'Cellf' Expression

Picower Institute scientists are using single cell genomics techniques to measure gene expression and produce unique insights into nervous system biology and diseases.
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Neuroscience News

WINTER 2023

THE PICOWER INSTITUTE
FOR LEARNING AND MEMORY

DIRECTOR'S **MESSAGE**

Dear Friends,

The nervous system, and our curiosity about how it works, have always been in place. What enables us to gain ever deeper insight is the new knowledge we've accumulated and new technologies that enable us to test our hypotheses. In this edition of *Neuroscience News* we feature multiple ways in which technology and discovery go together.

On page 7 we celebrate the arrival of a new member of our faculty, Linlin Fan. Linlin's graduate and postdoctoral work perfectly embody this partnership of technology and discovery. To make new discoveries about the nature of memory, she has been at the forefront of developing new ways of employing light to both experimentally manipulate and measure brain activity. We are excited that she is establishing her first independent lab with us at MIT.

In our cover story (p. 9) we examine how multiple Picower Institute investigators have seized the opportunities for discovery created by a different emerging technology: Single cell genomics including single-cell RNA sequencing. Because it provides a measure of how each cell in a sample makes use of its DNA (by transcribing genes into RNA), the technology provides us with a unique indication of each cell's identity and its function, both in healthy or disease conditions. You can read about how our labs have used this capability to study neurodegenerative disease and the amazing diversity of the nervous system. This approach was pivotal, for instance, in the Alzheimer's discoveries reported on this page and in the RNA editing findings of the Littleton lab on page 5.

More examples of new technologies follow in our news pages. Emery Brown and Earl Miller's labs teamed up to develop a new way to optimize the dosing of anesthesia drugs (p.3). They also collaborated with the lab of another MIT colleague to advance the utility of a new flexible fiber that can simultaneously manipulate and measure the brain (p.6).

It can be thrilling when new methods bring new opportunities to explore our scientific curiosity. And we are thrilled that by reading on, you are indulging your curiosity about those pursuits.

LI-HUEI TSAI, DIRECTOR

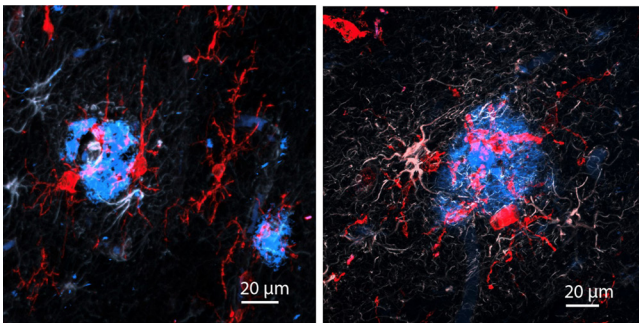
The Picower Institute for Learning and Memory

Decoding **Alzheimer's** disease

To discover new Alzheimer's treatment targets, MIT researchers have performed the broadest and most detailed analysis yet of the genomic, epigenomic, and transcriptomic changes that occur in the brains of Alzheimer's patients. The results appeared in four companion papers in *Cell*.

Using more than 2 million cells from 427 postmortem brain samples, the researchers analyzed how gene expression is disrupted as Alzheimer's progresses. They also tracked changes in cells' epigenomic modifications, which help to determine which genes are turned on or off in a particular cell. Together, these approaches offer the most detailed picture yet of the genetic and molecular underpinnings of Alzheimer's.

The studies were led by Picower Professor Li-Huei Tsai, who directs The Picower Institute and MIT's Aging Brain Initiative, and Manolis Kellis, a professor of computer science. Their findings suggest that an interplay of genetic and epigenetic changes feed on each other to drive the pathological manifestations of the disease.



Researchers tracked changes in microglia early (left) and late (right) in Alzheimer's disease. Microglia (red) surround an amyloid plaque (blue). The microglia appeared more activated, with larger cell bodies, on the right. The amyloid plaque is more diffused.

"It's a multifactorial process," Tsai said. "These papers together use different approaches that point to a converging picture of Alzheimer's where the affected neurons have defects in their 3D genome, and that is causal to a lot of the disease phenotypes we see."

Many efforts to develop drugs for Alzheimer's disease have focused on the amyloid plaques that develop in patients' brains. In their new set of studies, the MIT team sought to uncover other possible approaches by analyzing the molecular drivers of the disease, the cell types that are the most vulnerable, and the underlying biological pathways that drive neurodegeneration.

Using Single-cell RNA sequencing, the team analyzed how 54 types of brain cells each expressed their genes and identified cellular functions that were most affected in Alzheimer's patients. Among the most prominent, they found impairments gene expression involved in mitochondrial function; synaptic signaling, which governs how well cells communicate; and maintenance of the structural integrity of the genome. They also found that genetic pathways related to lipid metabolism were highly disrupted.

The analysis also revealed that cognitively resilient people had larger populations of two subsets of inhibitory neurons in the prefrontal cortex.

Another paper revealed that every type of cell in the brain undergoes a phenomenon known as "epigenomic erosion" as Alzheimer's progresses, meaning that the cells' normal pattern of accessible genomic sites is lost, which contributes to loss of cell identity.

In a third paper, the researchers used RNA sequencing to classify microglia into 12 different states, based on hundreds of genes that are expressed at different levels during each state. They showed that as Alzheimer's progresses, more microglia enter inflammatory states. The Tsai lab is now exploring ways to activate implicated transcription factors, in hopes of treating Alzheimer's by programming inflammation-inducing microglia to switch back to a homeostatic state.

In the fourth paper the researchers examined how DNA damage contributes to the development of Alzheimer's. As more DNA damage accumulates in neurons, it becomes more difficult for them to repair the damage, leading to DNA rearrangements and 3D folding defects. Repair mistakes also lead to a phenomenon known as gene fusion, which occurs when rearrangements take place between genes, leading to dysregulation of genes. Alongside defects in genome folding, these changes appear to predominantly impact genes related to synaptic activity, likely contributing to the cognitive decline seen in Alzheimer's disease, as afflicted neurons struggled to transmit information in the brain.

Anesthesia technology precisely controls **unconsciousness**

Researchers at The Picower Institute have invented a closed-loop anesthesia delivery system that accurately controls unconsciousness by automating doses of the anesthetic drug propofol every 20 seconds. By optimizing drug dose they hope to reduce post-operative side effects in patients.

The scientists describe the new system and its performance in animal testing in the journal *PNAS Nexus*.

