INSIDE

Human

MODELS *of* DISEASE

New technologies enable human brain cell cultures

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Neuroscience News

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THE PICOWER INSTITUTE FOR LEARNING AND MEMORY

DIRECTOR'S **MESSAGE**

Dear Friends,

With subject matter as complex and consequential as the brain, neuroscientists have no shortage of questions. We employ myriad methods to answer them... but we could always use more.

In mid-October, we convened a gathering of leading neuroscientists at our Fall Symposium (see p. 5). With the theme, "Neural Mechanisms of Memory and Cognition," the event evoked especially fascinating fundamental questions about the neurobiology underlying the mind. Just listening to nine speakers it was clear how diverse the field can be, both in what people are studying and how they go about it.

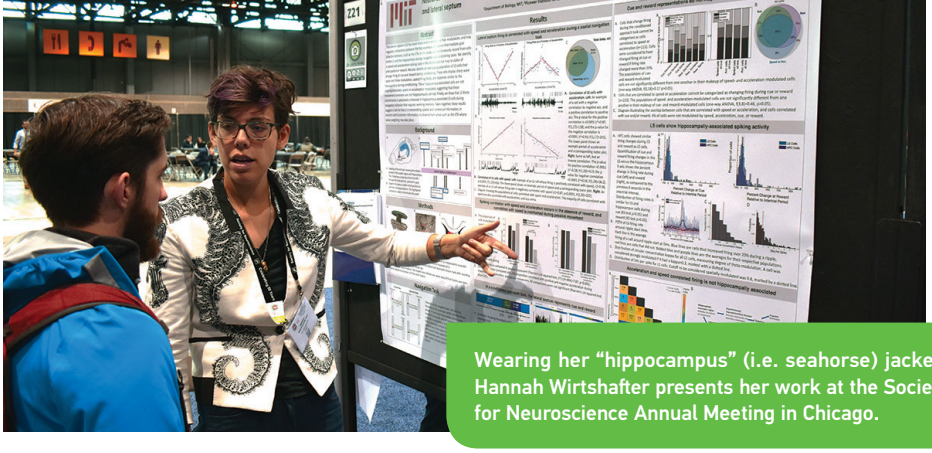
But then, just a few days later, many of us headed to Chicago to attend the vast Society for Neuroscience Annual Meeting. Among thousands of colleagues, about 50 of our researchers presented their work. Our wrap-up coverage (see p. 6) offers a sweeping survey of what we're doing across the Institute to understand the fundamental workings of the brain, to reveal the roots of mental illness, and to develop new tools and techniques for the field.

An amazing array of work is underway. If you read this newsletter cover to cover, you'll learn at least a bit about scores of current projects. In our cover story (see p.8) we've chosen to highlight one especially exciting new area – human models of disease.

A trio of innovations has given us unprecedented genetic, molecular and cellular access to the human brain. A person's brain cells are, of course, not available to research, but stem cell technologies allow us to transform cells that a patient can easily spare, for instance from skin, into neurons and other cells that can be cultured into personalized models, including complex brain tissue "organoids." We can conduct experiments we couldn't before and test potential therapies. Meanwhile new gene editing tools enable us to precisely swap disease-causing mutations in or out of these models to understand their impact.

Picower labs are embracing this extraordinary opportunity. It's a powerful new way we can ask and answer questions about the brain.

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



Wearing her "hippocampus" (i.e. seahorse) jacket, Hannah Wirtshafter presents her work at the Society for Neuroscience Annual Meeting in Chicago.

Region Links Movement With Motivation

Our everyday lives rely on planned movement through the environment to achieve goals. A new study by Picower Institute neuroscientists identifies a well-connected brain region as a crucial link between circuits guiding goal-directed movement and motivated behavior.

The research in *Current Biology* shows that the lateral septum (LS), a region considered integral to modulating behavior and implicated in many psychiatric disorders, directly encodes information about the speed and acceleration of an animal as it navigates and learns how to obtain a reward in an environment.

"The LS represents place, movement, and motivational information and that may enable it to help you integrate or optimize performance across considerations of place, speed, and other environmental signals." said Hannah Wirtshafter, the study's lead author who recently earned her PhD working on the research in the lab of senior author Matthew Wilson, Sherman Fairchild Professor of Neurobiology.

Previous research has attributed important behavioral functions to the LS, such as modulating anxiety, aggression, and affect. It is also believed to be involved in addiction, psychosis, depression, and anxiety. Neuroscientists have traced its connections to the hippocampus, a crucial center for encoding spatial memories and associating them with context, and to the ventral tegmental area (VTA), a region that mediates goal-directed behaviors via the neurotransmitter dopamine. But until now, no one had shown that the LS directly tracks movement or communicated with the hippocampus, for instance by synchronizing to certain neural rhythms, about movement and the spatial context of reward.

Wilson said the study helps to illuminate the importance of the LS as a crossroads of movement and motivation information between regions such as the hippocampus and the VTA.

"The discovery that activity in the LS is controlled by movement points to a link between movement and dopaminergic control through the LS that that could be relevant to memory, cognition, and disease," he said.

Wirtshafter was able to directly observe the interactions between the LS and the hippocampus by simultaneously recording the electrical spiking activity of hundreds of neurons in each region in rats both as they sought a reward in a T-shaped maze, and as they became conditioned to associate light and sound cues with a reward in an open box environment.

