Anthony Zador

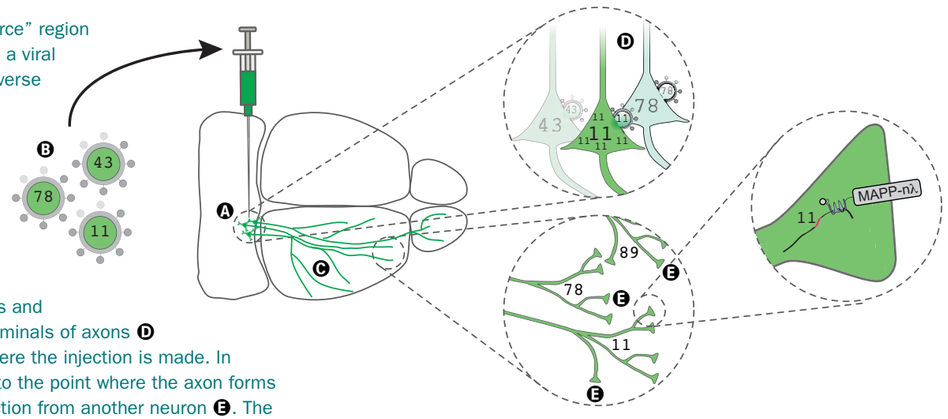
The signals travel along a network, sometimes connecting, other times going their own way, passing like unconnected thoughts in a cerebral night. A fabulously complex network of neurons that carries this information somehow represents everything in our minds, from our thoughts to our perceptions to our plans for action.

Anthony Zador, M.D., Ph.D., the Program Chair of Neuroscience and the Alle Davis Harrison Professor of Biology at Cold Spring Harbor Laboratory, would like to know how we go from what's in our heads to what's in our thoughts.

His overarching goal, he says, is to "understand how neural circuits form the basis for behavior." He wants to use the grey matter in his own brain to grasp how people "go from a circuit of 100 billion neurons, to complex behaviors, and, ultimately, to subjective experience."

He recently took an important step toward tracking the connections between those neurons, brain-wide, when a technology he has developed with several graduate students over the last 4 years bore fruit. Other researchers have gone to great lengths to see where individual neurons are located, using techniques like electron microscopy to map these fundamental units of the brain. While effective, these high-resolution approaches are laborious, expensive, and focus on tiny sections of the brain [facing page].

Zador's new approach [below] is revolutionary, mapping the brain not optically but with the help of gene sequencing technology. He has invented a way to hitch unique barcode-like identifiers to individual neurons. Those barcodes, made of long strings of RNA "letters," not only tag brain cells distinctively but also travel throughout the length of each cell's thread-like axonal projections, riding these all the way to the synapses where they connect with projections sent by other neurons. Using RNA sequencing, Zador can map the route through the brain that each of these axons travels.



An injection into a "source" region Ⓐ of the brain contains a viral library Ⓑ encoding a diverse collection of barcode sequences, which are hitched to an engineered protein that is designed to carry the barcode along axonal pathways Ⓒ. The barcode RNA is expressed at high levels and transported into the terminals of axons Ⓓ in the source region where the injection is made. In each neuron, it travels to the point where the axon forms a synapse with a projection from another neuron Ⓔ. The barcodes are sequenced when the brain is later dissected.

His team performed a proof of principle in the locus coeruleus (LC), a small group of neurons located in the brain stem. In a single experiment, they were able to trace axons from hundreds of LC neurons to their destinations in the cerebral cortex. The same technique can be used to trace projections from any neuron in the brain to any other region. The method's most immediate application—and advantage—is to trace the destinations of hundreds, thousands, even millions of neurons at a time, in a single, inexpensive and rapidly performed experiment.

Marrying molecular biology & neuroscience

Zador's talent and creativity has been recognized both in and out of science. He has received generous support from the Allen Brain Institute, and was selected in the inaugural class of Allen Distinguished Investigators. In 2015, he received an unexpected honor from *Foreign Policy* magazine, which named him one of 100 Leading Global Thinkers.

