute researchers explore the brain at multiple scales, from genes and molecules, to cells and synapses, to circuits and systems, producing novel insights into how disruptions in these mechanisms can lead to developmental, psychiatric, or neurodegenerative disease.

SUPPORT THE PICOWER INSTITUTE

For more information on our research or how to make a gift to the Picower Institute for Learning and Memory, please contact:
Asha Bhakar, PhD, abhakar@mit.edu, Tel: 617-258-0759.

HOW TO SUBMIT & SUBSCRIBE

Subscriptions to Neuroscience News are available at no charge in print or PDF form. To subscribe or submit story ideas and comments, send your mailing or email address to: David Orenstein, davidjo@mit.edu, Tel: 617-324-2079.

EDITORIAL CONTRIBUTORS

David Orenstein and Anne Trafton

CONTACT THE PICOWER INSTITUTE

The Picower Institute for Learning and Memory
Massachusetts Institute of Technology,
77 Massachusetts Avenue, Building 46, Room 1303,
Cambridge, MA 02139-4307, Tel: 617-324-0305 picower.mit.edu

TOP ROW: **Mark F. Bear**, Picower Professor of Neuroscience, Department of Brain and Cognitive Sciences, Investigator, Howard Hughes Medical Institute (HHMI); **Emery Brown**, Edward Hood Taplin Professor of Computational Neuroscience and Health Sciences & Technology, The Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology; **Gloria Choi**, Samuel A. Goldblith Career Development Assistant Professor of Applied Biology, Department of Brain and Cognitive Sciences; **Kwanghun Chung**, Associate Professor, Departments of Chemical Engineering and Brain and Cognitive Sciences, Institute of Medical Engineering and Science core faculty; **Steven Flavell**, Lister Brothers Career Development Assistant Professor of Neuroscience, The Picower Institute for Learning and Memory, Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology; **Myriam Heiman**, Associate Professor of Neuroscience, Department of Brain and Cognitive Sciences, Broad Institute core member; **Troy Littleton**, Menicon Professor of Biology and Neuroscience, Departments of Biology and Brain and Cognitive Sciences.

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; **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; **Matthew Wilson**, Sherman Fairchild Professor in Neurobiology, Departments of Brain and Cognitive Sciences and Biology, Associate Director, The Picower Institute for Learning and Memory.

Choi photo by Justin Knight