

nerve cell navigation

Nerve cells exploit a complex set of cues to wire up properly in developing organisms. A closer view of this process may ultimately help to correct neurological problems that lead to schizophrenia and other disorders.

by R. John Davenport | illustration by Jon Han





For a human embryo, fashioning a nervous system is a daunting challenge. It must direct a tube of cells to morph into the brain and spinal cord and form the one hundred billion neurons, or nerve cells, that twist and turn through the body to link the brain to every limb and organ.

Each neuron sends a long cable—an axon—to branch toward its destination, connecting to as many as a thousand other cells along the way. These branches enable every muscle, organ, gland, and bit of skin to transmit control signals. Spindly arms at the opposite end of the neuron, called dendrites, receive information from other neurons.

How this maze of neurons establishes itself seems mind-boggling. Yet scientists are beginning to discover the navigational signs that guide neurons and help them compete for survival. This knowledge not only sheds light on the fascinating problem of how brains establish their wiring but also reveals how faults can lead to problems. “Understanding normal wiring will help us understand miswiring,” says former HHMI investigator Marc Tessier-Lavigne, now executive vice president of research drug discovery at Genentech, Inc., in South San Francisco. “And how it might be possible to rewire the brain following injury or disease.”

Sniffing Out a Wiring Diagram

Since the late 1800s, neuroscientists have used Golgi staining, named for its inventor, Italian Nobel laureate Camillo Golgi, to trace the paths of neurons. In fixed tissue, Golgi staining randomly colors a small number of neurons and reveals their complex structure: the bulbous cell body in the middle, a bushy canopy of dendrites on one side, and the long sinewy cable-like axon on the other. But Golgi staining has

its limitations. For example, it cannot tint specific types of neurons, or neurons in which a particular gene is mutated.

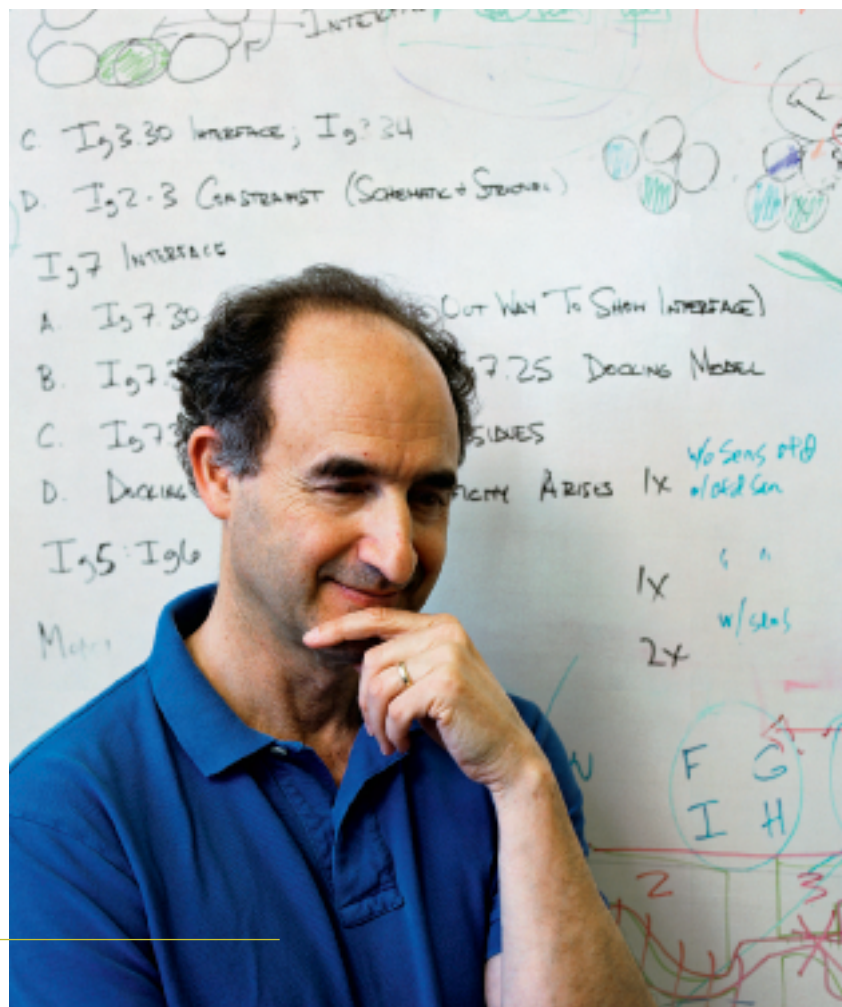
To improve on the method, HHMI investigator Liqun Luo of Stanford University has devised a way to label individual neurons by using genetics. In his MARCM technique, short for “mosaic analysis with a repressible cell marker,” Luo engineers fruit flies so that a small number of neurons express a fluorescent protein and consequently light up under a microscope. Moreover, Luo can restrict this labeling to particular types of neurons, so he knows exactly what kind of cell he’s observing.

