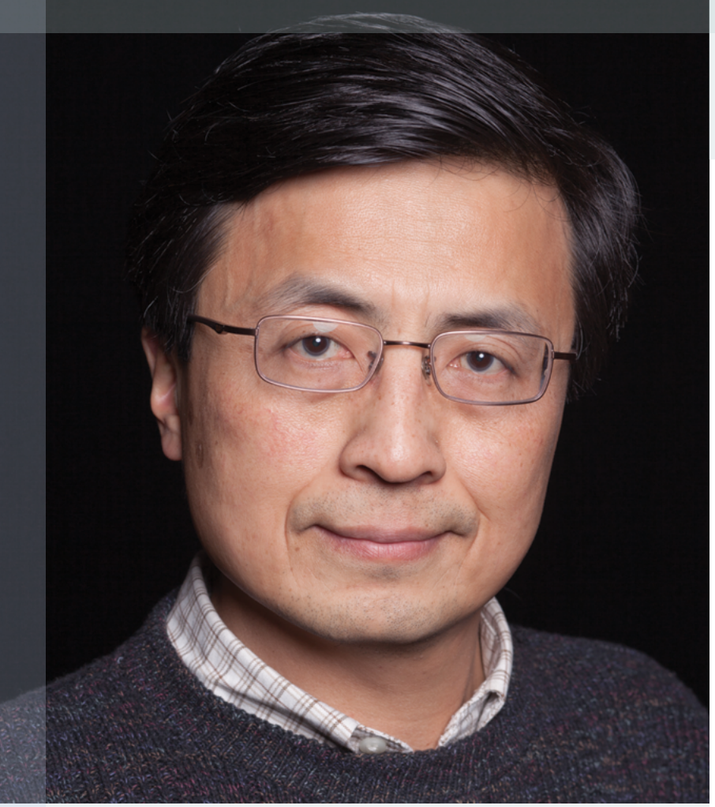


RESEARCH PROFILE

Z. Josh Huang

Exploring the cerebral cortex

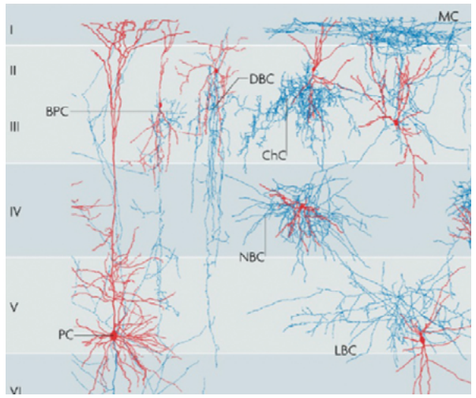
"An engineer will tell you that in order to understand how an electronic circuit works, you need to know the components. Well, that's not too difficult if you know resistors, transistors, etc. But in the human brain, things are pretty murky. We are only just beginning to get a fix on the types of cells that it contains."

The speaker, Z. Josh Huang, is the Marie and Charles Robertson Professor of Neuroscience at Cold Spring Harbor Laboratory. He explores the magnificent and profoundly complex territory of the cerebral cortex. Accounting for about 80% of the human brain's mass, the wrinkly cortex, the cerebrum's outer layer, is the site of neural processing that gives rise (in *homo sapiens*) to species-defining qualities such as consciousness, memory, attention, thought, and language. Over 3.5 billion years of life on Earth, says Huang, the human cerebral cortex is evolution's "supreme achievement."

It's also a place where small-scale anomalies can have outsized, often tragic, consequences. Huang is studying the cortex, typically in mice, our mammalian relatives, to learn more about neuronal subtypes: how they originate, develop, and form functional circuits. This basic research crucially informs efforts to discover what goes wrong, for instance, in autism spectrum disorders such as Rett syndrome and in schizophrenia, both of which Huang studies with collaborators.

"Studying the structure and function of the cortex has been a big problem for a very long time," says Huang. "It's like being in the Amazonian jungle, surrounded by all kinds of trees and vines and other plants that are different but look similar. How do you keep from getting hopelessly lost? How do you make sense of it?" In 2011 his lab crowned five years of often frustrating effort with a great success: they delivered to the worldwide neuroscience community a series of genetically engineered mouse lines, each of which enables investigators to isolate, identify, and track in living animals one of a dozen distinct cortical cell subtypes.

All of the subtypes rendered visible in Huang's engineered mice (the series continues to expand) are similar in one important respect. All are inhibitory neurons, one of the two basic neuronal types. Inhibitory neurons are sometimes called GABAergic cells after the neurotransmitter (gamma aminobutyric acid, or GABA) that they release and that triggers inhibition. They have the crucial role of modulating circuits composed of the other basic neuronal type, excitatory cells, which dominate the cortex. Despite the inhibitory function they have in common, each GABA cell subtype is in some way distinct, and until dissemination of Huang's mouse lines, they were impossible to study cortex-wide in a systematic and consistent manner.