"One of the ways to improve anesthesia care is to give just the right amount of drug that's needed," said corresponding author Emery N. Brown, Edward Hood Taplin Professor of Medical Engineering and Computational Neuroscience at MIT and an anesthesiologist at Massachusetts General Hospital. "This opens up the opportunity to do that in a really controlled way."

In the operating room, Brown monitors the brain state of his patients using electroencephalograms (EEGs). He frequently adjusts dosing based on that feedback, which can cut the amount of drug he uses by as much as half compared to if he just picks a constant infusion rate and sticks with that. Nevertheless, the practice of maintaining dose rather than consciousness level is common because most anesthesiologists are not trained to track brain states and often don't have time in the operating room to precisely manage dosing.

The new system is not the first closed-loop anesthesia delivery (CLAD) system, Brown said, but it advances the field in critical ways. Some prior systems merely automate a single, stable infusion rate based on general

patient characteristics like height, weight and age but gather no feedback about the actual effect on unconsciousness. Others use a proprietary control system where "black box" markers of unconsciousness vary within a wide range.

The new CLAD system developed by Brown and his team at the MIT and MGH Brain Arousal State Control Innovation Center, enables very precise management of unconsciousness by making a customized estimate of how doses will affect the subject and by measuring unconsciousness based on brain state. The system uses those measures as feedback to constantly adjust the drug dose.

In the paper, the team demonstrates that the system enabled more than 18 hours of fine-grained consciousness control over the course of nine anesthesia sessions with two animal subjects. Picower Professor Earl K. Miller is the paper's co-senior author.

The way **anesthesia** blocks sensation helps explain consciousness

How does general anesthesia disrupt consciousness, including sensory perception, and what might that say about the nature of consciousness? A new study led by Picower Institute researchers provides evidence in animals that consciousness depends on properly synchronized communication across the brain's cortex and that the anesthetic drug propofol cancels sensory processing by cutting it off.

In the *Journal of Cognitive Neuroscience*, the researchers reported clear evidence that in anesthetized animals, sounds and tactile sensations still produced neural activity in an area of the cortex that receives incoming sensory information. But just as clearly, measurements of neural spiking and broader oscillatory activity showed that those signals failed to propagate to three other cortical regions with higher-level processing and cognitive responsibilities, as seen during normal wakefulness.

"What this study shows is that the cortex isn't getting on the same page," said Picower Professor Earl K. Miller. "Information is making it to the cortex. It's being registered in primary sensory areas. It's just not reaching the rest of the cortex. Because of the anesthesia, it only makes it part of the way through."

The significance of that, said co-senior author Emery N. Brown, Edward Hood Taplin Professor of Medical Engineering and Computational Neuroscience, is that "the study suggests that consciousness requires coordination of activities among cortical regions. Simply activating one or more of these regions is not sufficient."

Study lead author John Tauber, who recently earned his PhD at MIT in Brown's lab, said the study could aid efforts to improve anesthesiology care. Brown is an anesthesiologist at Massachusetts General Hospital.

"We hope our paper further highlights the importance of actively monitoring what is happening in the brain during anesthesia," Tauber said. "Future studies in this direction will help us develop clear indicators of whether a patient is still processing sensory information. This would allow anesthesiologists to adjust drug dosage and prevent intraoperative awareness from occurring."

Study advances understanding of visual recognition memory

Because figuring out what is new and what is familiar in what we see is such a critically important ability for prioritizing our attention, neuroscientists have spent decades trying to figure out how our brains are typically so good at it. Along the way they've made key observations that seem outright contradictory, but a new study shows that the mystifying measures are really two sides of the same coin, paving the way for a long-sought understanding of "visual recognition memory" (VRM).

VRM is the ability to quickly recognize the familiar things in scenes, which can then be de-prioritized so that we can focus on the new things that might be more important in a given moment.

"Yet we do not yet have a clear picture of how this foundational form of learning is implemented within the mammalian brain," wrote Picower Professor Mark Bear and fellow authors of the new study in the *Journal of Neuroscience*.

As far back as 1991 researchers found that when animals viewed something familiar, neurons in cortex, or outer layer of their brain, would be less activated than if they saw something new. But in 2003, Bear's lab observed the opposite: Mice would actually show a sharp jump in neural activity in the primary visual region of the cortex when a familiar stimulus was flashed in front of the animals. They called these jumps "visually evoked potentials," or VEPs.

Now the Bear lab's rigorous and precise new recordings of neural electrical activity in the visual cortex have revealed a potential resolution to the contradiction between the VEPs and other measures of overall decreased activity.

Visual Recognition Memory helps you recall and ignore what's familiar so that you can focus on what's new. When you enter your home office some evening, VRM will ensure that you'll focus on the burglar, not your book shelves or your desk lamp

How so? The new data show that VEPs are very pronounced but transient spikes of neural electrical activity that occur amid a broader, overall lull of activity. Previous studies have reflected only the overall decrease because they have not had the temporal resolution to detect the brief spike. Bear's team, meanwhile, has seen the VEPs for years but didn't necessarily focus on the surrounding lull.

The new evidence suggests that what's happening is that the VEP is a sign of the activity of the brain quickly recognizing a familiar stimulus and then triggering a subsequent inhibition of further activity related to it.

Study deciphers surprising mouse learning style

Neuroscience discoveries ranging from the nature of memory to treatments for disease have depended on reading the minds of mice, so researchers need to truly understand what the rodents' behavior is telling them during experiments. In a new study that examines learning from reward, MIT researchers decoded some initially mystifying behavior, yielding new ideas about how mice think and a mathematical tool to aid future research.

The task the mice were supposed to master is simple: Turn a wheel left or right to get a reward and then recognize when the reward direction switches. When neurotypical people play such "reversal learning" games they quickly infer the optimal approach: stick with the direction that works until it doesn't and then switch right away. Notably, people with schizophrenia struggle with the task. In the new study in *PLOS Computational Biology*, mice surprised scientists by showing that while they were capable of learning the "win-stay, lose-shift" strategy, they nonetheless refused to fully adopt it.

"It is not that mice cannot form an inference-based model of this environment—they can," said corresponding author Mriganka Sur, Newton Professor in The Picower Institute. "The surprising thing is that they don't persist with it. Even in a single block of the game where you know the reward is 100 percent on one side, every so often they will try the other side."

One possibility is that mice don't commit to the human approach because they don't trust that their circumstances will remain stable or predictable, said graduate student and lead author Nhat Le. Instead, they might deviate from the optimal regime to test whether the rules have changed. Natural settings, after all, are rarely stable or predictable.

A key advance in the study was developing a mathematical model capable of identifying and tracking the more mixed tactics of the mice.