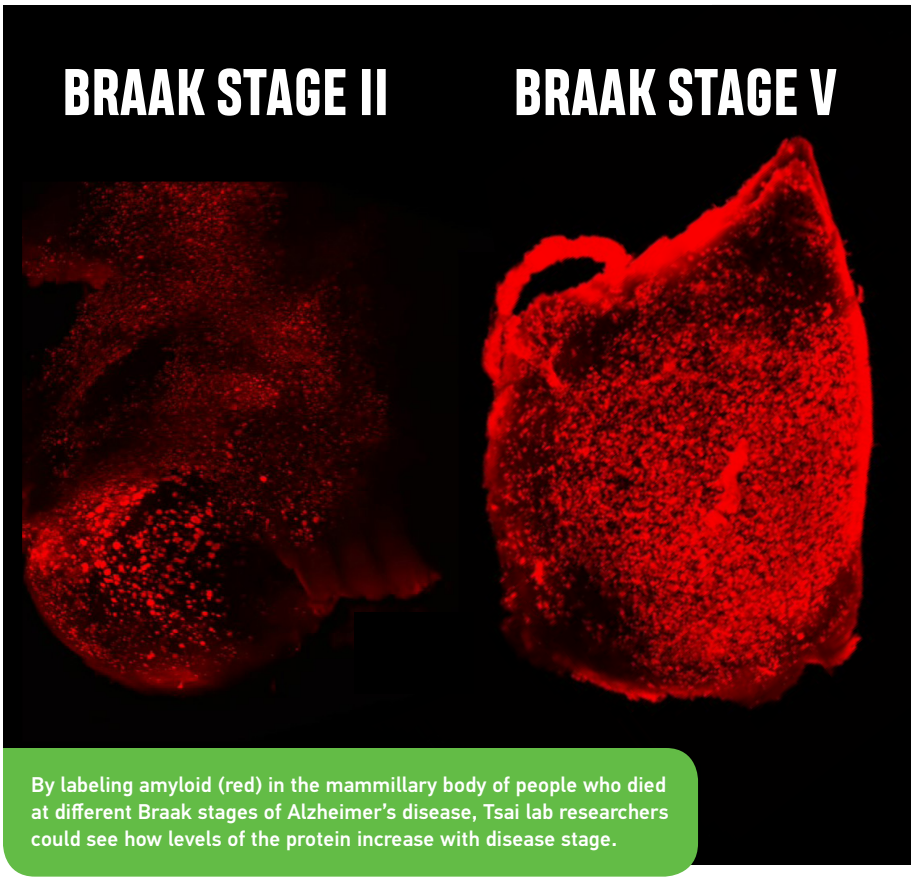
In that data, she and Wilson observed a speed and acceleration spiking code in the dorsal area of the LS, and saw clear signs that an overlapping population of neurons were processing information based on signals from the hippocampus, including spiking activity locked to hippocampal brain rhythms, location-dependent firing in the T-maze, and cue and reward responses during the conditioning task. Those observations suggested to the researchers that the septum may serve as a point of convergence of information about movement and spatial context.

Wirtshafter's measurements also showed that coordination of LS spiking with the hippocampal theta rhythm is selectively enhanced during choice behavior that relies on spatial working memory, suggesting that the LS may be a key relay of information about choice outcome during navigation.

ABOUT THE COVER: In the developing brain and 3D brain organoid cultures, cells self-organize into patterns. How do we interpret these patterns? In this visualization created by members of the Chung Lab, different cell types (color-coded dots) surround the ventricles (grey blobs). Different regions of the ventricle will produce specific patterns (colored on the right) based on the presence of cell types and their migration away from the ventricle.

Study Pinpoints Early Emergence of Alzheimer's Plaques

Long before symptoms like memory loss even emerge, the underlying pathology of Alzheimer's disease, such as an accumulation of amyloid protein plaques, is well underway in the brain. A longtime goal of the field has been to understand where it starts so that future interventions could begin there. A new study by Picower Institute scientists could help those efforts by pinpointing the regions with the earliest emergence of amyloid in the brain of a prominent mouse model of the disease.



By labeling amyloid (red) in the mammillary body of people who died at different Braak stages of Alzheimer's disease, Tsai lab researchers could see how levels of the protein increase with disease stage.

Notably, the study also shows that the degree of amyloid accumulation in one of those same regions of the human brain correlates strongly with the progression of the disease.

"Alzheimer's is a neurodegenerative disease so in the end you can see a lot of neuron loss," said Wen-Chin "Brian" Huang, co-lead author of the study and a postdoc in the lab of co-senior author Li-Huei Tsai, Picower Professor of Neuroscience and director of the Picower Institute. "At that point it would be hard to cure the symptoms. It's really critical to understand what circuits and regions show neuronal dysfunction early in the disease. This will in turn facilitate the development of effective therapeutics."

In addition to Huang, the study's co-lead authors are Rebecca Canter, a former member of the Tsai lab, and Heejin Choi, a former member of the lab of co-senior author Kwanghun Chung, associate professor of chemical engineering and a member of the Picower Institute and the Institute for Medical Engineering and Science.

TRACKING PLAQUES

Many research groups have made progress in recent years by tracing amyloid's path in the brain using technologies such as positron emission tomography and by looking at brains post-mortem, but the new study adds substantial new evidence from the 5XFAD mouse model because it presents an unbiased look at the entire brain as early as one month of age. The study reveals that amyloid begins its terrible march in deep brain regions such as the mammillary body, the lateral septum and the subiculum before making its way along specific brain circuits that ultimately lead it to the hippocampus, a key region for memory, and the cortex, a key region for cognition.

The team used SWITCH, a technology developed by Chung, to label amyloid plaques and to clarify the whole brains of 5XFAD mice so that they could be imaged in fine detail at different ages. The team was consistently able to see that plaques first emerged in the deep brain structures and then tracked along circuits such as the Papez memory circuit to spread throughout the brain by 6-12 months (a mouse's lifespan is up to three years).

The findings help to cement an understanding that has been harder to obtain from human brains, Huang said, because post-mortem dissection cannot easily account for how the disease developed over time and PET scans don't offer the kind of resolution the new study provides from the mice.

KEY VALIDATIONS

Importantly, the team directly validated a key prediction of their mouse findings in human tissue: If the mammillary body is indeed a very early place that amyloid plaques emerge, then the density of those plaques should increase in proportion with how far advanced the disease is. Sure enough, when the team used SWITCH to examine the mammillary bodies of post-mortem human brains at different stages of the disease, they saw exactly that relationship: The later the stage, the more densely plaque-packed the mammillary body was.

"This suggests that human brain alterations in Alzheimer's disease look similar to what we observe in mouse," the authors wrote. "Thus we propose that amyloid-beta deposits start in susceptible subcortical structures and spread to increasingly complex memory and cognitive networks with age."

The team also performed experiments to determine whether the accumulation of plaques they observed were of real disease-related consequence for neurons in affected regions. One of the hallmarks of Alzheimer's disease is a vicious cycle in which amyloid makes neurons too easily excited and overexcitement causes neurons to produce more amyloid. The team measured the excitability of neurons in the mammillary body of 5XFAD mice and found they were more excitable than otherwise similar mice that did not harbor the 5XFAD set of genetic alterations.