Schematic of 6-layered mouse cerebral cortex. Cell labeled 'PC' (far left) is a pyramidal neuron, the most common excitatory cell type. Its dendrites (output channels, in red) cut across many layers. Inhibitory cells feature profuse axons (input channels, blue) whose reach is local, often confined to a single cortical layer.

"Our method brings the study of cortical cell types from out of the realm of art and into that of science, where everyone can be sure they are working on the same kind of cells," Huang says. He likens his team's achievement to the building of a GPS system for cortical inhibitory neurons, but better: "because GPS can only show you where you are, while in our mice you can observe how the cells got to where they are—you can see where they are born and how they migrate into their characteristic positions in the maturing cortex."

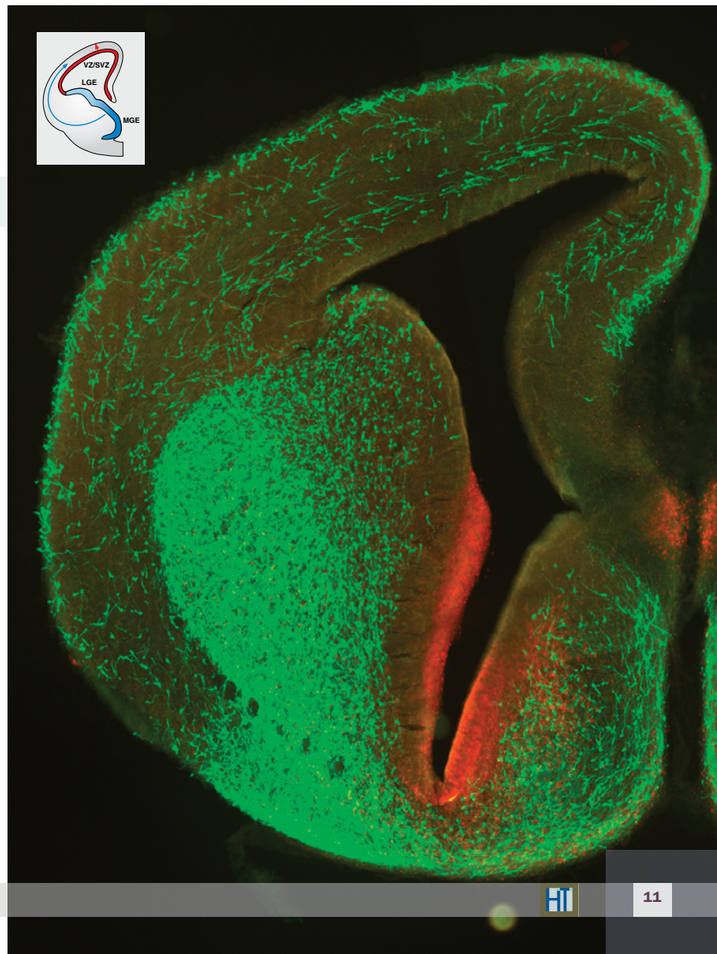
Genetic handle

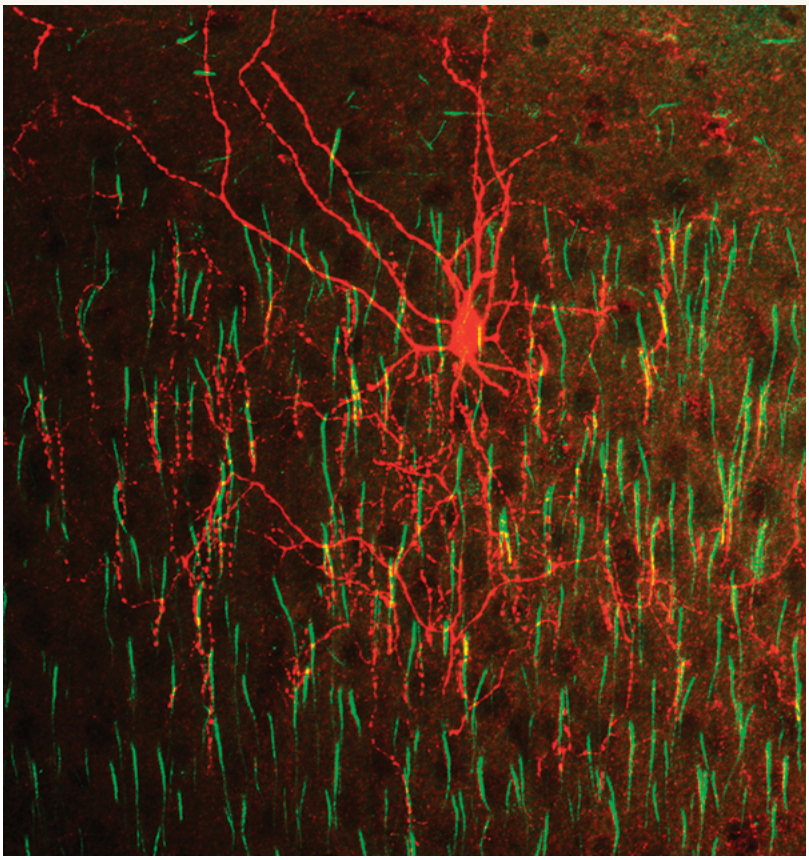
A native of Beijing who came to America in 1986, Huang appreciates the advantage of having grown up in the age of molecular biology. By the time he reached MIT as a postdoctoral researcher in 1995 after earning his Ph.D. at Brandeis, he knew he "wanted to build tools that would enable me to systematically study a problem that had been very difficult for classical neuroscience to address." His GPS system for inhibitory neurons is precisely this kind of advance, for it provides a handle with which to tag or manipulate specific subtypes of neurons. Importantly, the handle is a genetic one. This makes it very powerful.