Luo also uses MARCM to spur mutations, so he can manipulate genes and then probe how a neuron grows or makes connections in response. The approach offers several benefits. First, it allows researchers to identify mutated cells, because each mutated cell glows. Second, scientists can probe how a genetic change

influences a single neuron among a group of normal neurons.

Luo has used the technique to understand how the brain wires up its smell sensors—olfactory neurons—each of which carries a single olfactory receptor on its surface. Because flies carry 50 different odor receptors, they have 50 different types of olfactory neurons, with each one tuned to grab onto a corresponding kind of odor molecule. In the brain, all the neurons that produce the same receptor stem from a single spot, called a glomerulus. So the fly brain has 50 glomeruli, one for each receptor type. At each glomerulus, axons of the olfactory neurons relay smell information to dendrites of another type of neuron—a projection neuron—which transmits the information throughout the brain.

Using MARCM, Luo has investigated how olfactory neurons and projection neurons find each other during development of the fly brain. “It’s like 50 men and



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50 women on a dance floor, each needing to find dancing partners,” he says. His team has found that the dance floor isn’t mapped out before the neurons get there. Instead, one partner—a projection neuron—sets up where each match will take place. Then olfactory neurons come in and find the right partner. Luo and his colleagues have discovered many of the guidelines for accomplishing this quest. Neurons seem to divide up the problem. First, one set of molecular cues directs neurons to the general vicinity, like a dancer looking for a partner near the bandstand and ignoring the rest of the room. Then, another set of cues helps neurons move systematically from cell to cell in the vicinity until finding the exact meeting spot.

Luo and his team are also mapping how projection neurons connect olfactory neurons with other parts of the brain. They mark projection neurons that link to particular olfactory receptor neurons and then take snapshots of fly brains with those neurons lit up. So far they’ve mapped 35 of the 50 types of fly olfactory receptors. They now want to overlay the maps of different neuron types, but each brain is subtly different from another in the size and arrangement of its parts. So they use computer programs to align each image with a “master brain,” from which they are creating a unified map of the fly’s olfactory system.

Luo knows whether a particular olfactory receptor detects, for example, fruit (the fly’s food source) or pheromones (chemicals one individual emits to spur behaviors, such as mating, in another). As a result, the map illuminates not just where neurons go but what kind of smell information each one communicates. The method has already yielded important findings. Neurons that detect signals related to fruit cluster on one side of the lateral horn, a brain center for interpreting smell data, Luo’s team reported in March 2007 in *Cell*. Neurons that twitter in

response to pheromones cluster on the other side of the lateral horn.

“You have a special fruit-processing area and a special pheromone-processing area,” says Luo. “From our mapping, this becomes very clear.” According to the smells that neurons sense, the cells separate into areas that control different biological functions. Presumably, other neurons use information from the “fruit area” to spur the fly to eat or information from the “pheromone area” to tell the fly to start a courtship dance.

Turn Signals

Researchers also want to understand how neurons decide to change direction at waypoints during the developmental journey. David D. Ginty, an HHMI investigator at Johns Hopkins University, is studying the mouse to examine a set of neurons whose axons travel along arteries away from the spinal cord to the face. At one point, these projections hit a fork in the road: one group of axons follows the external carotid artery to the salivary glands, while another meanders along the internal carotid artery bound for the eye, pineal glands, and other destinations.

Something must tell the axons to go one way or the other. To identify possible signals, Ginty and his team extracted bits of each of the arteries and looked for genes activated in the external carotid artery, but not in the internal carotid artery; they pinpointed particular genes that encode members of the endothelin protein family. Although this group of molecules is involved in regulating blood pressure, the finding that more of the molecules appeared in one artery than in the other suggested that the proteins might direct axons down the external carotid artery, the researchers reported in the April 2008 issue of *Nature*.