In an experiment that may suggest a future therapeutic strategy, when the researchers used a genetic approach to silence the neurons in the mammillary body of some 5XFAD mice but left neurons in others unaffected, the mice with silenced neurons produced less amyloid.

BRAIN Grant Funds New Tools to Study Astrocytes

While neuroscientists have begun to appreciate how closely astrocyte cells support the development and function of neural circuit connections, or synapses, they haven't been able to investigate many of the cells' specific contributions because they have lacked the tools to manipulate them. With a new $1.5 million, three year grant from the U.S. government's BRAIN Initiative, Mriganka Sur will lead a three-lab partnership to develop new tools to study astrocytes.

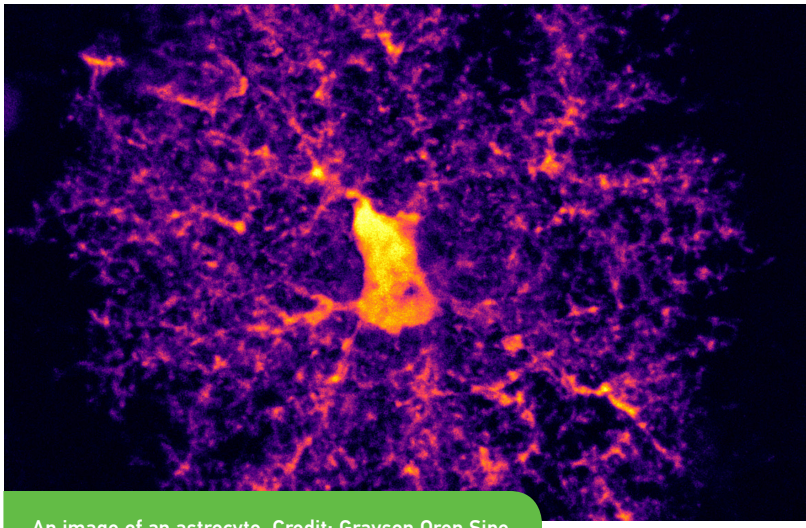
"A great many genes of brain disorders involve astrocytes," said Sur, Newton Professor of Neuroscience in the Picower Institute. "It's of great importance to be able to study and manipulate astrocytes in order to understand their role in modulating neurons."

Sur, with postdoc Grayson Oren Sipe and their collaborators, propose to create three tools to give scientists the ability to experimentally manipulate key properties of astrocytes in living animals wherever in the brain they want (spatial control) and with the timing of their choosing (temporal control).

The first tool, to be developed with Professor Rudolf Jaenisch of the Whitehead Institute and MIT Department of Biology, will give scientists a new way to manipulate astrocytes genetically. Sipe and Jaenisch lab postdoc Xin Tang will use the CRISPR/Cas9 gene editing technique to knock out with spatial and temporal precision two genes that allow astrocytes to receive the neuromodulator noradrenaline, which is important in arousal.

To create two other tools, Sur's lab will work with both Jaenisch's lab and the lab of Ed Boyden, Y. Eva Tan Professor of Neurotechnology. The tools will adapt for astrocytes a technology Boyden co-invented called optogenetics. Optogenetics changes cells to become responsive to flashes of visible light.

One tool will give them temporal control over receptors that the cells use to regulate calcium levels around neurons, for instance to intentionally increase intercellular calcium levels. The other tool will allow scientists to experimentally disrupt the astrocytes' uptake of the neurotransmitter glutamate by creating an ion channel called ChromeQ, that alters sodium ion currents within astrocytes. Manipulating ChromeQ to alter those currents will allow them to reduce glutamate uptake, which in turn will reduce intercellular calcium levels. In the motor cortex, Sur's team will be able to investigate the influence astrocytes have on neurons at the synaptic level in the process of motor learning in lab mice.



An image of an astrocyte. Credit: Grayson Oren Sipe

Modeling Alzheimer's on a Chip

With a new $5.6 million, five-year grant from the National Institutes of Health, Picower Institute neuroscientists will develop a "brain-on-a-chip" to model Alzheimer's disease, providing a powerful testbed for personalized disease and treatment research.

The lab of Picower Professor Li-Huei Tsai will develop their miBrain-chip platform starting from a crucial, but often overlooked component: blood vessels with a functional blood-brain barrier (BBB), which stringently filters the material that enters or leaves the brain through the circulatory system. Recently, Tsai lab postdoc Joel Blanchard successfully engineered and validated such an *in vitro* BBB from patient-derived stem cells.

> We can make all the cells from any individual and then modify their genes or test potential therapeutics. It could be akin to an in vitro clinical trial.
>
> ***Joel Blanchard***

"We are going to start off with the BBB, which is rather novel," Tsai said. "We will build on top of that, adding other cell types in the system. There are a lot of questions we can ask with this system that we could not with others."

Working with Robert Langer, David H. Koch Institute Professor in the Departments of Chemical Engineering and Biological Engineering and the Koch Institute at MIT, Tsai and Blanchard will integrate the BBB into a chip system including neurons, microglia immune cells, and oligodendrocytes, which maintain the efficiency of neural circuits through a process called myelination.

"This new model includes most of the cell types of the human brain so it will provide insight into how they interact and how those interactions change during the progression of the disease," Blanchard said. "We can make all the cells from any individual and then modify their genes or test potential therapeutics. It could be akin to an *in vitro* clinical trial."

The team plans to make miBrain-chips from tissue samples of people who lived with Alzheimer's disease. Collaborator and computer science Professor Manolis Kellis will build computational models that track changes in gene expression, cell dynamics and other data so that they can analyze the disease progression in the chip system, comparing it to related data in the individuals' medical histories. They can also examine the effects of potential treatment interventions in copies of those systems and track their effects.

They also plan to engineer chips in which the sole difference is the presence of the typical APOE3 gene variant or the APOE4 variant that increases disease risk.

Fall Symposium Explores Fundamental Questions of Memory, Cognition

Though the research find- ings presented at The Picower Institute's Fall Symposium, "Neural Mechanisms of Memory and Cognition," were novel, the questions they help to answer were as old as humanity's wonder about the brain: How can such a compact, physical thing produce and control infinitely flexible thoughts and behaviors? How does the brain store all our memories and associate them with meaning? How do we make our way through an ever-changing world? How did humans evolve a unique intelligence?