Now that the researchers have decoded the peculiar approach mice take to reversal learning, they are planning to look more deeply into the brain to understand which brain regions and circuits are involved.

By examining reversal learning circuits in detail, Sur said, it's possible the team will gain insights that could help explain why people with schizophrenia show diminished performance on reversal learning tasks. Sur added that some people with autism spectrum disorders also persist with newly unrewarded behaviors longer than neurotypical people, so his lab will also have that phenomenon in mind as they investigate.

Mice playing a learning game often continued to explore different options (symbolized by the ?) even after learning the optimal strategy (light bulb)

How a **single neuron** can coordinate many aspects of behavior

A new Picower Institute study that focuses on a single cell in one of nature's simplest nervous systems provides an in-depth illustration of how individual neurons can use multiple means to drive complex behaviors.

In the *C. elegans* worm, the neuron HSN releases several chemicals and makes multiple connections along its length to not only control the animal's instantaneous egg laying and locomotion, but also to then slow the worm down for several minutes after the eggs are laid. To control that latter phase of the behavior, HSN transfers the neurotransmitter serotonin to a fellow neuron, which re-releases it to influence behavior minutes later.

The neuron HSN in the body of a *C. elegans* worm

"Our results reveal how a single neuron can influence a broad suite of behaviors over multiple timescales and show that neurons can 'borrow' serotonin from one another to control behavior," wrote Associate Professor Steven Flavell, postdoc and lead author Yung-Chi Huang, and colleagues in *Current Biology*.

HSN's cell body is in the midbody of the animal. To drive egg-laying, it forms synapses with the egg-laying circuit in the midbody. Then its axon continues to the head where it connects with other neurons to coordinate increased locomotion with egg-laying. It drives locomotion by releasing two neuropeptides, called FLP-2 and FLP-28.

HSN, meanwhile, slows the worm by supplying another neuron with serotonin. Flavell's team had previously shown that the neuron NSM uses serotonin when a worm is feeding to inhibit motor circuits and slow the worm down for the meal. In this study, the team showed that NSM uses the serotonin transporter SERT (called MOD-5 in *C. elegans*) to take up HSN's serotonin and re-release it.

The finding that neurons can borrow serotonin from other neurons to control behavior reveals a novel feature of serotonin signaling that could have important medical implications, Flavell said. The molecule that takes up the serotonin, SERT/MOD-5, is the target of serotonin-specific reuptake inhibitor drugs like Prozac. This study raises the possibility that SSRIs may influence how neurons share serotonin with one another, which could be relevant for their mode of action in treating psychiatric disorders.

Neurons mix multiple **RNA edits** of key synapse proteins

Neurons communicate with fellow neurons, muscles or other cells by releasing neurotransmitter chemicals at "synapse" junctions, ultimately producing functions ranging from emotions to motions. But even neurons of the exact same type can vary in their conversational style. A new study in *Cell Reports* by Picower Institute neurobiologists highlights a molecular mechanism that might help account for the nuanced diversity of neural discourse.

The scientists made their findings in neurons that control muscles in *Drosophila* fruit flies using the neurotransmitter glutamate. In the lab of Menicon Professor Troy Littleton, which uses these models to study neural communication, researchers frequently see that individual neurons vary in their release patterns. Some "talk" more than others.

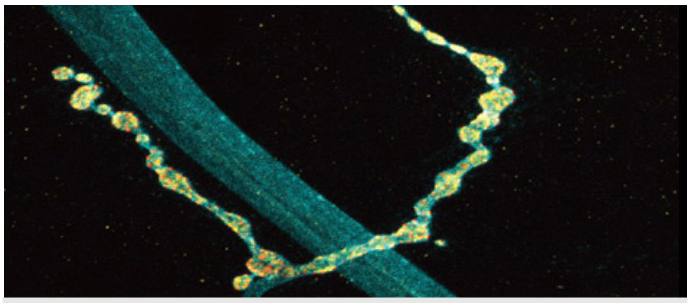
Littleton's lab has shown that a protein called Complexin has the job of restraining spontaneous glutamate chatter. It clamps down on fusion of glutamate-filled vesicles at the synaptic membrane to preserve a supply of the neurotransmitter for when the neuron needs it.

In the new study, led by former graduate student Elizabeth Brija, the lab discovered that RNA editing of a form of Complexin has a significant impact on how well the protein prevents glutamate release. Moreover, they found that this varies widely among individual neurons because they can stochastically mix and match up to eight different editions of the protein. Some edits were much more common than others on average, but 96 percent of the 200 neurons the team examined had at least some editing.

Experiments to test some of the consequences of this editing showed that different editiions can dramatically affect the level of electrical current measurable at different synapses. That varying level of activity can also affect the growth of the synapses the neurons make with muscle. RNA editing of the protein might therefore endow each neuron with fine degrees of communication control.

"What this offers the nervous system is that you can take the same transcriptome and by alternatively editing various RNA transcripts, these neurons will behave differently," Littleton said.

Moreover, Littleton and Brija's team found that other key proteins involved in synaptic glutamate release, such as Synapsin and Syx1A, are also sometimes edited at quite different levels among the same population of neurons. This suggests that other aspects of synaptic communication might also be tunable.



Yellow staining highlights a particular edition of the protein Complexin. Other editions resulted in different physical distributions within neuronal axons

Flexible fibers to monitor and manipulate neural activity

A team of researchers at MIT's McGovern and Picower Institutes has advanced the clinical potential of a thin, flexible fiber designed to simultaneously monitor and manipulate neural activity at targeted sites in the brain. The collaboration's improvements on an earlier model of the multifunctional fiber enabled exploration of dynamic changes to neural signaling as large animals engaged in a working memory task. The results appeared in *Science Advances*.

The new version, developed by Indie Garwood, who recently received her PhD in the Harvard-MIT Program in Health Sciences and Technology, includes four microelectrodes for detecting neural activity and two microfluidic channels through which drugs can be delivered. This means scientists can deliver a drug that alters neural signaling within a particular part of the brain, then monitor the consequences for local brain activity. This technology was a collaborative effort between the groups of McGovern Institute Investigator Polina Anikeeva, who invented the earlier version, and Picower Institute Investigators Emery N. Brown and Earl Miller, who jointly supervised Garwood to develop a multifunctional neurotechnology for larger and translational animal models. This is necessary to investigate the neural circuits that underlie high-level cognitive functions.