While marrying molecular biology and neuroscience is enabling Zador to tackle important questions about the brain, by his own admission he was an unlikely candidate to develop such an approach. "It's pretty ironic that I would undertake this," he acknowledges. In his earlier training, he had focused on developing other skills, and paid little attention to molecular biology.

He studied physics and linguistics at UC Berkeley, then went on to earn Ph.D. and M.D. degrees at Yale while conducting research in theoretical neuroscience and neural networks. During his medical training, he considered becoming a neurosurgeon before dedicating himself to research.

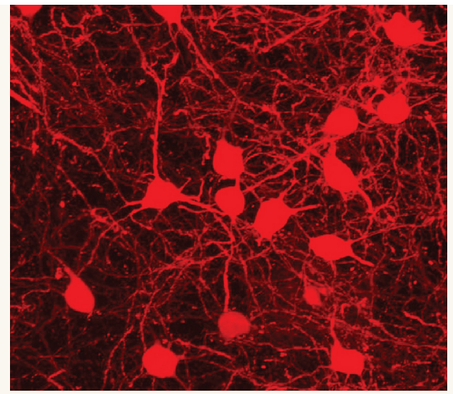
Zador conducted postdoctoral research at the Salk Institute in La Jolla. Arriving at CSHL in 1999, he put his lab to work on how the brain "computes"—how, for example, neurons represent sounds and how the processed signals are harnessed by other parts of the brain as a basis for acting and deciding. Over time, this work broadened to consider how neural circuits underlie cognition itself.

Zador's plunge into molecular biology began about 6 years ago, thanks, he says, to the support of faculty member Joshua Dubnau, who conveyed some of his expertise during their daily runs in the backroads behind the Lab. For an hour a day, four times a week, Dubnau served as an "advisor, sounding board and tutor."

More recently, working with talented graduate students and postdocs, including Hassana Oyibo, Ian Peikon, and Justus Kebschull, among others, Zador developed his novel neuron-mapping method, dubbed MAPseq (Multiplexed Analysis of Projections by Sequencing).

Visualizing the projectome

Early work with MAPseq set the stage for a broader effort to map the entire "projectome," Zador's first draft of the widely popularized concept of the "connectome." The latter—a wiring map of the brain—has animated neuroscience researchers and helped to inspire President Obama's "Brain Initiative," announced in 2013.



This high-resolution image of neurons and their projections shows the obvious limitations of optical technology in tracing individual neuronal paths.

While the object of a connectome is to map every connection in the brain, the projectome is a major step toward it. "In a limited number of inexpensive experiments, we are already able to indicate the destinations of axons from a vast numbers of neurons. We've focused first on neurons in the cerebral cortex," Zador says.

These data—from the cortex and a few related subcortical regions—have been processed by his team in the form of

a digital 3D rendering of the brain that can be oriented by dragging a computer mouse. These detailed maps are based on data obtained in just a few experiments, performed over several weeks, at a cost of a few thousand dollars.

Moving from a projectome to a connectome—in which not only the destinations but also the connections between nerve-cell projections will be registered—will not be easy, but it is a goal Zador defends as worthy. To date, scientists have generated a full connectome for just one living creature, the tiny roundworm *C. elegans*, with a mere 302 neurons connected by 7,000 synapses. Knowledge of this connectome has not had a transformative impact. But this is precisely because of its simplicity, Zador argues. "When you scale up to a brain with 100 billion neurons, there must be systematic rules and principles that generate the wiring, and knowing these rules is going to be extremely valuable."