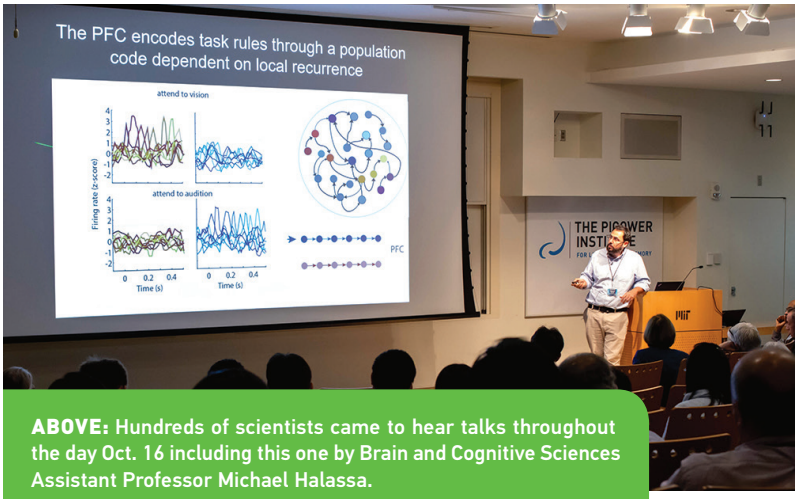
The ability of the brain to change and adapt with experience, a flexibility that neuroscientists call "plasticity," begins with the connections, or "synapses," between neurons. Picower Institute Professor Elly Nedivi, William R. (1964) & Linda R. Young Professor, described how her research group literally watches plasticity happen by individually labeling different types of synapses under the microscope and tracking how they come and go on a daily basis as a mouse has experiences. The research has enabled her to see how neurons try out new connections to later be discarded or cemented, and how cells appear to fleetingly form inhibitory synapses next to permanent excitatory ones perhaps as a way of taking them offline when needed.

To understand the circuitry of cognitive flexibility, Yale Professor Jessica Cardin, a former Picower Institute postdoc, has tracked how an animal's behavioral state – highly aroused or calm – varies the ability of neurons in circuits of the visual cortex to encode information. She has showed how a particular neuron type helps to regulate this state dependence and is also associated with widespread activation of cortical circuits. Using tools to image circuit activity across the cortex, she studies how the circuits of cognitive flexibility differ in autism model mice.

To control attention, the cortex interacts with a deeper brain region called the thalamus, explained Michael Halassa, an assistant professor in MIT's Brain and Cognitive Sciences Department. His model of that interaction suggests that to pay attention to one thing vs. others, different sets of neurons in a region called the mediodorsal thalamus respectively influence the coordination of prefrontal neurons and help configure prefrontal circuits to inhibit sensory inputs that compete with the stimulus of interest. Halassa, a psychiatrist, noted these mechanisms might be implicated in autism or schizophrenia.

Interregional relationships also help endow memory with meaning. The hypothalamus, for instance, helps the hippocampus integrate social or spatial novelty with memories, said Thomas McHugh, a Professor at the RIKEN Center for Brain Science in Japan who was one of the earliest Picower Institute faculty members. His team has tracked how cells in a hypothalamic region transmit novelty information via circuit connections with separate parts of the hippocampus.

Martin Furhmann, a group leader at the German Center for Neurodegenerative Diseases, described how misregulation of novelty and memory in Alzheimer's disease may present a conflict. Mice genetically engineered to model Alzheimer's store memories in ensembles of hippocampus neurons just like healthy mice, but later struggle with recall. The trouble, he's found, is potential interference from an overlapping ensemble of neurons encoding novelty.



ABOVE: Hundreds of scientists came to hear talks throughout the day Oct. 16 including this one by Brain and Cognitive Sciences Assistant Professor Michael Halassa.

LEFT: Professor Elly Nedivi delivers a talk on synapse research. Images by Rose Lincoln

Memory helps us navigate to get what we need. Christopher Harvey of Harvard University described a model of how regions of the cortex process sensory information about an area, factor that into a plan for motion, and then send that information elsewhere to effect that motion. In particular, the retrosplinial cortex is an apparent locus of making choices and planning, he's found.

Lisa Giocomo of Stanford University, meanwhile, focused on the medial entorhinal cortex (MEC). She showed that the region maintains multiple maps that can account for changes in environment and behavior. When mice learned the location of a reward within an environment, for instance, MEC neurons restructured their mappings to account for it.

In the keynote talk, Mu-ming Poo of the Institute of Neuroscience in Shanghai argued that to understand how human intelligence evolved and what neural substrates enable it, scientists must study non-human primates, which can model higher-level cognition in ways that rodents cannot. Moreover, he said, non-human primates can provide better models of diseases affecting cognition, such as autism. He described his efforts to study self-awareness in rhesus macaques and to develop genetic models of disease in cloned animals. He acknowledged that there is an ethical debate among people who have different opinions on such research.

Picower Institute Associate Director Matt Wilson, Fairchild Professor of Neurobiology, said such questions often arise when a field is at its frontier.

"This has really been really remarkable to be going from synapses to circuits to systems and the incredible sophistication and combination of tools involved," he said. "There are a whole host of issues and questions about how we can apply this science and where the science is going."

Picower Scientists Shine in Chicago at 'SfN'

Professor Li-Huei Tsai delivers her featured talk in the main hall of the conference on her research using gamma rhythm stimulation to potentially treat Alzheimer's disease.

MORE ONLINE

For a full listing of all Picower presentations at SfN see:

http://bit.ly/PILMatSFN

For five days in Chicago, the so-called "Second City," neuroscience came first as 30,000 brain scientists flooded the city for the 2019 Society for Neuroscience Annual Meeting Oct. 19-23. For the 50 Picower researchers among them who presented findings on topics as varied as autism, anxiety and anesthesia, they each had a moment, large or small, to convene an audience around their work.

Young-Gyun Park of Kwanghun Chung's lab explains his poster on brain-wide mapping of a specific memory.

Postdoc Omar Costilla Reyes of Professor Earl Miller's lab co-organized a "minisymposium" on intersections between neuroscience and artificial intelligence. The morning-long meeting Monday Oct. 21 drew perhaps a couple of hundred people. They heard from a series of experts, including Reyes, who discussed using machine learning to help analyze the importance of different neural signals recorded in the cortex of experimental animals as they carried out cognitive tasks.

