INSIDE

Immune & Inflamed

Neuroscientists are finding that immune system activity within the brain and the body has important impacts on mental health and behavior

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Neuroscience News

MIT SPRING 2023

THE PICOWER INSTITUTE
FOR LEARNING AND MEMORY

DIRECTOR'S **MESSAGE**

Dear Friends,

A virtue of science is that it enables openness to overcome orthodoxy. When evidence emerges that contradicts a canon, scientists (eventually) dispense with the dogma.

This issue of *Neuroscience News* happens to contain several current examples of such rational revolts. Our cover story (page 8) highlights the emerging realization among neuroscientists that immune responses, both within the brain and the broader body, can have enormously consequential influences on brain function and health. Our research, and that of colleagues elsewhere, is finding many examples in which immune cells and their molecular signals affect the brain at stages ranging from development to degeneration. The vibrancy of research in this area is a distinct change from decades ago when the brain was assumed to be aloof from the immune system and very few neuroscientists talked about immune signals.

Earl Miller and Emery N. Brown, as well, are challenging status quo notions in their fields. Scientists who study working memory – the way we hold information such as directions "in mind" as we use it—long have thought that neurons maintain it by staying constantly active. Earl is showing instead that working memory works very differently (page 4). And based on observations from sources as diverse as patients under Covid care and turtles under frozen lakes, Emery and a colleague recently proposed that the human brain has a previously unknown hibernation-like state (page 7).

These pages also feature an example from my work. Whereas neuroscientists long assumed that brain rhythms (or "waves") were mere byproducts of neural activity with no causal role in function or health, my lab has found that 40Hz rhythms may matter greatly in the brain's response to Alzheimer's disease. After several mouse studies, we just published our first results in human volunteers. The sample sizes are small but the results suggest that using light and sound to increase 40Hz power and synchrony may meaningfully impact disease (page 3).

Read on, not only for our latest news, but also some of our latest new thinking.

LI-HUEI TSAI, DIRECTOR

The Picower Institute for Learning and Memory

Alzheimer's risk gene undermines insulation of brain's "wiring"

Carrying one copy of the APOE4 gene variant increases Alzheimer's disease risk threefold and two copies about tenfold, but the fundamental reasons why and what can be done to help patients remain largely unknown. A study published by an MIT-based team in *Nature* provides some new answers as part of broader research on APOE4's consequences cell type by cell type in the brain.

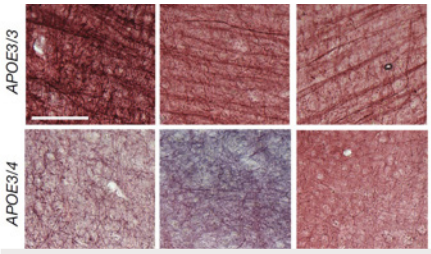
The new study combines evidence from postmortem human brains, lab-based human brain cell cultures, and Alzheimer's model mice to show that when people have one or two copies of APOE4, rather than the more common and risk-neutral APOE3 version, cells called oligodendrocytes mismanage cholesterol, failing to transport the fat molecule to wrap the long vine-like axon "wiring" that neurons project to make brain circuit connections. Deficiency of this fatty insulation, called myelin, may be a significant contributor to the pathology and symptoms of Alzheimer's disease because without proper myelination, communications among neurons are degraded.

Recent studies by the research group of Picower Professor Li-Huei Tsai, director of The Picower Institute and the Aging Brain Initiative, have found distinct ways that APOE4 disrupts how fat molecules, or lipids, are handled by key brain cell types including neurons, astrocytes and microglia. In the new study as well as in those, the team has identified compounds that appear in the lab to correct these different problems, yielding potential pharmaceutical-based treatment strategies.

The new study extends that work not only by discovering how APOE4 disrupts myelination, but also by providing the first systematic analysis across major brain cell types using single nucleus RNA sequencing (snRNAseq) to compare how gene expression differs in people with APOE4 compared to APOE3.

"This paper shows very clearly from the snRNAseq of postmortem human brains in a genotype specific manner that APOE4 influences different brain cell types very distinctly," Tsai said. "We see convergence of lipid metabolism being disrupted, but when you really look into further detail at the kind of lipid pathways being disturbed in different brain cell types, they are all different.

"I feel that lipid dysregulation could be this very fundamental biology underlying a lot of the pathology we observe," she said.



"Black Gold" staining shows people with two copies of the APOE3 variant have much more myelin than people (bottom row) with a copy of APOE4.

The paper's lead authors are Joel Blanchard, an assistant professor at Mt. Sinai's Icahn School of Medicine who began the work as a postdoc in Tsai's MIT lab, Djuna Von Maydell and Leyla Akay, who are graduate students in Tsai's lab, and Jose Davila Velderrain, a research group leader at Human Technopole and former postdoc in the lab of co-corresponding author Manolis Kellis, Computer Science Professor at MIT.

The team found that APOE4-carrying oligodendrocytes exhibited greater expression of cholesterol synthesis genes and disruptions to cholesterol transport. The more APOE4 copies people had, the greater the effect. Using a variety of techniques to look directly at the tissue, the team saw that in APOE4 brains, aberrant amounts of cholesterol accumulated within cell bodies, especially of oligodendrocytes, but was relatively lacking around neural axons.

Applying the drug cyclodextrin to APOE4 oligodendrocytes cultured in a dish reduced accumulation of cholesterol within the cells and improved myelination in co-cultures with neurons. Moreover, it also had these effects in APOE4 mice.

Small **40Hz** sensory stimulation studies confirm safety, suggest Alzheimer's benefits

A pair of early stage clinical studies testing the safety and efficacy of 40Hz sensory stimulation to treat Alzheimer's disease (AD) has found that the potential therapy was well tolerated, produced no serious adverse effects and was associated with some significant benefits among a small cohort of participants.