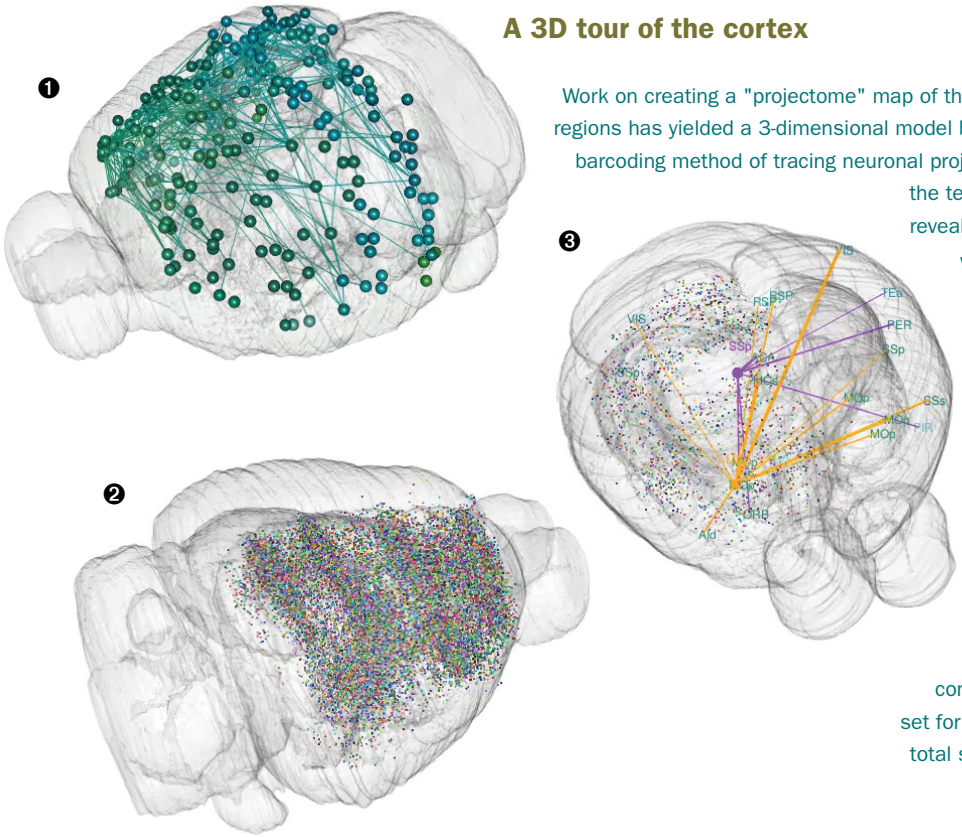
Hence his goal of revealing the mouse connectome, a project with likely implications for numerous human health challenges. "We think important neuropsychiatric disorders like autism and schizophrenia and conditions like addiction derive in part from mis-wiring the brain," he says.

Once researchers have fuller grasp on the range of natural variation in the connectome for healthy mice, they may find specific, characteristic differences in those of mice that model a variety of neuropsychiatric conditions, ranging from autism to schizophrenia to bipolar disorder. At some point down the road, this could lead to therapeutic targets or novel interventions.

More broadly, Zador expects that building a connectome will lead to a specific understanding of how the brain works. He likens attaining this architectural and structural awareness to the ground-breaking discovery of the double-helical shape of DNA. "How does the genetic material replicate? The *structure* of DNA provided the answer to that fundamental question." So, too, Zador hopes, will the structure of the mammalian brain suggest answers to questions about how we go from the material in our heads to the thoughts and inspirations in our minds.

Daniel Dunaief

A 3D tour of the cortex



Work on creating a "projectome" map of the cerebral cortex and several important subcortical regions has yielded a 3-dimensional model based on a single set of experiments using Zador's barcoding method of tracing neuronal projections. These images are stills from an animation the team made, taking viewers on a tour of the cortex as revealed by the approximately 50,000 individual neurons whose axonal paths were traced. In image ❶, each of the green spheres represents a site in the cortex where barcodes were extracted and sequenced. Each contains ~500 to 1000 neurons. The green lines between the spheres represent the strongest connections emanating from each of the spheres. In image ❷, the cloud of multicolored dots—each a barcoded neuron—reminds us that there are actually ~50,000 neurons represented in the green spheres shown in image ❶. In image ❸, the team selected 2 out of the 50,000 neurons and rendered the path of each cell's strongest connections. This is merely a demonstration; the data set for these experiments makes it possible to render the total set of connections for each of the 50,000 neurons.