"I saw the call for mini-symposiums after SfN 2018 and decided to apply with a proposal since I did not see many events at the intersection of AI and Neuroscience last year," Reyes said. "I was expecting a large group to attend the minisymposium since the intersection of AI and neuroscience is getting attention from both the computer science community and neuroscience."

In all, Picower presenters may have reached thousands of colleagues. That number almost certainly became locked in by Tuesday Oct. 22, when Professor Li-Huei Tsai – after an introduction by Professor Emery N. Brown – took the conference's main stage for a featured lecture on stimulating the brain's 40Hz "gamma" rhythms with light, sound or both to combat Alzheimer's disease, a technique she calls "GENUS," for Gamma Entrainment Using Sensory stimuli.

In multiple mouse models GENUS stimulates microglia cells to aid brain health, reduces amyloid and tau proteins, prevents neuron death and preserves memory and cognition. Testing has recently begun in humans.

"We are eager to learn as much as we can about GENUS for two main reasons," Tsai said. "We hope our findings in mice will translate to helping people with Alzheimer's disease, though it's certainly too soon to tell. But there also may be exciting implications for fundamental neuroscience in understanding why stimulating a specific rhythm via light or sound can cause profound changes in multiple types of cells in the brain."

Tsai lab graduate student Scarlett Barker was among several other Picower researchers who delivered podium talks. She described her studies of a gene whose enhanced expression amid environmental enrichment may provide a mechanistic explanation for why some people remain resilient to a buildup of Alzheimer's pathology. Peter Finnie of Professor Mark Bear's lab discussed the lab's work to determine if differences in visual recognition memory – the ability to recognize things as familiar – could prove to be a biomarker for autism spectrum disorders. And Mikael Lundqvist of the Miller lab told his audience that the same specific interplay of neural rhythms the lab has documented in the prefrontal cortex during working memory appears to be a general feature across other cortical areas as well.

POSTER OUTPOSTS

At dozens of posters throughout the conference, Picower Institute researchers held court for hundreds of visitors. Often, they were grouped together forming temporary Midwestern outposts of the Institute.

Associate Professor Kwanghun Chung's lab drew a steady crowd of onlookers to a strip of eight posters presenting their latest technology to clarify, enlarge, preserve and label whole brains and tissues to enable unprecedentedly rich imaging and

analysis of neurophysiology, development and function. Several lab members showed how their technologies are applied to fascinating neuroscience questions. Minyoung E. Kim presented a scalable, end-to-end framework to predict morphological features of microglia and analyze distribution of their subtypes, at whole-brain scale, during development and after GENUS. Young-Gyun Park showed how the lab's technologies allowed the labs of Chung and Professor Susumu Tonegawa to track down the ensemble of neurons that form a contextual memory, or "engram," even though it is distributed among many different brain regions.

The Sur lab presented five posters Tuesday afternoon describing new insights into how cortical circuits and cells represent sensory information and use it to guide movement and behavior. Elie Adam, for example, detailed the multi-regional circuit required for a mouse to halt a sprint as quickly as possible given a visual cue. That's a behavior familiar to anyone who has watched a baseball player run to a base and stop without going over, or anyone who has poured a drink and stopped before it overflowed. Similarly Vincent Breton-Provencher demonstrated how noradrenergic neurons in the locus coeruleus help produce the focus of attention needed to distinguish visual cues amid high uncertainty – much like people must do when driving in bad weather.

The Miller lab, too, occupied a ward of boards on Wednesday morning detailing how neural rhythms reflect and influence cognitive behavior. Lab member Roman Loonis demonstrated the neural correlates of keeping an open mind with a poster that tracked how oscillations and neural signals characteristically changed when monkeys tasked with choosing whether one of two images belonged in a category surveyed both options, rather than just one, before making the choice. Scott Brincat, meanwhile, showed how he's tracked down the means by which the brain's working memory system rapidly transfers information about a visual stimulus of interest when it moves from the domain of one hemisphere to the other.

HOT TOPICS

Even when they weren't grouped, many other posters showed off the breadth and depth of Picower's work on a variety of important topics in mental health and fundamental neuroscience.

The poster of Hannah Wirtshafter (see page 2), formerly of Professor Matt Wilson's lab, was ordained by the Society for Neuroscience as an official "hot topic."

Both the Miller and Brown labs presented several posters on anesthesia. Sourish Chakravarty, for example, detailed the Brown lab's efforts to create a closed-loop anesthesia delivery system, based on monitoring brain rhythms, that can help regulate real-time dosing of anesthetic drugs. Pegah Kahali, meanwhile, traced the thalamocortical connectivity patterns that could support the dynamics of coherent alpha rhythms across posterior sensory and frontal cortices as people lose consciousness under propofol anesthesia.

Three posters showed how work in the Tsai lab supported by the new Alana Down Syndrome Center is progressing (see cover story page 8) and seven other Tsai lab posters detailed aspects of her Alzheimer's work. In work to determine the key cell types that allow for visual stimulation of 40Hz rhythms, Chinnakkaruppan Adaikkan presented research indicating that parvalbumin neurons are "indispensable" and somatostatin neurons may play a regulatory role. Brennan Jackson and Noah Milman, members of the team testing 40Hz stimulation with humans, reported early observations from those studies including that the stimulation produces a steady increase in gamma power and functional connectivity in the brains of healthy volunteers and is well tolerated in the trials. Karim Abdelaal, meanwhile presented initial findings in mice that two weeks after stimulation stops, there are still positive effects, including a healthier state of microglia and reduced DNA damage.

Gwyneth Welch and Liwang Liu also presented research on DNA damage in Alzheimer's in the form of double-stranded breaks (DSBs). Welch's work has provided new insight into the fate of diseased neurons, including their increased likelihood of an inflammatory response and death in the Ck-P25 mouse model. Liu examined an association between DSBs in pyramidal neurons in the hippocampus of Tau P301S mice and hyperexcitability.

Wen-Chin Huang, meanwhile, showed research explaining new findings about the early effects of Alzheimer's. Following up on a recent paper showing that it first emerges in deep region called the mammillary body (see page 3), Huang examined specific neuron types there and found that one in particular exhibits hyperactivity in the 5XFAD model of the disease.