"In these clinical studies we were pleased to see that volunteers did not experience any safety issues and used our experimental light and sound devices in their homes consistently," said Li-Huei Tsai, Picower Professor in the The Picower Institute and senior author of the paper describing the studies in *PLOS ONE*. "While we are also encouraged to see some significant positive effects on the brain and behavior, we are interpreting them cautiously given our study's small sample size and brief duration. These results are not sufficient evidence of efficacy, but we believe they clearly support proceeding with more extensive study of 40Hz sensory stimulation as a potential non-invasive therapeutic for AD."

Dr. Diane Chan (left) meets with Professor Li-Huei Tsai (right).

In three studies spanning 2016-2019, Tsai's lab discovered that exposing mice to light flickering or sound clicking at the gamma-band brain rhythm frequency of 40Hz – or employing the light and sound together – produced widespread beneficial effects. Treated mice modeling AD pathology experienced improvements in learning and memory; reduced brain atrophy, neuron and synapse loss; and showed lower levels of the hallmark Alzheimer's proteins amyloid beta and phosphorylated tau compared to untreated controls. The stimulation appears to produce these effects by increasing the power and synchrony of the 40Hz brain rhythm, which the lab has shown profoundly affects the activity of several types of brain cells, including the brain's vasculature.

Based on those encouraging results, Diane Chan, a neurologist at Massachusetts General Hospital and a postdoctoral clinical fellow in Tsai's lab, led the two new clinical studies. The "Phase 1" study enrolled 43 volunteers of various ages including 16 people with early stage AD to confirm that exposure to 40Hz light and sound was safe and test whether it increased 40Hz power and synchrony after a few minutes of exposure, as measured with EEG electrodes. The study also included two patients with epilepsy at the University of Iowa who consented to having measurements taken in deeper brain structures during exposure to 40Hz sensory stimulation while undergoing epilepsy-related surgery.

The second set of tests, a "Phase 2A" pilot study, enrolled 15 people with early stage Alzheimer's disease in a blinded, randomized, controlled study to receive stimulation for an hour a day for at least three months. They underwent baseline and follow-up visits including EEG measurements during stimulation, MRI scans of brain volume, and cognitive testing. Volunteers used stimulation devices in their homes (a light panel synchronized with a speaker and video cameras to monitor device usage). Participants also wore sleep-monitoring bracelets.

The Phase 2A trial launched just before the onset of the Covid-19 pandemic in 2020, causing some participants to become unable to undergo follow ups after three months. The study therefore only reports results through a four-month period.

Phase 1 volunteers filled out a questionnaire on side effects, reporting a few minor but no major adverse effects. Measurements taken with EEG scalp electrodes clustered at frontal and occipital sites showed significant increases in 40Hz rhythm power at each cortical site among cognitively normal younger and older participants as well as volunteers with mild Alzheimer's. The readings also demonstrated significant increase in coherence at the 40Hz frequency between the two sites. In the two volunteers with epilepsy, measurements showed significant increases in 40Hz power in deeper brain regions such as the gyrus rectus, amygdala, hippocampus and insula with no adverse events including seizures.

In Phase 2A, neither treated nor control volunteers reported serious adverse events. Both groups used their devices 90 percent of the time. The eight volunteers treated with 40Hz stimulation experienced several beneficial effects that reached statistical significance compared to the seven volunteers in the control condition. Control participants exhibited two signs of brain atrophy as expected with disease progression: reduced volume of the hippocampus and increased volume of open spaces, or ventricles. Treated patients did not experience significant changes in these measures. Treated patients also exhibited better connectivity across brain regions involved in the brain's default mode and medial visual networks, which are related to cognition and visual processing respectively. Treated patients also exhibited more consistent sleep patterns than controls.

Neither the treatment and control groups showed any differences after just three months on most cognitive tests, but the treatment group did perform significantly better on a face-name association test, a memory task with a strong visual component.

Synapses help hold information in mind

How do we hold information, like a Wi-Fi password we've just been told, in mind? MIT neuroscientists have published a key new insight to explain how it works.

In a study in *PLOS Computational Biology*, scientists at The Picower Institute compared measurements of brain cell activity in an animal performing a working memory task with the output of various computer models representing two theories of the underlying mechanism for holding information in mind. The results strongly favored the newer notion that a network of neurons stores the information by making short-lived changes in the pattern of their connections, or synapses, and contradicted the traditional alternative that memory is maintained by neurons remaining persistently active (like an idling engine).

While both models allowed for information to be held in mind, only the versions that allowed for synapses to transiently change connections ("short-term synaptic plasticity," or STSP) produced neural activity patterns that mimicked what was actually observed in real brains at work. The idea that brain cells maintain memories by being always "on" may be simpler, acknowledged senior author Earl K. Miller, but it doesn't represent what nature is doing and can't produce the sophisticated flexibility of thought that can arise from intermittent neural activity backed up by STSP.

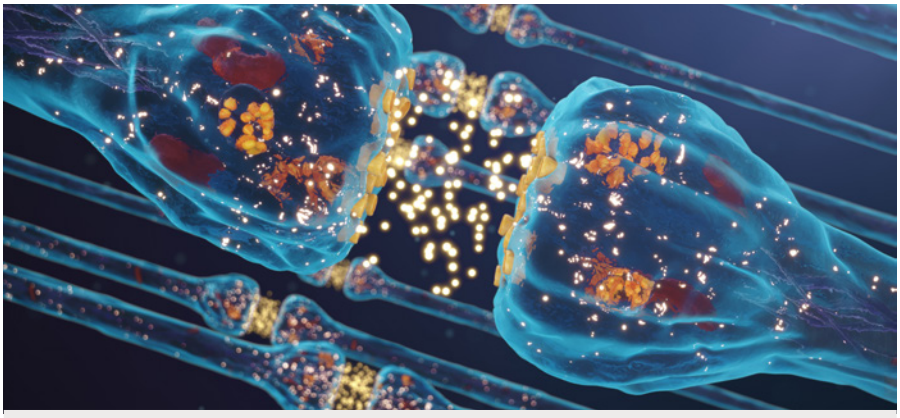
"You need these kinds of mechanisms to give working memory activity the freedom it needs to be flexible," said Miller, Picower Professor of Neuroscience. "If working memory was just sustained activity alone, it would be as simple as a light switch. But working memory is as complex and dynamic as our thoughts."