In mice that lacked certain members of the endothelin family, neurons did not course down the external carotid artery, according to the team’s findings. However, neurons grew down the internal carotid artery just fine, suggesting that endothelins beckon axons in one direction. The findings also support the idea that neurons use cues at various waypoints to navigate as opposed to taking a random path.

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It's an "unexpected and exciting discovery" that endothelins, which work in the circulatory system, help guide certain neurons, says Tessier-Lavigne. Ginty is now looking for related molecules, such as those that guide neurons down the internal carotid artery.

Mutual Avoidance

Once neurons arrive at their destinations, they split into a tree of branches that cozy up to the target tissue and make their final connections. Several research groups are discovering the cues that guide this phase.

Branches of a single neuron rarely cross. A protein called DSCAM keeps dendrites from snarling, according to a trio of studies last year: one from HHMI investigators Lily Jan and Yuh Nung Jan of the University of California, San Francisco; one from HHMI investigator Lawrence Zipursky of the University of California, Los Angeles, who collaborated with Wesley Gruber (Yuh Nung Jan's former postdoctoral fellow); and a third from a team headed by Dietmar Schmucker (Zipursky's former postdoctoral fellow) of Harvard University.

Eight years ago, Zipursky and his colleagues found—to their surprise—that a single DSCAM gene could generate a huge number of subtly different variants of the protein—38,000 variations, in fact. Aside from the vertebrate immune system, which can generate a vast array of antibody proteins by shuffling single genes, getting such a large number of different proteins out of the same gene is unusual. Zipursky wondered if this extraordinary capability related to DSCAM's role in organizing neuron growth.

Test-tube experiments revealed that each variant binds to itself but not to other variants. Each neuron makes a random assortment of these variants and neigh-

boring neurons are unlikely to make the same versions. Because dendrites from the same neuron would bear the same DSCAM variants, these molecules might allow the dendrites to recognize and grow away from each other, Zipursky proposed. Later experiments suggested that was the case.

In recent work, the three teams independently used Luo's MARCM technique to wipe out DSCAM in a small set of fly neurons. Dendrites of neurons lacking DSCAM frequently crossed, showing that the protein is required for them to avoid each other. DSCAM is made up of several parts, including one that protrudes inside the cell. Zipursky's team further showed that dendrites repel each other only when the protein bears this internal portion; without it, they cling together.

DSCAM also exists in mice, and a study earlier this year revealed that it might carry out a similar function: mice without it have neurons that clump and don't spread. "The molecule used for self-avoidance seems to have been conserved," says Yuh Nung Jan. But the mammalian version of DSCAM doesn't come in the plethora of flavors that

the fly version does, and how rodent neurons establish independent identities remains to be seen.

Many other important questions also remain, says Zipursky. Is DSCAM's sole job to tell a neuron to avoid itself or does it carry out other tasks too? Do cells always use a random assortment of DSCAMs or do particular variants identify subsets of neurons? To investigate those questions, his group is generating flies that are missing specific sets of DSCAM variants.