Once the new device was developed, Garwood and colleagues in the Miller and Brown labs put it to work. They used the tool to study changes in neural activity as an animal completed a task requiring working memory. The fluid channels in the fiber were used to deliver small amounts of GABA, a neurotransmitter that dampens neuronal activity, to the animal's premotor cortex, a part of the brain that helps plan movement. At the same time, the device recorded electrical activity from individual neurons, as well as broader patterns of activity in this part of the brain. By monitoring these signals over time, the team learned how neural circuits adapted to the local inhibition they had applied.

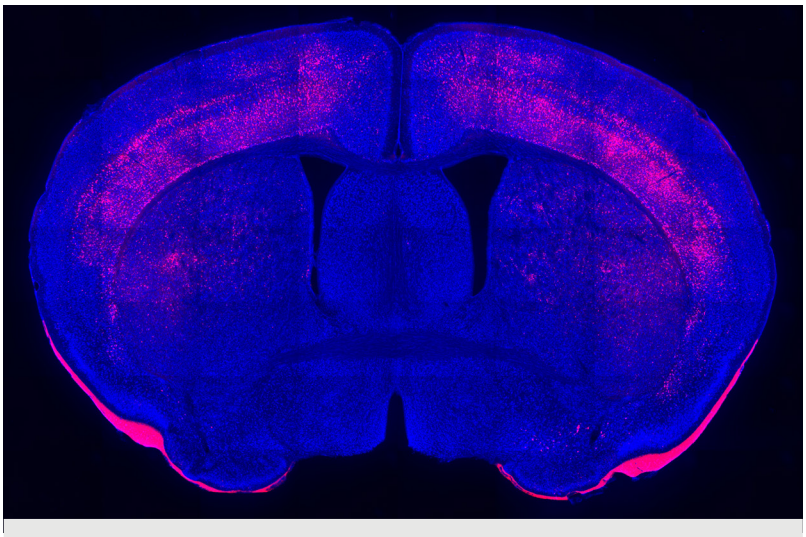
These successes are an important step toward the development of tools to modulate and manipulate neuronal activity in the human brain to benefit patients. For example, the researchers say, a multifunctional fiber might one day be used to more accurately pinpoint the origin of seizures in people with epilepsy, by testing the effects of activating or inhibiting specific brain cells.

New grant to study immunotherapy for **autism**

A new three-year research project, funded by the Simons Center for the Social Brain at MIT, posits that the immune system can be harnessed to help treat behavioral symptoms of autism.

In 2020, Associate Professors Gloria Choi of The Picower Institute and Jun Huh of Harvard Medical School showed that the immune system "cytokine" molecule IL-17a, when applied to a particular brain region in mice modeling autism, improved social behavior and reduced repetitive behaviors.

The study in *Nature* provided an explanation for reports by many pediatricians and parents that some people with autism improve in these ways when they happen to experience an infection. In mouse models, the team showed that peripheral immune cells, if they had been primed while *in utero* by a maternal infection, increased IL-17a when the mice sensed a new infection. In the brain, the molecule happens to calm the hyperactivity of neurons that produces the autism symptoms. The researchers also showed that even when other mouse models of autism did not have immune systems primed by maternal infection, their behavioral symptoms improved if IL-17a was directly injected into the brain.



An image from the Choi lab highlights receptors for the cytokine IL-17a in a cross-section of a mouse's brain

The study left open questions that, if answered, could enable the development of an immunotherapy for brain disorders. How do immune cells in the gut that produce IL-17a find their way to the brain? How does the molecule then get into the brain, which stringently filters what goes in or out? Once it gets in, how does IL-17a act on brain cells to achieve the symptom improvement? The new project will seek to identify the mechanisms that drive such steps so that they can then be targeted for therapeutic enhancement.

"This is a novel way to think about treating neurological symptoms: using the immune system," said Choi, who leads the collaboration. "The question is whether we can use this system to heighten it or dampen it to modulate the brain."

Four labs, including those of Choi, Huh, and Picower Institute investigators Myriam Heiman, Associate professor, and Mriganka Sur, Newton Professor of Neuroscience, will collaborate on the project, each bringing very different expertise but also collaborating closely and sharing ideas to tackle the larger problem.

New faculty member advances optical methods to study learning and memory

Like the beams of light she precisely patterns to probe and control the brain's electrical activity, Linlin Fan's research interests have developed a clear focus: advancing technology to make discoveries about how memory works. So where better to launch her career as a primary investigator than The Picower Institute for Learning and Memory and the Department of Brain and Cognitive Sciences at MIT.

"MIT's neuroscience community has a long history of studying learning and memory," said Fan, who joins the MIT faculty as an Assistant Professor in January 2024. "But it's also very interdisciplinary. What attracted me to MIT is not only its neuroscience community but also its engineering school, and chemistry and physics departments that are all close by."

Fan has combined biology, chemistry, and engineering ever since her undergraduate days on her way to becoming a pioneer of using all-optical techniques to experimentally precisely control and measure neural activity in the brain. As she establishes her new lab at MIT, Fan said she hopes to make an impact both by advancing the technology's utility for answering a wide variety of neuroscience questions, and by demystifying memory so that diseases such as Alzheimer's can be better understood.

"By continuing to develop these technologies and disseminating them, I hope that more neuroscientists can benefit from these tools," Fan said. "And by decoding fundamentally how memory works we hope to understand what goes wrong in disease and ultimately contribute to better treatments."

A sharpening focus

Fan grew up in central China with a strong interest in science, math and engineering, which ultimately earned her admission to Peking University in Beijing. In an undergraduate research project performed at Stanford, Fan united her interdisciplinary interests to engineer new light-controllable tools to optically control protein activity.

"That's how it all started, by doing well in school and getting into college, and having the experience of creating something that nature hasn't created before and feeling the joy of simply finding out the secrets of nature," she said.

Fan became inspired to apply optical technologies to neuroscience. So when she graduated, she crossed the world to join the Harvard lab of Adam Cohen, a physicist and chemist who was working to develop "genetically encoded voltage indicators." GEVIs genetically engineer neurons to emit light that instantaneously and precisely indicates the cells' most subtle changes in membrane voltage. The technology is ideal for genetically targeting specific neurons for fine-grained, long-term monitoring of not just when they "fire" to send electrical signals, but also all the "subthreshold" ebbs and flows of charge that build up to that. But when Fan arrived in Cohen's lab in 2014, the GEVIs were only useful in single cells in dishes. Fan worked on improving the technologies to measure the communication between neurons (both how they can electrically excite or inhibit each other) and to get them working in awake, behaving animals.