Co-lead author Leo Kozachkov, who earned his PhD at MIT in November for theoretical modeling work including this study, said matching computer models to real-world data was crucial.

"Most people think that working memory 'happens' in neurons—persistent neural activity gives rise to persistent thoughts. However, this view has come under recent scrutiny because it does not really agree with the data," said Kozachkov who was co-supervised by co-senior author Jean-Jacques Slotine, a professor in Brain and Cognitive Sciences and mechanical engineering. "Using artificial neural networks with STSP, we show that synaptic activity (instead of neural activity) can be a substrate for working memory. The important takeaway from our paper is these 'plastic' neural network models are more brain-like, in a quantitative sense, and also have additional functional benefits in terms of robustness."

Alongside co-lead author John Tauber, an MIT graduate student, Kozachkov's goal was not just to determine how working memory information might be held in mind, but to shed light on which way nature actually does it. That meant starting with "ground truth" measurements of the electrical "spiking" activity of hundreds of neurons in the prefrontal cortex of an animal as it played a working memory game. In each of many rounds the animal was shown an image that then disappeared. A second later it would see two images including the original and had to look at the original to earn a little reward. The key moment is that intervening second, called the "delay period," in which the image must be kept in mind in advance of the test.

The team consistently observed what Miller's lab has seen many times before: The neurons spike a lot when seeing the original image, spike only intermittently during the delay, and then spike again when the images must be recalled during the test (these dynamics are governed by an interplay of beta and gamma frequency brain rhythms). In other words, spiking is strong when information must be initially stored and when it must be recalled but is only sporadic when it has to be maintained. The spiking is not persistent during the delay.



Short-term changes in neural connections, called synapses, help us hold information in working memory.

Researchers have theorized that changes in the relative strength, or "weights," of synapses could store the information instead. The MIT team put that idea to the test by computationally modeling neural networks embodying two versions of each main theory. As with the real animal, the machine learning networks were trained to perform the same working memory task and to output neural activity that could also be interpreted by a software "decoder."

The computational networks that allowed for short-term synaptic plasticity to encode information spiked when the actual brain spiked and didn't when it didn't. The networks featuring constant spiking as the method for maintaining memory spiked all the time including when the natural brain did not. And the decoder results revealed that accuracy dropped during the delay period in the synaptic plasticity models, as it did in the natural data, but remained unnaturally high in the persistent spiking models.

Diverse neural connections help make **perception** reliable, efficient

The brain's cortex produces perception based on the sensory information it's fed through a region called the thalamus. Despite the importance of thalamic input to the cortex, neuroscientists have struggled to understand how it works so well given the relative paucity of observed connections, or "synapses," between the two regions.

To help close this knowledge gap, Elly Nedivi, William R. and Linda R. Young Professor, assembled a collaboration within and beyond MIT to apply several innovative methods. In *Nature Neuroscience*, the team reports that thalamic inputs into superficial layers of the cortex are not only rare but also surprisingly weak and quite diverse in their distribution patterns. Despite this, they are reliable and efficient representatives of information in the aggregate, and their diversity is what underlies these advantages.

By meticulously mapping every thalamic synapse on 15 neurons in layer 2/3 of the visual cortex in mice and then modeling how that input affected each neuron's processing of visual information, the team found that wide variations in the number and arrangement of thalamic synapses made them sensitive to different visual stimulus features. While individual neurons therefore couldn't interpret all aspects of the stimulus, a small population of them could together reliably and efficiently assemble the overall picture.

"It seems this heterogeneity is not a bug, it's a feature that provides not only a cost benefit, but also confers flexibility and robustness to perturbation" Nedivi said.

Aygul Balcioglu, the research scientist in Nedivi's lab who led the work, added that the research has created a way for neuroscientists to track all the many individual inputs a cell receives as that input is happening.

"Our techniques give us the ability to describe in living animals where in the structure of the single cell what kind of information gets incorporated. This was not possible until now," Balcioglu said.

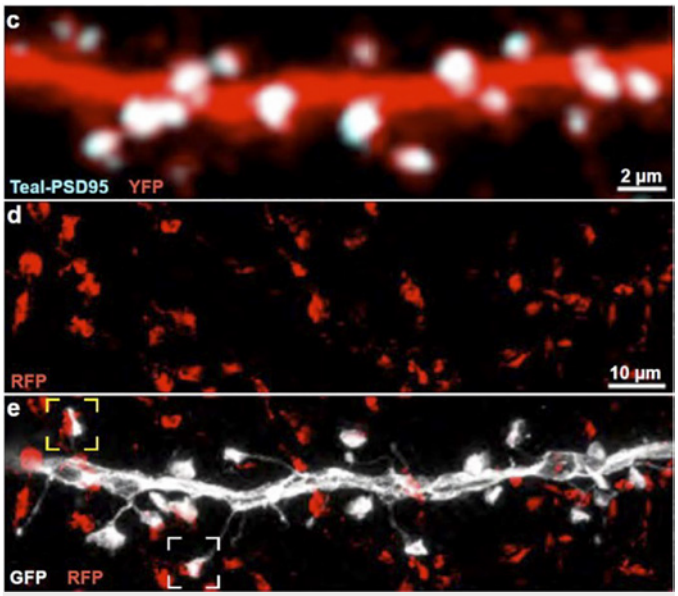
The team used a technique established in Nedivi's lab that enables observing whole cortical neurons under a two-photon microscope using three different color tags to label cortical neurons, their synaptic connections and inputs from the thalamus. Where ever the color of the thalamic inputs overlapped with the color labeling excitatory synapses on the cortical neurons, that revealed the location of putative thalamic inputs onto the cortical neurons.