To explain why, Huang points out that the architecture of the human brain is fundamentally similar in each individual. What makes each of us unique is partly a function of experience and how it tweaks nature's basic design for the brain, by making some circuits as well as synapses between nerve cells stronger or weaker than others. Yet the underlying structural and functional similarity across individual brains is a product of the genetic program that guides brain development. "Genes provide the finest scalpels for circuit dissection," he says.

In their GPS project—"a *gene*-based cell positioning system"—Huang's team figured out how to genetically engineer mice in which a particular gene or genes of interest, ideally gene(s) expressed by a single subtype of inhibitory cell, becomes a place in the genome where a molecular

Migratory route of a GABA cell subtype. Only cells expressing the gene *Dlx5* glow green. It's one of a family of regulatory genes switched on at various times in an inhibitory cell's life—here, to regulate the young cell's journey from its birthplace (area labeled MGE, inset) along a curving path (blue arrow, inset) into the cerebral cortex.





Master inhibitor: axons from a single chandelier cell (red) in the mouse cortex align precisely with green-labeled axon initial segments (AIS) emanating from many local excitatory neurons. By forming synapses at these points, one chandelier cell can halt or modulate signals coming from hundreds of excitatory cells.

tool can be attached. The tool might be a marker, like green fluorescent protein: in the animal bearing the modified gene, all brain cells expressing it will glow green. Or, one might splice in a gene that encodes a light-sensitive channelrhodopsin protein, so that any neuron expressing the protein can be activated or inactivated with a targeted beam of colored laser light.

Balancing inhibition and excitation

Neural circuits in the mature cortex depend on an exquisite balance between excitation and inhibition to function normally. "Keeping the balance is a very demanding task," notes Huang. "This is because the balance is not static; cortical cells are constantly receiving inputs, and the balancing process is going on all the time, responding on a time scale in the range of tens of milliseconds [thousandths of a second]."

If inhibitory cells were not present in cortical circuits, the circuits would seize up, as in epilepsy, due to an overload of excitation. Because they supply needed balance on a dynamic basis, inhibitory cells can be thought of as rendering local groupings of excitatory cells functional.

Since they enable the observation of specific cell types in living animals, Huang's technologies have opened a new window on disease processes. Some pathology in neuropsychiatric illnesses is assumed to be caused by errors in the way the cortex self-assembles; or by imbalances between excitation and inhibition that may originate in local circuitry in specific brain regions.

Huang and colleagues this past year solved a mystery about where in the brain a master-inhibitory cell type is born and how and when during development it navigates into the cortex. Called chandelier cells, each one of these rare gossamer structures "wires-up" to hundreds of excitatory cells, and acts as a kind of circuit breaker, capable of canceling out all of their input signals at once. Huang and Dr. David Lewis of the University of Pittsburgh are studying how schizophrenia pathology may be traceable to imbalances created by faulty or missing chandelier cells.

Recently, members of Huang's team have made progress in developing a set of markers and tools for excitatory neurons (also called pyramidal cells). This will provide long-sought information about their diversity and functional organization. There are probably hundreds of excitatory cell subtypes in the cerebral cortex, Huang says, but no one knows how many or how they are distinguished functionally.

Unlike inhibitory cells, which tend to act locally and therefore have short axonal projections that can be traced with comparative ease, excitatory neurons project great distances, often across brain hemispheres and all the way down to the spinal cord, rendering them impossible to follow at high resolution in real time as they interact with distant cortical regions.

Yet these are "the real information processing streams and output channels in our brain, and, in a sense, inhibitory cells are there mainly to help these excitatory cells work properly," Huang notes. "If we want to understand the cortex, we have to understand these cells. This will be a new chapter not just for my lab but for the entire field."

Peter Tarr