Incoming Assistant Professor Linlin Fan in The Picower Institute's 6th Floor "Reading Terrace"

By 2020 she had succeeded. In the journal *Cell* she demonstrated that she could not only use GEVIs to read out neural electrical activity in live mice, but also simultaneously manipulate neurons with a different color of light using a somewhat more established technology called optogenetics. The experiments revealed how the sense of feeling in mouse whiskers depends on a specific pattern of neural excitation and inhibition in the brain's outermost layer. Because both optogenetics and GEVIs require shining light onto target cells, and GEVIs then output light, Fan had to devise a way to maximize the signal and minimize any "cross-talk." Her set up employed distinct light colors and holographic patterning of the separate incoming light sources so they hit just the right parts of the right cells.

For her postdoctoral work Fan moved to the Stanford lab of Professor Karl Deisseroth, a psychiatrist/engineer and pioneer of optogenetics. There Fan hoped to study something that had struck her as more enduring in the brain and more profound for behavior than sensation: memory and knowledge.

At the beginning of 2023, as a capstone on her graduate and postdoctoral work, she published again in *Cell*, this time applying her all-optical methods to the hippocampus, a brain region central to memory. She showed that by optogenetically stimulating individual CA1 neurons when mice were in a specific place in an environment, she could induce those CA1 cells to become more responsive to those places, as indicated by GEVI measurements. She further developed all-optical interrogation of the connections between those CA1 cells and CA2/3 cells via junctions called "synapses," and revealed potentiation, or strengthening, of those connections. The study therefore demonstrated the synaptic basis for "behavioral time-scale plasticity," which is a fundamental transformation for neurons to encode memories, for instance of places.

Launching a lab

"We are only at the beginning of harnessing such technologies to establish direct and causal links between synaptic properties, and neural circuit dynamics, and behavior all in awake, behaving mammals," Fan said.

(Continued top of page 8)

In her new Picower Institute lab, Fan wants to expand that capability, for instance by pushing the technology to work in more connected pairs of neurons. She also wants to ensure it can work everywhere in the brain. Helping her get started is a new 2023 Career Award at the Scientific Interface from the Burroughs Wellcome Fund.

The young girl from central China who aspired to a career in science is now a leader at the forefront of all-optical techniques for probing the synaptic and neural basis of learning and memory. Her focus has sharpened, but she has retained all her desire for innovation and discovery.

"Bringing in these new techniques and seeing things no one has seen before is the thrill of innovation and discovery that drives us to continue," Fan said.

Awards recognize **Mark Bear**, **Elly Nedivi** for plasticity studies

Julius Axelrod Prize

Recognizing his research advancing understanding of how the brain changes with experience by altering the strength of connections among neurons, a phenomenon called "synaptic plasticity," the Society for Neuroscience named Picower Professor Mark Bear a co-recipient of the 2023 Julius Axelrod Prize.

The prize honors scientists with distinguished achievements in the broad field of neuropharmacology or a related area and exemplary efforts in mentoring young scientists.

In its announcement, SfN wrote: "Bear fundamentally advanced our understanding of experience-dependent plasticity in the mammalian brain…Additionally, Bear is an extraordinary mentor, with 18 of his 35 former postdoctoral researchers and 11 of his former PhD students now in tenure track positions. He is known for his positivity, optimism, and steadfast enthusiasm for science — even in the face of the challenges that research and funding present — and through his mentorship he passes these essential traits on to the next generation of scientists."

Bear said he is honored to receive this award.

"Recognition for mentorship is particularly meaningful," Bear said. "The greatest satisfaction of my career has been to help my undergraduate students, graduate students, and postdocs mature into the fantastic scientists they are today. I am proud of their many accomplishments, and very grateful for their key contributions to our studies of brain plasticity."

Krieg Cortical Kudos Discoverer Award

The Cajal Club has named Elly Nedivi, William R. (1964) & Linda R. Young Professor of Neuroscience, the 2023 recipient of the Krieg Cortical Kudos Discoverer Award.

The Club's award, first bestowed in 1987, honors outstanding established investigators studying the cerebral cortex, the brain's outer layers where circuits of neurons enable functions ranging from sensory processing to cognition. These circuits can constantly remodel their connections to adapt the brain to experience. This "plasticity" underlies learning and memory, and other properties of the brain.

With a focus on the visual cortex, Nedivi's lab investigates the molecular and cellular mechanisms that enable plasticity in the developing and adult brain, including identification of the genes whose expression is involved, characterization of the cellular functions of the proteins those genes encode, and studies of synaptic and neuronal remodeling as it happens in live, behaving animals. To enable those observations, Nedivi and longtime collaborator Peter So, Professor of Mechanical Engineering, have developed advanced microscopy systems that can image multiple components of neural connections in the cortex of live rodents.

In a message to Nedivi notifying her of the honor, Cajal Club president Leah Kurbitzer, Professor of Psychology at UC Davis, said: "This award recognizes your outstanding and continuous contributions to our understanding of fundamental aspects of cortical connectivity in the mammalian brain, and the cellular and molecular mechanisms underlying adult visual experience plasticity."

Nedivi said she was thrilled to receive the award.

"I am honored to be recognized with this award and to be following in the footsteps of many previous recipients whose work I admire and respect," Nedivi said.

Congratulations to new **Picower PhDs**

With studies ranging from the molecular roots of brain cell diversity to highly detailed characterization of brain tissues, to phenomena underlying behavior, learning and consciousness, five graduate students in Picower Institute labs have recently earned doctoral degrees.

Sur lab graduate student Dr. Gabi Drummond defends her doctoral thesis

CONGRATULATIONS TO THEM ALL:

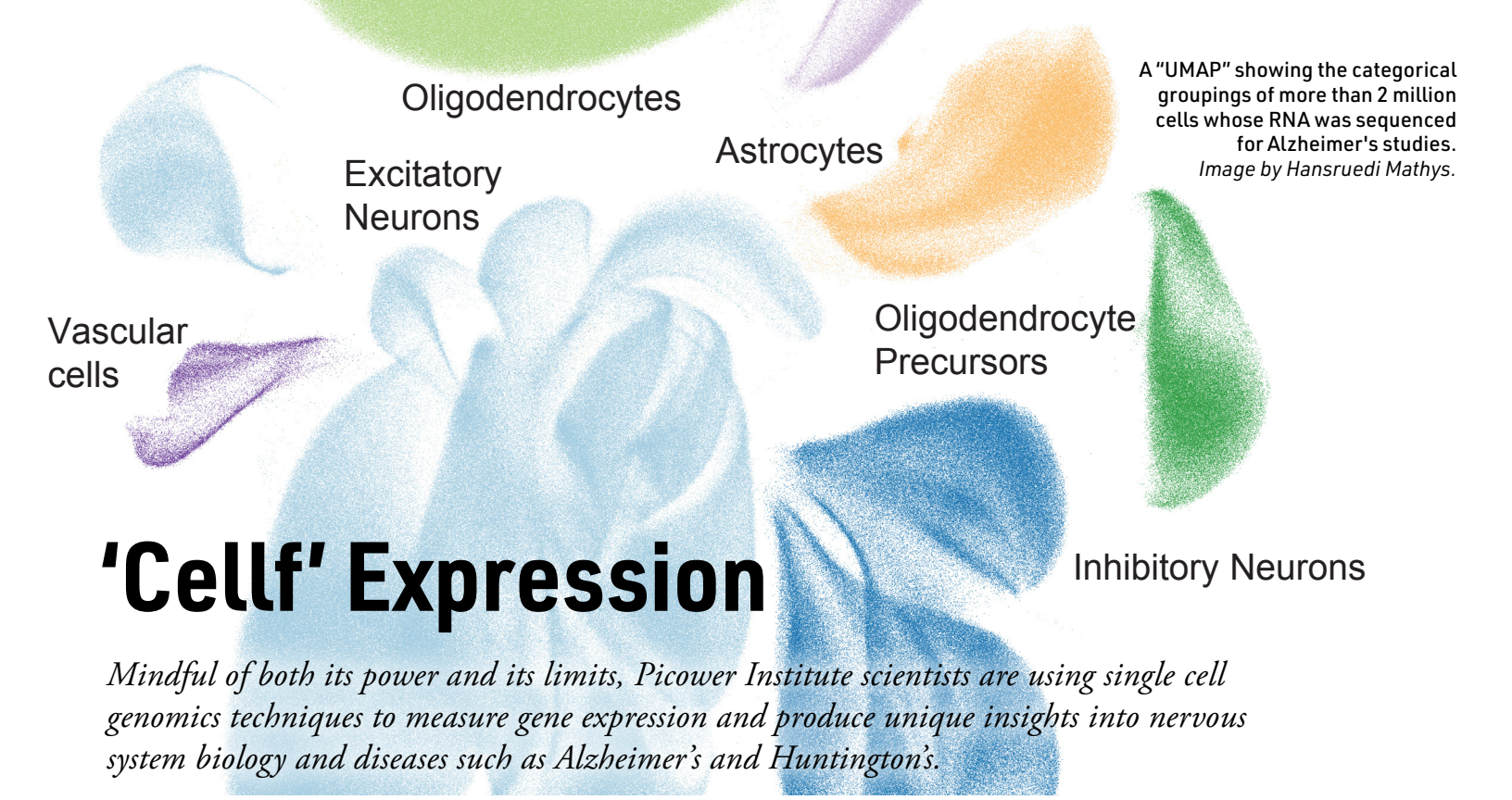
Dr. Seo Woo Choi, *Chung Lab,* "Multi-omic Characterization of Brain Models via Engineering of Tissue Physicochemical Properties"

Dr. Andrés Crane, *Littleton Lab,* "Characterization of the Role of Differential Gene Expression and RNA Editing in Drosophila Tonic and Phasic Motoneuron Diversity"

Dr. Gabi Drummond, *Sur Lab,* "The role of Locus Coeruleus Norepinephrine in Reinforcement Learning"

Dr. Gurrein Madan, *Flavell Lab,* "Genetic and Neural Circuit Analysis of Sickness and Foraging Behaviors in C. elegans"

Dr. John Tauber, *Brown Lab,* "Statistical Modeling of Disrupted Sensory Processing during Propofol-Mediated Unconsciousness"



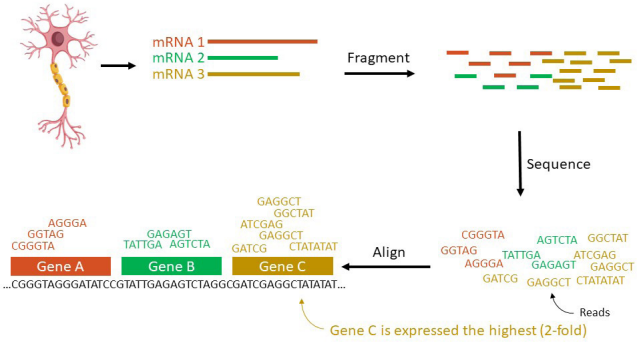
A "UMAP" showing the categorical groupings of more than 2 million cells whose RNA was sequenced for Alzheimer's studies. *Image by Hansruedi Mathys.*

'Cellf' Expression

Mindful of both its power and its limits, Picower Institute scientists are using single cell genomics techniques to measure gene expression and produce unique insights into nervous system biology and diseases such as Alzheimer's and Huntington's.

When Biology Department Assistant Professor Sara Prescott was a new postdoc at Harvard in 2016, her lab had made an important advance in studying the vagus nerve, a major conduit of signals between the brain and the body's organs. The lab had identified six different types of sensory neurons in the nerve bundle, validating the hypothesis that a diversity of vagal neurons sensed problems in the body and responded with appropriate reflexes.

When Prescott picked up the torch, she did so with a new tool called "single cell RNA sequencing" (scRNA-seq). Almost all cells in an organism have the same DNA, but each type of cell becomes distinct in its form and function by how it expresses those genes. scRNA-seq tallies up which genes a cell has transcribed into RNA, for instance to make a protein, and how often. At the time, scRNA-seq commercial systems weren't widely available. Using it took considerable work. But when Prescott was done, her team showed that there were not just six distinct types of sensory neurons in the vagus nerve, but 37. The results she published in *Cell* in April 2020 have already been cited more than 100 times. The results continue to propel her work at MIT as she investigates the function of those neurons.



In Single Cell RNA sequencing, the messenger RNA that a cell has transcribed from DNA is extracted and sequenced to form "reads." By matching reads to a reference genome, scientists can determine which genes are being expressed most. *Diagram courtesy of Andrés Crane*

Back then Picower Professor and Institute Director Li-Huei Tsai had also just begun to incorporate scRNA-seq into her Alzheimer's disease research. In 2017, she co-led its first use to analyze the brain's microglia immune cells in Alzheimer's disease model mice. Inflammation is a hallmark of Alzheimer's pathology and this study showed how microglia become more inflammatory as the disease progresses. Since then Tsai has become a leader of using scRNAseq and other single cell genomic measures to discover how different cell types and molecular pathways go awry in Alzheimer's disease, which ones contribute to resilience, and the effects of carrying Alzheimer's disease risk genes or experiencing age-related DNA damage. In some of her most recent studies, she and collaborators also employed single cell ATAC-seq, which measures, cell by cell, how accessible genes are for transcription, providing a deeper understanding of how cells are regulating gene expression.