Two-photon microscope resolution is not sufficient to confirm that the overlapping labels are indeed synaptic contacts. To confirm their first indications of thalamic inputs, the team turned to a technique called MAP invented in The Picower Institute lab of Associate Professor Kwanghun Chung. MAP enlarges tissues, effectively increasing the resolution of standard microscopes. Researchers used the techniques to resolve, count, map, and even measure the size of all thalamic-cortical synapses onto entire neurons.

Thalamic inputs were rather small (typically presumed to also be weak and maybe temporary), and accounted for between 2 and 10 percent of the excitatory synapses on visual cortex neurons. The variance in thalamic synapse numbers occurred between whole cells and across different "dendrite" branches of the same cells, accounting for anywhere between zero and nearly half the synapses on a given branch.

Nedivi's team faced a conundrum: If the thalamic inputs were weak, sparse and widely varying then how good could they be for reliable information transfer?

Nedivi turned to colleague Idan Segev, a professor at Hebrew University in Jerusalem specializing in computational neuroscience to create a biophysically faithful model of the cortical neurons.



A segment of a cortical neuron dendrite. Top: Two-photon image showing red cell fill and synapse label PSD-95 (teal). Middle: Same segment processed with MAP labeled with anti-RFP (red) to label thalamic boutons. Bottom: Same MAP-processed segment labeled with white cell fill. Boxes note thalamocortical synapses where red and white meet.

Segev's model showed that when the cells were fed visual information their electrical responses varied based on how their thalamic input varied. Some cells perked up more than others in response to different aspects of the visual information, such as contrast or shape, but no single cell revealed much about the overall picture. But with about 20 cells together, the whole visual input could be decoded from their combined activity—a so-called "wisdom of the crowd."

Segev compared the performance of cells with the weak, sparse and varying input to the performance of a group of cells that all acted like the best single cell of the lot. Up to about 5,000 total synapses, the "best" cell group delivered more informative results but after that level, the small, weak and diverse group performed better.

How **Huntington's disease** affects different neurons

In Huntington's disease patients, neurons in a part of the brain called the striatum are among the hardest-hit.

Neuroscientists at MIT have now shown that two distinct cell populations in the striatum are affected differently by Huntington's. They believe that neurodegeneration of one population, in a structure called the matrix, leads to motor impairments, while damage to the other population, located in structures called striosomes, may account for the mood disorders often seen in the early disease stages.

"As many as 10 years ahead of the motor diagnosis, Huntington's patients can experience mood disorders, and one possibility is that the striosomes might be involved in these," said Ann Graybiel, an MIT Institute Professor, and a study senior author.

Using single-cell RNA sequencing to analyze the genes expressed in mouse models of Huntington's and postmortem brain samples from Huntington's patients, the researchers found that cells of the striosomes and the matrix begin to lose their distinguishing features as the disease progresses. The researchers hope that their findings could help lead to new treatments that target specific cells.

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

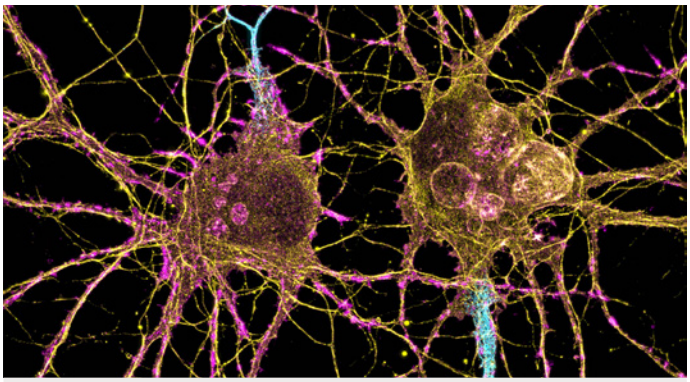
This kind of analysis could also shed light on other brain disorders that affect the striatum, such as Parkinson's disease and autism spectrum disorder, the researchers say.

Manolis Kellis, MIT professor of computer science is also a senior author. Ayano Matsushima, a McGovern Institute research scientist and Sergio Sebastian Pineda, a graduate student in the Heiman and Kellis Labs, are the lead authors of the paper in *Nature Communications*.

Within the striatum, neurons can be classified as either D1 or D2 neurons. D1 neurons are involved in the "go" pathway, which initiates an action, and D2 neurons are part of the "no-go" pathway, which suppresses an action. D1 and D2 neurons can both be found within either the striosomes or the matrix.

The analysis of RNA expression in each of these types of cells revealed that striosomal neurons are harder hit by Huntington's than matrix neurons. Furthermore, within the striosomes, D2 neurons are more vulnerable than D1.

The findings suggest that damage to the striosomes, which are known to be involved in regulating mood, may be responsible for the mood disorders that strike Huntington's patients in the early stages of the disease. Later on, degeneration of the matrix neurons likely contributes to the decline of motor function, the researchers say.



Two distinct cell populations in the striatum are affected differently by Huntington's disease. Image credit: Leterrier, NeuroCyto Lab, INP, Marseille, France.

Self-assembling proteins can store cellular **"memories"**

As cells perform everyday functions, they turn on a variety of genes and cellular pathways. MIT engineers have now coaxed cells to inscribe the history of these events in a long protein chain that can be imaged using a microscope.

Cells programmed to produce these chains continuously add building blocks that encode particular cellular events. Later, the ordered protein chains can be fluorescently labeled, allowing researchers to reconstruct the timing of the events. The technique could help reveal steps that underlie processes such as memory formation or response to drug treatment.

"There are a lot of changes that happen at organ or body scale, over hours to weeks, which cannot be tracked over time," said Edward Boyden, the Y. Eva Tan Professor in Neurotechnology at MIT and an affiliate member of The Picower Institute. If the technique could be extended to work over longer time periods, it could also be used to study processes such as aging and disease progression, the researchers say.