Several other posters addressed autism spectrum disorders. Patrick McCamphill of the Bear lab examined whether a promising treatment for Fragile X syndrome may have been hindered by tolerance at the point in the pathway affected by the drug. He described a new intervention that in mouse testing produced benefits without a tolerance effect. Michael Reed in Gloria Choi's lab described new research that might explain how an immune response to infection sometimes results in a temporary alleviation of symptoms in some individuals with autism.

Peter Finnie of Mark Bear's lab speaks on research to develop a new biomarker for autism spectrum disorders.

Two researchers from Tonegawa's lab presented studies on fear and anxiety. Andrea Bari described research in mice finding that stimulating neurons in the locus coeruleus could reduce anxiety and help maintain healthier levels of attention when this is impaired by stressful experiences. Xiangyu Zhang, meanwhile showed that fear extinction memories, which override original fear memories, are formed and stored in the basolateral amygdala neurons that are associated with reward.

A REWARDING TRIP

Several researchers reported that the trek to Chicago was worthwhile. Liu said poster visitors kept him talking non-stop on Saturday for four hours. Throughout the conference, he said, he got good feedback, new ideas and information and even scoped out some new equipment.

His fellow lab member and collaborator Huang said perhaps 20-30 people visited his Alzheimer's poster, often asking good questions and providing helpful feedback.

"It is quite an enriching experience," he said. "I got to know the updated research in the field of memory and Alzheimer's disease to understand the research directions other scientists are taking to tackle this devastating disease."

Human MODELS *of* DISEASE

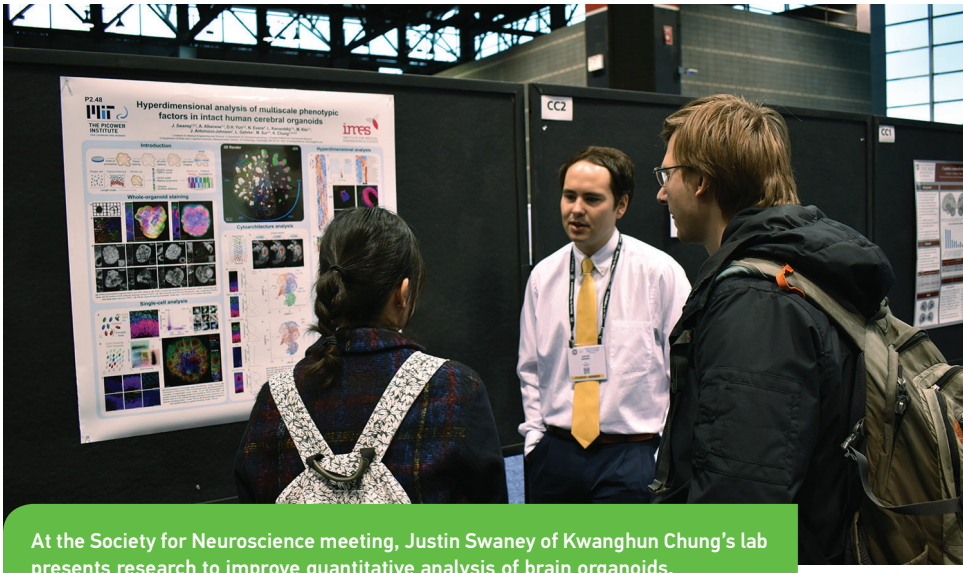
Stem cell, genetic technologies enable sophisticated studies of human brain cells and brain "organoids"

For decades, neuroscientists seeking to better understand human neurological disease and develop new therapies have worked with the obvious limitation that a living patient's brain is not open for investigation or experimentation at the genetic, molecular or cellular scale where many of the brain's mysteries hide. Nonetheless, they've made extraordinary progress with studies in animal models and post-mortem human tissue.

But now neuroscientists are in a whole new era. Three technological breakthroughs over the past 12 years have given them a revolutionary way to study human brain disease: They can create cultures of brain cells derived from other cells in individual patients, and even engineer complex, three-dimensional "organoids" that mimic key aspects of brain tissue. Scientists are only beginning to harness the potential of these new human cell and tissue models, and Picower Institute labs are helping lead the way. Professors Li-Huei Tsai, Mriganka Sur and Kwanghun Chung are among a global vanguard that is making and analyzing these new patient-derived testbeds and applying them to study conditions such as Down syndrome, Alzheimer's disease, Rett syndrome, and Zika virus infection.

While the whole field's progress with these new capabilities has been rapid, so has been the recognition of their limits. That's why rather than replacing animal models and other research methods, these new models are becoming integrated as powerful tools to complement broader research programs where findings from multiple methods often enhance each other's value.

The new wave of breakthroughs began in 2007 when scientists showed how to take a cell from an individual's body (often a skin cell) and to "reprogram" it to become an "induced pluripotent stem cell" (iPSC) that can then be biochemically guided to become any other cell, like a neuron or a supporting astrocyte or microglia. This development allowed scientists to make the brain cells that they of course would never directly extract from patients. By 2013, scientists began using iPSCs to grow 3D cultures of multiple brain cell types, or "organoids," that can reproduce key aspects of brain development and intercellular interactions. That same year, scientists demonstrated that a technique called CRISPR/Cas9, could be used for precise genetic editing. Scientists quickly began using that to manipulate the genes in their iPSC-grown cultures and organoids, creating "isogenic pairs" where two otherwise identical stem cells contain a disease-causing or healthy version of a gene.



At the Society for Neuroscience meeting, Justin Swaney of Kwanghun Chung's lab presents research to improve quantitative analysis of brain organoids.

Tsai and lab members Jay Penney and William Ralvenius summarized and celebrated the significance of these breakthroughs for Alzheimer's disease research in an August 2019 paper in *Molecular Psychiatry*.

"In little more than a decade since the advent of human iPSC technologies we have developed the ability to generate all the main brain cell types from pluripotent cells," they wrote. "Increasingly complex 3D co-culture systems are also emerging that allow us to reconstitute many of the key interactions between brain cells. These technologies have already contributed greatly to our understanding of human development and human disease."

Sur agreed, "It is perhaps the only way one can study a direct human model – it's astonishing to be able to grow brain cells from a patient with a disease, derived from that person's genetic material."