'A readout and a reference'

Increasingly Picower Institute scientists are embracing the power (and working through the limitations) of single cell genomics techniques to make many influential discoveries about the diversity of cells in the nervous system and the way they each function in health and disease. By tracking indicators of gene expression cell by cell, they allow scientists to not only discern the cell types in a tissue but also how they are uniquely functioning, or faltering.

"Having a reference atlas of what's in the brain is very important, not just for the sake of knowledge and cataloging, but also to understand changes that might be occurring in normal development as well as in disease," said Associate Professor Myriam Heiman, who has used scRNA-seq in several studies since 2020. These studies have advanced that have advanced understanding of which cells are especially vulnerable in several neurodegenerative diseases and what makes them so.

Prescott agrees: "It is both a description of the [cell-type-specific] data that you have but it's also a very valuable resource for doing follow-up studies on the functions of those cells. It's a readout and a reference."

The technique's popularity has surged as it has become much easier to obtain scRNA-seq data from a sample, said Menicon Professor Troy Littleton, whose lab published its first two RNA sequencing papers this year as the team sought to investigate why two similar-seeming types of neurons exhibit distinct communication properties. But it's no panacea, he said. The method can oblige users to perform detailed computational analyses and experimental investigation at the lab bench to, for instance, avoid falling for irrelevant hits that seem statistically significant but turn out to make no difference to a study's hypothesis. But given the relative ease of getting the data, he said, there's no reason not to take advantage of it when it can provide useful leads.

The promise and the pitfalls of scRNA-seq are explained by how it works. When scientists have a tissue sample, they first separate out each cell. The cells (or their nuclei) are then enveloped in a droplet and broken open. Pieces of the RNA that were being transcribed at the time are then "barcoded" with synthetic RNA that labels it uniquely and notes which cell it came from. The RNA fragments are then sequenced, which allows for identifying which gene they were transcribed from. In this way, for the organism's whole genome, scientists get an unbiased accounting of which genes each cell in the sample was actually making use of, or "expressing," and how much they were doing it.

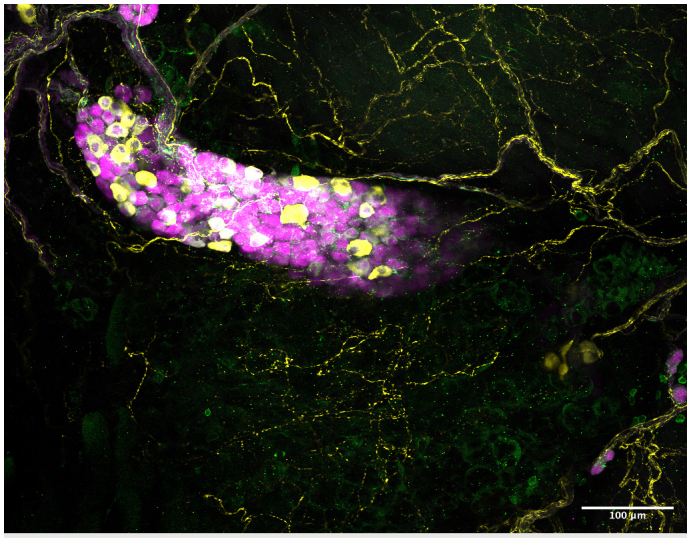
In a review paper in *Nature Neuroscience* in 2023, Tsai and graduate student Mitch Murdock noted that Alzheimer's disease studies using postmortem human brains require careful thought about which samples to sequence. What stage of disease was the tissue obtained during? What are the demographics and lifestyle factors of the donors? Are the samples of good quality and are they processed right? The larger and more variable the samples are, the more tricky and difficult analysis will be to ensure that the results are biologically meaningful and not artifacts of flawed input. Tsai and Heiman, for instance, frequently work with MIT computer science Professor Manolis Kellis who is a globally renowned computational biology expert.

Even after expert analysis, one should still be skeptical of single cell genomics data alone. A significant difference in gene expression could arise for many reasons. Littleton found hundreds of differentially expressed genes between the two neurons his team was comparing, but in many cases the expression differences did not explain any of the differences between neurons he was interested in. Moreover, proteins his lab had already shown to be consequentially different between the two cells showed no difference in RNA levels. The differences arose from some other mechanism scRNA-seq doesn't detect.

With the caveats in mind, Picower scientists have succeeded in using single cell genomics to catalog and understand the diversity of cells in the nervous system and to uncover mechanisms of how specific cellular functions become compromised in neurodegenerative diseases.

Cell diversity

Discovering and cataloging the diversity of cell types in a tissue is often called making a map or an atlas. With about 25,000 vagal sensory neurons from 40 mice, Prescott's postdoctoral team was able to define their distinct expression profiles and discern which "markers" sorted them into 37 distinct clusters. Then they exploited those markers to distinctly label each kind of neuron and to experimentally manipulate them. Those steps allowed her to show that one of the neuron types she identified was critical for triggering life-saving swallowing and coughing reflexes when water or acid entered the throat.



Different color stains label some of the diversity of neurons found in the mouse airway. Single cell sequencing provides a means for distinguishing among the cell types and giving scientists genetic access to them. *Image courtesy of Sara Prescott*

In her new work at MIT, she is not only following up on the functions of other vagal neurons, but also atlasing another novel population of neurons: the neurons intrinsic to the lungs and airways. No one has characterized them before.

"It's shocking to me that we are still discovering cell types in the body," Prescott said. "It's really revolutionized a lot of cellular biology and molecular neuroscience."

In a preprint posted in 2021, Heiman and Kellis produced one of the first atlases of the cell types of the human motor cortex. The survey of gene expression in 380,000 cells identified 46 distinct cell types. And in 2022 in *Nature*, they atlased the diversity of cells in the brain's vasculature. They obtained more than 100 human postmortem samples, and 17 healthy brain tissue samples removed during surgery to treat epileptic seizures. They were therefore able to sequence more than 16,000 brain vasculature cells from people of different ages and genders. They discerned 11 distinct cell types, observed how gene expression changes based on where cells are in the vasculature (a property called "zonation"), and noted important differences in zonation between humans and mice that likely affect properties including what each species will allow to enter the brain through the blood-brain barrier.