Boyden is the senior author of the study in *Nature Biotechnology*. Changyang Linghu, a former J. Douglas Tan postdoc at the McGovern Institute for Brain Research is the lead author.

One way to study cellular functions is to image proteins, RNA, or other molecules inside the cells, which provide hints to what the cells are doing. However, most methods for doing this offer only a glimpse of a single moment in time, or don't work well with very large populations of cells.

"The human brain has 86 billion cells," Linghu said. "To understand those kinds of biological systems, we need to observe physiological events over time."

To achieve that, the research team came up with the idea of recording cellular events as a series of protein subunits that are continuously added to a chain. To create their chains, the researchers used engineered protein subunits, not normally found in living cells, that can self-assemble into long filaments.

Are **covid** 'comas' signs of a protective hibernation state?

Many Covid-19 patients who have been treated for weeks or months with mechanical ventilation have been slow to regain consciousness even after being taken off sedation. An article in the *Proceedings of the National Academy of Sciences* offers the hypothesis that this peculiar response could be the effect of a hibernation-like state invoked by the brain to protect cells from injury when oxygen is scarce.

A very similar kind of state is observed in cardiac arrest patients treated by chilling their body temperature, a method called "hypothermia," and in the painted turtle, which has evolved a form of self-sedation to contend with long periods of oxygen deprivation, or "hypoxia," when it overwinters underwater.

"We propose that hypoxia combined with certain therapeutic maneuvers may initiate an as yet unrecognized protective down-regulated state (PDS) in humans that results in prolonged recovery of consciousness in severe COVID-19 patients following cessation of mechanical ventilation and in post-cardiac arrest patients treated with hypothermia," wrote authors Nicholas D. Schiff and Emery N. Brown. "In severe Covid-19 patients we postulate that the specific combination of intermittent hypoxia, severe metabolic stress and GABA-mediated sedation may provide a trigger for the PDS."

Brown is the Edward Hood Taplin Professor of Medical Engineering and Computational Neuroscience in The Picower Institute. Schiff is a Jerold B. Katz Professor of Neurology and Neuroscience in the Feil Family Brain Mind Research Institute at Weill Cornell Medicine.

Cardiac arrest patients treated with hypothermia, Covid-19 patients with prolonged wakeups after sedation and ventilation, and the hibernating painted turtle all exhibit a brain rhythm pattern called "burst suppression." A decade ago ShiNung Ching, Brown and colleagues described a model suggesting that burst suppression is an activity pattern signaling that the brain is reducing energy use when sufficient supplies are not available. In this way, the brain limits the damage that neurons could otherwise endure by trying to operate at full power.

The turtles appear to achieve this state by rapidly ramping up the release of GABA, a neurotransmitter chemical known to reduce neural activity, hours after oxygen becomes scarce. This GABA release reduces the energy demand of brain cells. The authors see a direct parallel in Covid patients who are often given sedatives whose effects are mediated by GABA.

If Brown and Schiff's hypotheses are correct, they write, then there may be a principled approach for better reviving Covid patients from unconsciousness after ventilation is removed.

Technology reveals cross-cutting breakdowns in **Alzheimer's**

The new research capability of single-cell profiling has allowed the Alzheimer's field to rapidly achieve long-sought insights with strong potential for both explaining Alzheimer's and doing something meaningful about it, according to a new review paper by Picower Institute scientists. For instance, results from such research show that the disease's disruptions converge on five main areas of cellular function, or "pathways," in each of five major brain cell types.

Single-cell profiling technologies produce comprehensive measurements of genetic activity in individual cells, such as levels of RNA which is transcribed from DNA, so that the cell's functions and roles in the biology of the brain, and the pathology of disease, can be assessed. Single-cell profiling technologies go beyond genome sequencing, which catalogs the DNA present in most every cell of a person, by revealing how each cell is uniquely making use of that common set of instructions. In studying Alzheimer's, scientists have been using single-cell profiling to see how various brain cells, such as distinct types of neurons, microglia, and astrocytes, act differently in disease compared to how they behave in a healthy brain.

In the article, doctoral student Mitch Murdock and Picower Professor Li-Huei Tsai, director of The Picower Institute and Aging Brain Initiative, write that while the findings of single-cell profiling studies confirm that the disease's terrible effects are complex and far-reaching, there appear to be common pathways. Investigating these pathways, they write, could produce valuable biomarkers of disease and yield meaningful targets for therapeutic intervention:

- Inflammation and immune response
- Lipid (fat molecule) signaling and metabolism
- Metabolic stress and protein folding
- DNA damage and cellular senescence (aging)
- Interactions with brain vasculature (blood vessels)

For each of these pathways in neurons, microglia, astrocytes, oligodendrocytes and oligodendrocyte precursor cells, Tsai and Murdock highlighted specific differences in gene regulation, identified in single-cell studies, that significantly occur in brains of Alzheimer's patients or mouse models compared to healthy control samples.

"By identifying vulnerable cell types and the molecular programs that give rise to them, therapeutic interventions might reverse aberrant cellular trajectories," Murdock and Tsai wrote in *Nature Neuroscience*. "While many transcriptional alterations are cell-type specific, these changes ultimately might converge on shared signaling pathways across cell types that might represent targets for new therapeutic strategies."

On the Cover: A cartoon depicts microglia, the brain's immune cells (green) amid globs of amyloid protein (yellow) and inflamed neurons (red). Image by Sputnik Animation

Immune & Inflamed

Neuroscientists are finding that immune system activity within the brain and the body has important impacts on mental health and behavior

When Associate Professor Myriam Heiman began studying neuroscience as a postdoc, scientists interested in neurodegenerative diseases had long reported evidence of inflammation, or the activation of immune responses, but few were claiming it was an early driver of disease.

"It was initially thought it might be perhaps more of a secondary response to neural damage," Heiman said.

Similarly, in the late 1990s as Picower Professor Li-Huei Tsai began focusing her research on understanding Alzheimer's disease, she had no inkling of how deeply she would now be delving into the central nervous system's immune activity.