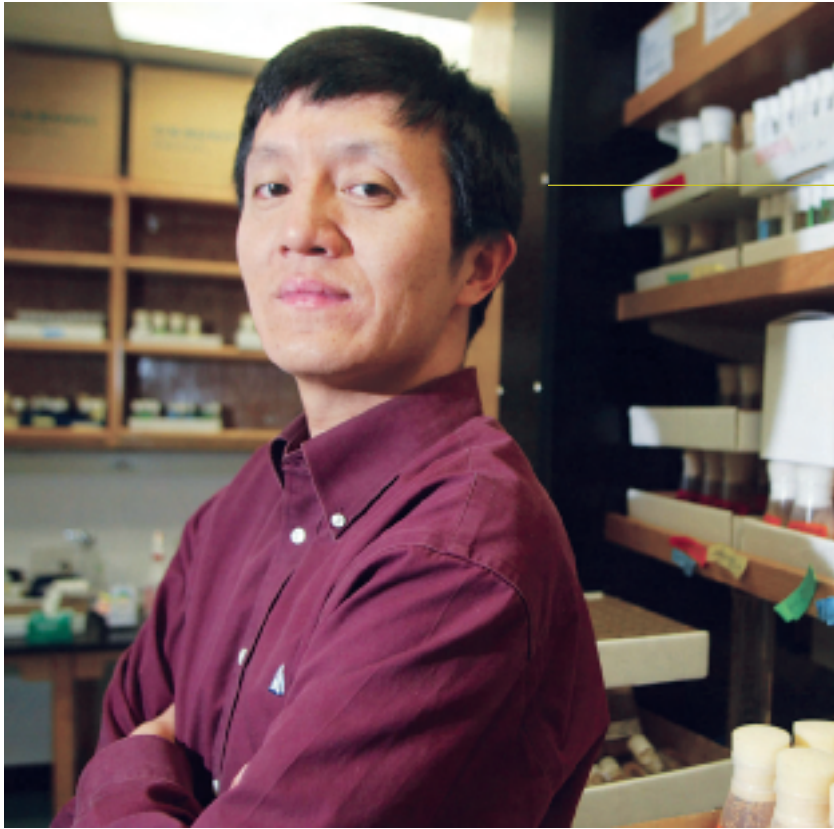
The Right Connections

To make connections correctly, neurons also need signals from skin, muscle, or other tissues they ultimately connect to. Ginty knew that such target tissues release a molecule called NGF, or nerve growth factor, which prompts certain kinds of neurons to branch and supports their survival. He then found that neurons exposed to NGF ramp up production of a gene-controlling molecule called SRF. Further genetic studies revealed that SRF relays NGF's order for a neuron to branch and penetrate the target tissue. However, SRF is not necessary to

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communicate NGF's survival message—without SRF, neurons exposed to NGF survive. Some other molecules must prevent cells from dying.

The role of SRF is intriguing, says Ginty, because the molecule exists only in a neuron's nucleus—not in its growing tip, where the neuron first encounters NGF. He is keen to understand how NGF communicates with the nucleus. In Ginty's preferred model, the cell membrane at the neuron's end pinches off, forming small spheres that capture NGF and transport the growth factor along with its receptor all the way back to the cell body, where the nucleus resides. The model makes sense because defects in this transport system are implicated in neurodegenerative disorders such as Parkinson's disease and amyotrophic lateral sclerosis, or ALS (often referred to as Lou Gehrig's disease).

What is remarkable, says Ginty, is that NGF influences an axon's growth and branching by tweaking genes in the cell's

nucleus. Because cells see NGF at their growing end, "it wasn't intuitive that you'd need signaling all the way to the nucleus." One reason for such an ornate process might be that neurons must shift modes. "Until it reaches the target region, the role of a neuron is to grow, grow, grow," he says. But once it arrives, it must penetrate the tissue, branch, and form connections. NGF might be the trigger that tells the neuron to switch on all the genes required to perform the new gymnastics.

Ginty's work is also defining how sets of connections involving many neurons are fine-tuned. As an organism establishes its nervous system, it sends more neurons than necessary to a particular place. Ginty and his team have found that neurons use a series of genetic feedback loops to determine which cells live and which ones die. As a result, when a group of neurons see NGF, some of them become more sensitive to the molecule and consequently become robust survivors, Ginty and his colleagues reported in the April 18, 2008, issue of *Science*.

In addition, the hearty cells churn out molecules that kill off neighboring neurons. Those neighbors see NGF, but

the death molecules appear to override the NGF signal. The right balance between the forces that make neurons strong and those that kill them helps an organism wire up just the right number of connections.

Studies such as these are revealing new clues about how normal brains and nervous systems develop as an embryo matures. But the research may also illuminate causes of disease. Problems in wiring can crop up, resulting from genetic miscues or other causes, as an embryo develops. Growing evidence suggests that this kind of faulty wiring may underlie neurological disorders such as schizophrenia. Understanding how and why wiring gets fouled up during development might lead to ways to correct the problem before it's too late.

In addition, some of the strategies that help an embryo set up a working nervous system might also keep the nervous system in proper order during adulthood; defects that develop later in life could contribute to neurodegeneration. Finally, one way to fix a damaged nervous system might be to reactivate the processes an embryo uses and spur the body to regrow parts of the system.

The new insights about how neurons find their path, avoid themselves, and establish appropriate connections reveal many of the instructions for weaving the intricate tapestry of the nervous system. "We already know a lot of the molecules that are involved," says Tessier-Lavigne, "but these studies highlight the fact that many additional players remain to be discovered." Many of the overarching navigation strategies likely remain undiscovered as well, he suggests. Further efforts will provide even more nuanced views, not only of the journey that each neuron takes to create a working nervous system but also of ways to preserve the wiring's integrity and to keep brains healthy. ■