Rather than a broad-scale atlas, Littleton's recent studies focused on comparing gene expression between exactly two different cell types. They each connect to muscles to help control motion in a fly. His lab's guiding question was why one type of neuron makes stronger connections, or synapses, than the other. Littleton's lab extracted RNA from each type of cell in 100 flies, already knowing exactly which ones they were. His lab instead focused on determining how their gene expression differed and then investigating in the flies how those measured differences might matter to the cells' synaptic properties. While many differences weren't relevant, many were. In papers in *Neuron* and in *Cell Reports* this fall his lab not only reported the important differences they'd discovered, but also showed that the cells edited their RNA extensively to produce even more fine-grained control over synaptic communication (see p. 5).

Mechanisms of disease

The power of single cell genomics to offer clues about how cells respond to disease has been the focus of Tsai's use of the technique in at least 10 studies over the last several years. In 2019 in *Nature*, she and Kellis published the first comprehensive analysis of differential gene expression in healthy and Alzheimer's disease postmortem brains by sequencing 80,000 cells from 24 people who had Alzheimer's disease and 24 otherwise similar people who did not. The research revealed that not only neurons but also microglia, astrocytes and oligodendrocytes showed important differences. In particular, oligodendrocytes have the responsibility of insulating the wiring that neurons grow to connect with each other. The study indicated that this insulation process, or "myelination," was compromised. The data also showed sex differences in response to Alzheimer's.

In 2023 the labs combined for a massive update. In four companion papers in *Cell* in September (see p. 2), the labs sequenced more than 2 million cells of 54 distinct types from 427 brain samples—an unprecedented effort. Through careful analysis and experimental follow-up, the labs revealed numerous insights including targets for potential therapies. Alzheimer's disease afflicted cells struggled with mitochondrial function, lipid metabolism, synaptic signaling and maintenance of their genome. In one paper, however, they showed that when comparing people who remain cognitively resilient to those with cognitive impairment, a key difference was the relative abundance of specific types of inhibitory neurons.

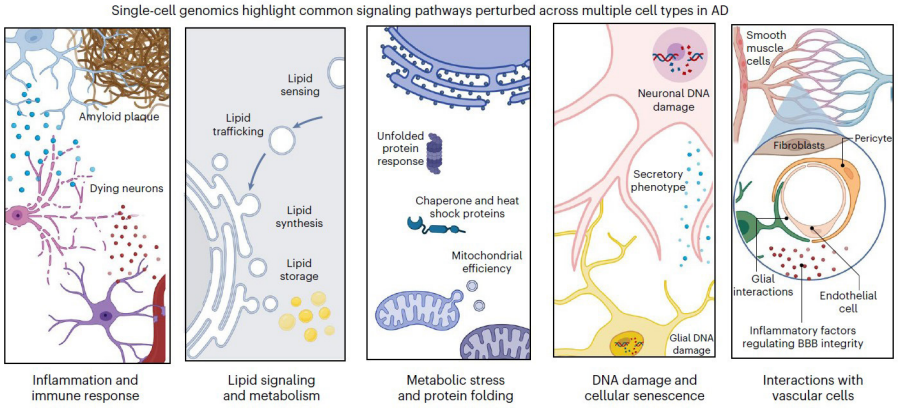
Another paper focused on how age-related lapses in DNA repair led to genome rearrangements and 3D folding defects. Yet another paper focused more deeply on microglia, showing that they assume 12 different states amid disease and that as more become inflammatory, it hinders both neural communication and the effectiveness of the blood brain barrier. But the study also pointed to transcription factors that, if altered, could reduce microglial inflammation. The fourth paper in the set combined scRNA-seq and scATAC-seq to delve into why some genes are expressed more than others and showed how the process goes awry in Alzheimer's

In a 2023 study in *Science Translational Medicine*, Tsai's lab incorporated scRNA-seq to pinpoint that an exact type of neuron in the brain's mammillary body exhibits aberrant activity especially early in disease, leading to memory loss. And by comparing gene expression in cells with the biggest genetic risk factor for Alzheimer's disease, the APOE4 gene variant, to those harboring the normal APOE3 version, the lab has identified how distinct cell types falter. For instance, in *Nature* in 2022, scRNA-seq helped Tsai's lab show that APOE4 oligodendrocytes mishandle cholesterol, leading to myelination failures.

In the *Nature Neuroscience* review, Tsai and Murdock reported that single cell genomic studies from multiple labs worldwide have converged on five major pathways that become disturbed in major cell types in the Alzheimer's disease (AD) brain (see graphic).

"Single-cell profiling facilitates a nuanced portrait of the diverse cellular processes perturbed in the AD brain," they wrote. "These varied molecular programs help explain the divergence between healthy aging and cognitive decline, and highlight cell-type-specific molecular programs involved in AD."

Heiman's scRNA-seq-aided studies have focused on other age-related neurodegenerative diseases. Her 2020 study in *Neuron* was one of the first to use the technique to characterize how cells respond to Huntington's disease. The findings included that many aberrations in gene transcription may be related to a few factors that could be targeted with drugs. Another finding was that an especially vulnerable type of neuron mounts a misguided and potentially fatal innate immune response to unusual levels of mitochondrial RNA.



Single cell genomics by labs around the world have revealed five major common molecular pathways affected by Alzheimer's disease. *Image by Mitch Murdock and Li-Huei Tsai*

Signs of a similar problematic immune response also emerged in the 2022 study that atlased the vasculature. As part of that study, Heiman and Kellis compared gene expression in the vascular cells of people with and without Huntington's disease. They found that an altered innate immune response and other factors may contribute to the blood-brain barrier becoming more permeable than it should be.

Similarly, the preprint featuring an atlas of the motor cortex features comparisons among the brains of people with ALS, Frontotemporal Lobar Degeneration (FTLD), or neither of those motor disorders. Their findings included an interesting overlap: Betz cells (most vulnerable in ALS) and von Economo neurons (VENs; most affected in FTLD) were among the most affected in terms of gene expression, and turned out to have an almost identical basal gene expression profile, which might explain why the cells are especially vulnerable to the diseases.

In January 2023, Heiman teamed up with Kellis and Institute Professor Ann Graybiel in a study in *Nature Communications* that employed scRNA-seq to pinpoint how two distinct cell populations in a brain region called the striatum were affected differently by Huntington's disease. The findings suggested that the disease's damage to a population in the striatum's matrix leads to motor impairments, while damage to the other population, located in structures called striosomes, may account for the mood disorders that manifest in early stages of the disease.

At the time, Heiman said: "This study addresses an important outstanding question in the field, how striosome-matrix striatal projection neuron identity is affected in Huntington's disease. The use of single-cell RNA profiling has allowed us to address this question for the first time in a comprehensive manner."

Across many studies in tissues as diverse as the lungs and regions deep in the brain, scRNA-seq has become a valuable tool.

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