But over the last several years research has increasingly shown that immune activity may have a core role in the progression of neurodegenerative diseases. Even when researchers don't set out in search of immune impacts, they find them anyway. Using broad, unbiased methods to detect ways in which cellular and molecular activity is altered by disease, Tsai and Heiman have each recently discovered specific new ways in which immune activity appears to be more prominent and consequential in Alzheimer's and Huntington's disease respectively.

"Our recent studies indicate it might be more of an initial contributor to pathogenesis," Heiman said. "There's a theme emerging across the studies of our labs and others in the community that perhaps neural inflammation isn't just a secondary reaction, but maybe among the primary toxicity mechanisms as well."

Tsai agrees: "Neural inflammation is a defining feature of Alzheimer's disease."

And while neuroscientists once insisted that the brain was "immune privileged," which is to say they thought it did not interact with the rest of the body's immune system, Mark Hyman Jr. Associate Professor Gloria Choi is among a small community of neuroscientists who are finding exactly such connections between peripheral immune system activity and the brain, including a link between maternal infection and the development of autism-like social deficits in offspring. By dedicating her lab to the study of how the immune and central nervous systems interact to influence brain function, disorders and social behavior, Choi has joined the vanguard of a relatively new field: "neuroimmunology."

Activity in Alzheimer's

In a recent review paper co-authored with graduate student Mitch Murdock, Tsai analyzed a recent outpouring of evidence neuroscientists have produced about Alzheimer's using "single-cell" techniques, in which they can assess how cellular activities such as gene expression differ in healthy brains vs. ones afflicted with disease (see p. 7). Tsai and Murdock found that in each of five major brain cell types, including neurons, one of the five most prominent changes were increases in immune and inflammatory activity. Moreover, Tsai says, other studies are showing that expression of genes associated with increased Alzheimer's risk, including the one of the most common, APOE4, is pronounced in many immune cells.

Tsai's own research employing broad-based single-cell sequencing techniques to better understand Alzheimer's has been revealing the importance of immune activity in the brain for years. In 2015 her lab collaborated with that of MIT computer scientist Manolis Kellis to show that the brains of Alzheimer's patients and lab mice modeling the disease both developed heightened genetic predisposition to inflammatory activity, particularly because of the enhancement of a gene transcription factor called PU.1 (now seen as a promising therapeutic target).

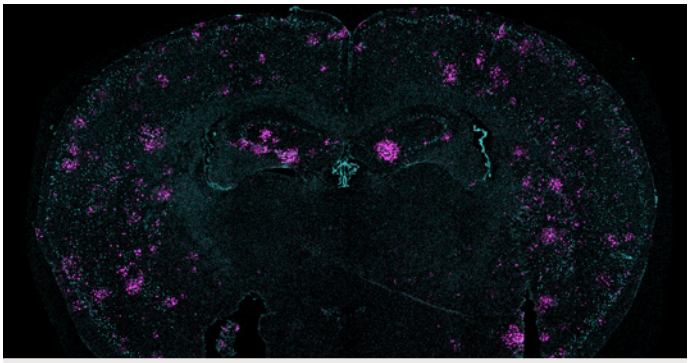
"We showed that the immune response is an extremely early event way before neurodegeneration," Tsai said.

Indeed in 2017 the lab followed up with a study focusing on how gene expression changed in the brain's resident immune cells, called microglia, as disease progressed. They found evidence that some microglia change their activity early and dramatically in disease, potentially triggering a cascade of inflammation that leads to neuron death.

And two studies published by Tsai's lab in just this past year illustrate new complexities of immune activity's integral role in Alzheimer's disease.

Postdoc Matheus Victor led a study published in August in which the lab sought to understand how APOE4 affects microglia. The APOE gene, of which APOE4 is a harmful variation, determines how cells handle fat molecules called lipids. APOE4 microglia, they found, are prone to becoming inflammatory, rendering them less adept at processing lipids. They accumulate more of the molecules, clear away less, and that, in turn, impedes the ability of nearby neurons to communicate by disrupting the potassium ion receptors they use to regulate electric charge.

Just a month later, graduate student Gwyneth Welch led the Tsai lab's discovery of another problematic immune interaction between neurons and microglia. When encoding memories, neurons normally snap open their DNA for quick gene expression. Tsai's lab has found that over time and with age, these breaks aren't repaired as well as they should be and therefore accumulate. Welch found that when this happens neurons emit molecular immune activation signals as if calling to microglia for help, a phenomenon that had not been observed before in Alzheimer's disease. The microglia respond to this neural instigation, but in an inflammatory way that leads to a degradation of neural circuit connections called synapses.



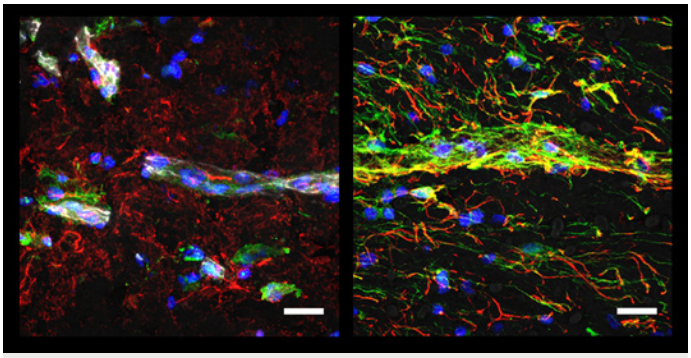
Mouse brain cross section shows a marker of double-stranded DNA breaks in teal and the immune system cytokine Cxcl10 in magenta.

It's not clear why immune responses, which are surely meant to protect health, can instead come to undermine it, but Tsai notes that the classic immune role of fighting off a pathogen is meant to be a short-term battle. In cases such as the double-stranded breaks associated with neurodegeneration, the problem is problematically prolonged.