ABUNDANT APPLICATIONS

Picower labs have embarked on numerous studies using the models. In 2018, Tsai's lab, led by Yuan-Ta Lin and Jinsoo Seo, published a paper in *Neuron* in which they used 2D single-cell-type iPSC cultures and 3D mixed-cell-type organoids to study the differences made by two versions of the leading risk gene for Alzheimer's disease, APOE. People with the APOE4 variant are at much higher risk for the disease than APOE3 carriers but scientists haven't been sure why. Tsai and Lin's team used CRISPR/Cas9 to make isogenic pairs of neurons, astrocytes and microglia and spotted several key differences that likely help explain how APOE4 raises disease risk. For instance, APOE4 neurons secreted more potentially harmful amyloid proteins, APOE4 astrocytes showed dysregulated cholesterol metabolism and cleared less amyloid. APOE4 microglia, too, did a poorer job of getting rid of amyloid buildup. Using CRISPR to change APOE4 to APOE3, meanwhile, improved cell activity.

Elsewhere in the the Tsai lab, Joel Blanchard is using iPSCs to model the blood-brain barrier, which stringently filters what comes into and goes out of the brain through the blood stream, so he can see how APOE variants affect that. Researchers are also looking at how myelination – the process of wrapping neurons in a fatty sheath to improve their electrical conductivity – may differ in Alzheimer's.

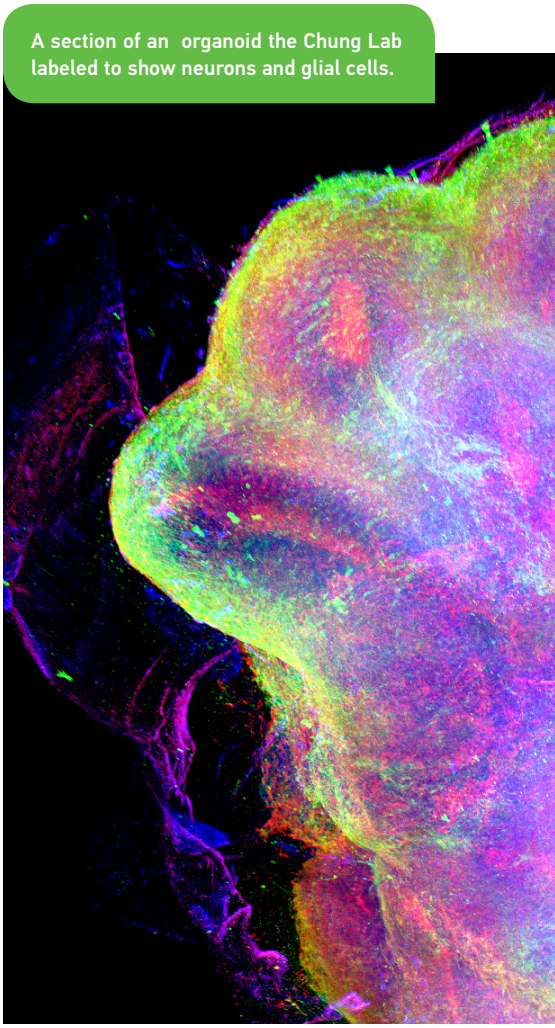
As part of the work in the Alana Down Syndrome Center, Tsai's lab is also using iPSC-based cultures to study the difference that having a third copy of chromosome 21 makes in gene expression in a variety of brain cells and in the development of organoids. At October's Society for Neuroscience (SfN) annual meeting, the team presented some initial results. Hiruy Meharena observed significant physical changes within chromosomes in various brain cell types with the syndrome's three copies of chromosome 21 (trisomy) vs. when they have two copies (disomy). These changes, which appear especially prevalent in neural progenitor cells, occur genomewide and result in substantial differences in gene expression that are associated with brain development. Meanwhile, Lin showed how organoids grown from trisomy iPSCs vs. disomy ones have smaller diameters after 30 days of growth and show gene expression differences that may hinder development. Elana Lockshin focused on differences in glial cells, finding, for instance, that trisomy astrocytes don't migrate during development as readily as in disomy ones.

Sur's lab has been able to make important findings by using iPSCs as part of its studies of Rett syndrome, an autism-like disorder. In a study published in 2017 in *Molecular Psychiatry*, a team led by Nikolaos Mellios used isogenic 2D iPSC cultures to find that the disease-causing mutation in the gene MeCP2 led to misregulated forms of RNA that alter a key molecular pathway in early neural and brain development. They also used organoids to show that if they corrected regulation of those RNAs, they could restore healthier development. The study helped to show that Rett syndrome may begin to affect health even earlier than the onset of systems in toddlerhood.

> Increasingly complex 3D co-culture systems are also emerging that allow us to reconstitute many of the key interactions between brain cells. These technologies have already contributed greatly to our understanding of human development and human disease.
>
> ***Jay Penney, William Ralvenius and Li-Huei Tsai***

At SfN, Sur lab member Vincent Pham showed how he and Chloe Delepine are carrying on this work. Isogenic organoids with vs. without the MeCP2 mutation show distinct differences in development. They don't grow as large and the open spaces within, called ventricles, are longer and thinner. The team also documented comparative reductions in the number of newly born neurons. Using a sophisticated imaging method developed by labmate Murat Yildirim, they observed that young neurons in Rett model organoids also struggle to migrate from the ventricles where they are born, to outer layers where they are supposed to settle into cortical circuits. Typically newborn neurons would follow a straight line outward along a pathway provided by radial glia cells. In Rett organoids the radial glia are normal, but the team watched young neurons zig zag along those paths, taking longer to arrive than they should. By applying sophisticated molecular analysis techniques to the organoid testbed, they were able to investigate the molecular pathway to account for the misguided migration. Certain adhesion molecules that are supposed to keep the new neurons on track are altered, they found.