"A pathogen invasion is an acute event," Tsai said. "The pathogen's there, microglia come in, get rid of whatever, and it's over. But I think the DNA breaks are pervasive. It's a chronic condition. So that leads to a cascade of events and it almost becomes the immune system attacking the self."

Happening in Huntington's

In her Huntington's disease research, Heiman has found evidence of what appears to be self-inflicted neuron death. In a 2020 study led by postdoc Hyeseung Lee, she teamed up with Kellis to assess gene transcription and translation in Huntington's disease patients and in model mice in neurons that have proven to be especially vulnerable in



In vessels of people with Huntington's disease (right image) researchers saw much higher indications of innate immune signaling (green) and much lower levels of a protein associated with blood-brain barrier integrity (white), than in people who did not have the disease (left image).

the disease. To their surprise they saw RNA not only from the nucleus, but also leaking from cells' damaged mitochondria. They also saw evidence of a highly elevated immune response to this misplaced genetic material that, to the cell, might have looked foreign. The inwardly turned immune response, signaled at least in part by the protein PKR, is likely toxic to the cell although the lab is following up to determine if it's really causal, Heiman said. But what's remarkable already, she said, is that this was the neuron's own "innate" immune response to its own mitochondrial RNA, which had not been appreciated before.

"What was surprising was how early we saw this innate immune activation in neurons in Huntington's disease," Heiman said.

In a 2022 study led by graduate student Francisco Garcia that mapped cell types in the brain's vasculature, Heiman and Kellis's labs found that in Huntington's disease PKR was also elevated in the endothelial cells that constitute the brain's blood vessels. The increased immune activity was strongly correlated with a loss of proteins that help form the blood-brain barrier, which could interfere with the brain's ability to keep out harmful substances.

Heiman hypothesizes that knocking down expression of PKR in cells might tamp down these potentially toxic immune responses.

Heiman's lab has also found other indications of immune response problems in Huntington's disease. In another study in 2020 led by postdoc Mary Wertz, her lab became the first to perform a genome-wide screen in mice of the consequences of knocking out each of 22,000 genes in neurons to see which genes, when lacking, proved lethal. The screen therefore revealed which genes were essential for neuronal survival. The lab then also did the screen in mice modeling Huntington's disease so that they could see, by comparison, whether knocking out each gene hurt neurons or helped. Among the "hits" in that work was the gene for a signal transducer for the immune signaling molecule Interleukin-6 (IL-6) among other molecules. Knocking it out seemed to benefit neural survival in Huntington's disease.

That result seemed to square with clinical reports suggesting that Huntington's disease patients experience elevated levels of IL-6 years before they show clinical symptom onset. Researchers including Heiman hypothesized that knocking out or blocking IL-6 receptors would therefore make Huntington's disease model mice healthier, but in a study later that year led by Wertz the lab surprisingly showed the opposite. Digging deeper into gene expression differences made by

having IL-6 vs. not having it, Heiman's lab noted that in a normal (non diseased) context the molecule may promote healthier synaptic connections among neurons. It may be that normal levels of IL-6 aren't the problem but that higher levels in disease are. Unresolved though it may be, the finding is still a lead that could yield a therapeutic target.

"The important thing to keep in mind is that these are new hypotheses in fields that have been wanting for therapeutics for decades," Heiman said. "It could be that there are immune relevant pathways that were underappreciated."

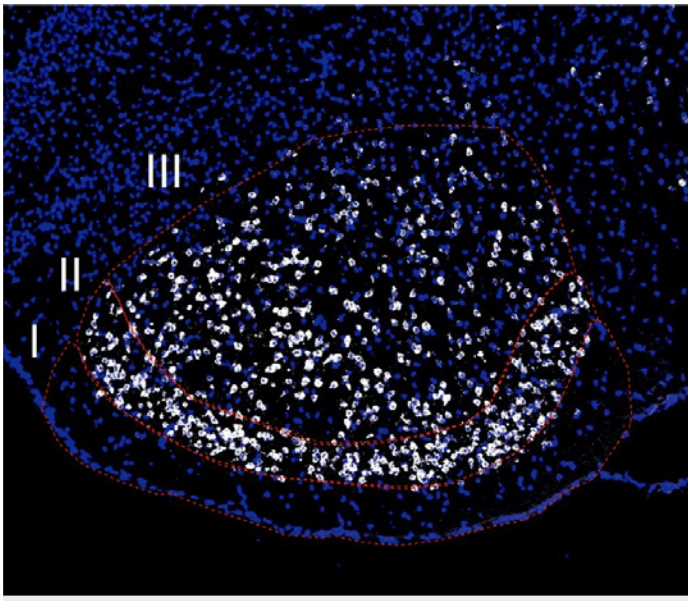
Bearing on behavior

Choi's work, meanwhile, is showing that immune pathways from beyond the brain (and maybe from the brain back out to the body) have profound impacts on brain functions, especially including social behavior.

Choi launched into neuroimmunology not long after starting her MIT lab in 2013. She and her husband Jun Huh, an immunology associate professor at Harvard Medical School, decided to tackle a puzzling clinical question: Why is infection during pregnancy associated with the development of autism in the child? Over several years the couple's labs found the link in mouse models: When a pregnant mouse has certain bacteria in her microbiome and becomes infected, immune cells overproduce the cytokine IL-17a. The molecule reaches receptors in the fetal brain. Cortical development in the fetus becomes disrupted and is characterized later in life by neural hyperactivity in circuits leading from the S1DZ region of the cortex to areas affecting social behavior, producing autism-like symptoms. Choi is now looking more deeply at IL-17a's role in the S1DZ region, as well as its links to social behavior.

In 2021 Choi's lab also found a specific brain circuit in a region called the amygdala that enforces a form of social distancing. Led by postdoc Jeongtae Kwon, the lab showed that when a male mouse smells that a potential mate is ill, he will restrain that mating instinct. Published amid peaks of Covid-19 surges, the study provided a timely link between social behavior and immune behavior that the lab is continuing to study intently. In early-stage experiments the lab is finding that some degree of social interaction (but not mating) between apparently sick and well cage mates "primes" the immune system of well mice such that when they are then challenged with infection they fare better than cage mates who don't have any interaction with a sick cage mate. Relatedly, postdoc Yire Jeong recently earned a competitive grant from the MIT School of Science John W. Jarve (1978) Seed Fund for Science Innovation, to investigate why group-housed mice appear to fight off sickness better than isolated mice upon exposure. Jeong's hypothesis, Choi said, is that social interaction may affect immune pathways from the brain to perhaps prime immune cells.

"There's a benefit to social interaction, but also there's a risk that comes from interacting with the others in terms of increased exposure to infections," Choi said. "Many of the interactions between the immune system and brain may have evolved to modulate social behaviors because social behaviors, I think have such a profound impact on how we deal with pathogens."



White staining in the mouse amygdala highlights the hormone TRH, which mediates a circuit that prevents mating when a partner smells sick.

Choi is also embarking on new studies to systematically survey the brain for other receptors for immune system molecules—cytokines and chemokines—like the ones that give IL-17a a foothold in the brain. With funding from MIT's Aging Brain Initiative and the Ludwig Family Foundation, she's also looking at how this changes with age.

"One of the major things we're trying to do is to map out all the communication channels through which the immune system can influence brain function," Choi said. "By doing that we've been discovering how different cytokines and chemokines can modulate different brain regions in neural circuits for specific behaviors or internal states."

For instance, early data indicates that immune system molecules in the brain can affect anxiety and feeding behaviors, Choi said.

Such studies by Choi, Tsai and Heiman are all among the forefront of a transition in neuroscience in which the field has been moving from dismissing or merely noting immune activity in the brain to exploring, appreciating and understanding its considerable depth and importance in health and behavior.

"The important thing to keep in mind is that these are new hypotheses in fields that have been wanting for therapeutics for decades. It could be that there are immune relevant pathways that were underappreciated.

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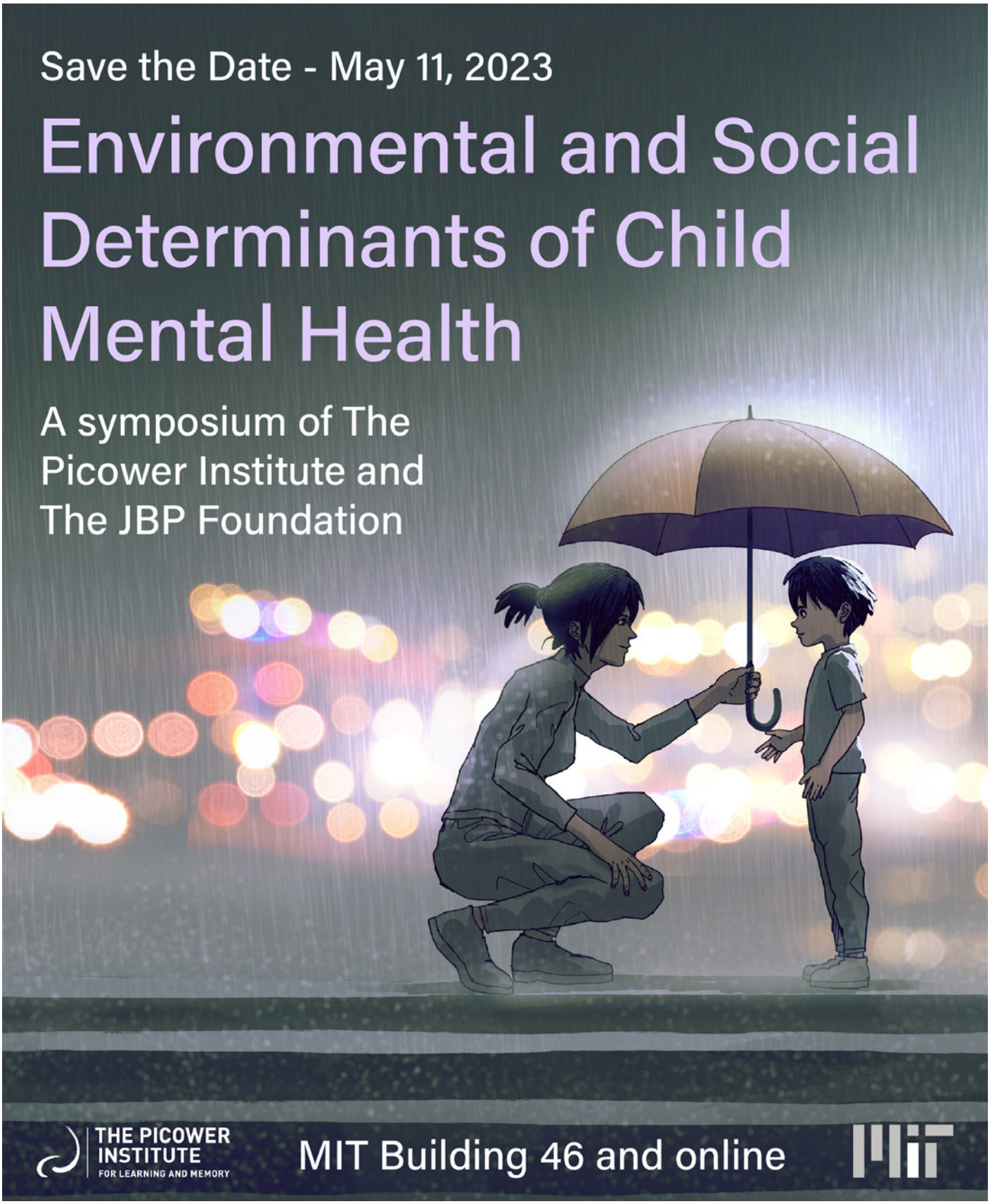

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Neuroscience News Spring 2023

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