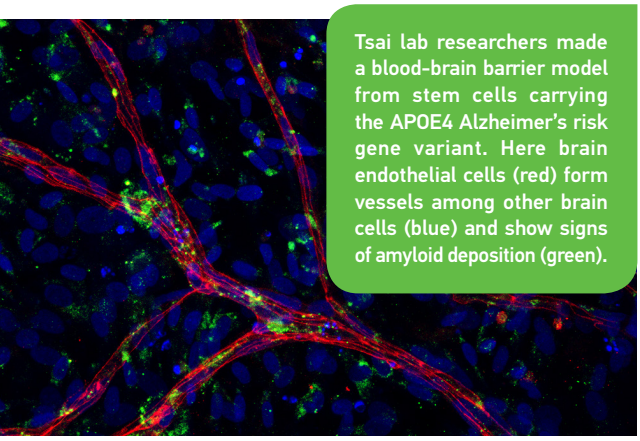
Both the Tsai and Sur labs frequently collaborate with Kwanghun Chung, whose research group is dedicated to developing tools and technologies to help fellow scientists better visualize and quantitatively analyze tissues from the scale of whole human brains down to subcellular components like the synaptic connections between neurons. Chung has not only aided the Sur' lab's Rett syndrome studies but has also been working with MIT



A section of an organoid the Chung Lab labeled to show neurons and glial cells.

Health Sciences and Technology Professor Lee Gehrke in his lab's efforts to quantify differences that may help explain what hinders the growth of organoids – and brains – with Zika infection.

"Organoids were pivotal in helping scientists discover how Zika causes microcephaly," said Chung lab postdoc Alex Albanese. "We are taking a more in depth look at how Zika is actually modifying the structure and cell populations inside the organoids."



Tsai lab researchers made a blood-brain barrier model from stem cells carrying the APOE4 Alzheimer's risk gene variant. Here brain endothelial cells (red) form vessels among other brain cells (blue) and show signs of amyloid deposition (green).

INNOVATING PAST LIMITS

A large part of the work in Chung's lab, which is led by Albanese and Justin Swaney, aims to overcome some key fundamental limits of organoids. Though they are sometimes called "minibrains," they really aren't lab replicas of the real thing. A human brain, while extraordinarily complex, has a well-defined geography. Even though organoids are much simpler – with thousands of cells rather than about 100 billion – they are much more variable in how they turn out. While a real brain will develop highly distinct regions and exactly the right number of ventricles in the right places, organoids will recapitulate only an approximation of, say, cortical structure and may have enough ventricles to look more like Swiss cheese than a brain. Albanese calls this the "snowflake" problem, alluding to how organoids can differ so widely.

So how can they still be valuable models? One has to know how to assess them. Chung's lab has developed advanced tissue processing technologies that can clarify, preserve, label and enlarge tissues, including organoids, so that properties like physiology and cellular function can be highlighted at all scales. Moreover, his lab has developed an imaging pipeline using light-sheet microscopy that is capable of capturing enormous amounts of data quickly (15 minutes per organoid), so that technicians can thoroughly image many organoids in a day.

Beyond that, Swaney and Albanese are developing algorithms and other analytical tools to quantify a wide array of measurements in organoids, such as the physical form, distribution and numbers of cell types. They examine how cells are arranged in the 3D context of each other, which tells them important information about their type and function. All of these advances work in combination to help researchers rigorously assess which differences that they are measuring in their organoids are attributable to their experimental variable – like a genetic disease mutation – or just to the natural variance in how organoids turn out. This improved analysis is part of the Chung lab's Rett and Zika collaborations, Swaney said in presenting his poster at the Society for Neuroscience meeting (that same week Albanese gave a podium talk on their work at the Biomedical Engineering Society in Philadelphia).

Another limitation of organoids is that they aren't actually that small. A millimeter or two of diameter may seem tiny, but that's still big enough to present challenges. Traditional microscopes can't image far enough into them to resolve what's going on with deeper cells. Chung's technologies overcome that problem both by clarifying tissue and labeling cells and proteins, but that requires chemically fixing the organoids. Yildirim's "three photon" microscope technology doesn't label cells as richly, but it can image all the way through organoids while they are alive and active. That's how Sur's lab was able to witness the erratic migration of new neurons. They've developed microfluidic multiple-well housings for organoids and devised methods for holding them perfectly steady for long-term imaging while still allowing nutrients and oxygen to circulate around them.

Indeed a significant problem associated with large size is that with no blood vessels to carry oxygen and nutrients in or to take waste out, the innermost cells can die. Keeping either the nutrients and oxygen, or the organoid, in motion helps keep them healthier than just growing them in dishes, research has shown. The Tsai lab recently began growing organoids by the thousands using a clever bioreactor device that keeps them spinning in the incubator. Postdoc Ping-Chieh Pao said the system saves space and uses less growth media, while yielding higher quality organoids with more mature neurons and less cell death.

Blood vessels are also integral to brain function. For instance, dysfunction in the blood-brain barrier plays a potentially pivotal role in Alzheimer's. To build a human model of Alzheimer's that explicitly accounts for vasculature in sickness and in health, Tsai and Blanchard recently secured a grant from the National Institutes of Health (see p. 4) to build a "brain on a chip" that will engineer connections to unify their blood-brain barrier model with a co-culture of many brain cell types. The addition of vasculature and cell types like the oligodendrocytes that produce myelination will simulate a richer degree of Alzheimer's complexity than human iPSC-based models have to date.

Yet another limitation of organoids has been that they don't mimic multiple brain regions all that clearly, though they can be chemically coaxed to trend toward one or another. For that reason, organoids don't provide much of a testbed for understanding the functional significance of inter-regional circuits. Delepine said the Sur lab is interested in experimenting with growing multiple organoids of different regional character on a chip and then nurturing connections between them to see if they can replicate such circuits.

MULTIPLE MODELS TOGETHER

While Picower researchers and colleagues elsewhere work to make the most of human disease models, there are some limits that seem sure to endure, both for technical and ethical reasons. That means that traditional models, including animals, will remain integral to neuroscience research.

After all, organoids don't think, behave or remember. They can't gather any sensory input and do not exhibit consciousness. Lacking all these basic capabilities, they naturally can't provide any data about how disease affects those vital functions of daily existence. In a recent study where Sur collaborated with MIT biologist Rudolf Jaenisch to test new drugs with the potential to treat Rett syndrome, Jaenisch's lab screened the compounds on stem-cell derived human neurons, but Sur's lab tested the most promising ones in mouse models, because that was the only way to see if they improved cognition and behavior.

"The question was, were they valid in a much more functional system," Sur said.

Mellios's study, too, drew upon and also utilized findings in Rett model mice.

"Our work in these two studies shows how mouse work can complement human work," Sur said.

Especially when fighting disease, researchers will always draw upon whatever systems they can to help them find answers.

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Choi photo by Justin Knight