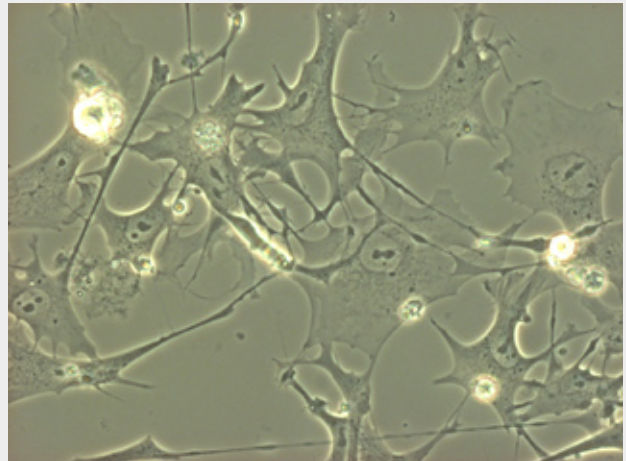


Neurons: A fish-eye view of the brain

Many scientists consider the human brain the most complex object in the known universe. Within three pounds of tissue, one hundred billion neurons, each with tens of thousands of connections to others, engage in busy electro-chemical conversations. The signals they send result in our thoughts, actions, words, and emotion; at some level, they are the stuff of consciousness. How does a collection of cells the size of a cantaloupe perform such magic? Surprisingly, we're finding some answers to that question by studying the tiny, 10,000-neuron brains of the developing *Danio rerio*, commonly known as the zebrafish.



Neurons grown from mouse embryonic stem cells.

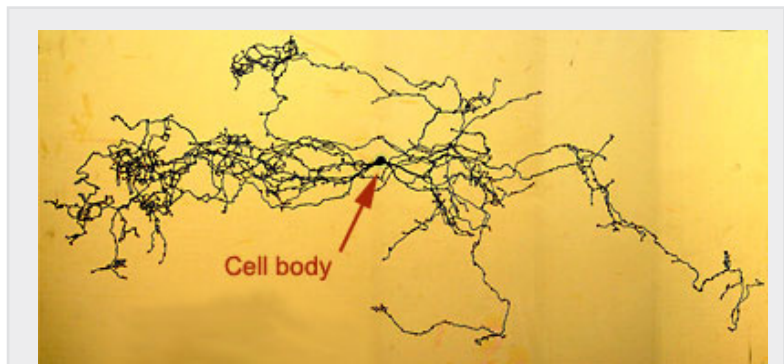
The stuff of brains

What makes a brain powerful isn't just its size or the number of neurons it contains, but the way those cells grow and connect to each other. Wiring a human brain correctly takes the effort of about 10,000 genes. About 80 percent of these genes are absolutely essential: if their expression is flawed, the whole system will be thrown off, and the resulting brain will function improperly (if at all). The body accomplishes a major feat in each of us when it correctly uses the products of these 10,000 genes to power the creation of more than 100 trillion neural connections.

The cells usually associated with functions like thought and action are neurons, electrically active cells that process and transmit information (Other supporting cells, known as glial cells, are also believed to play a supporting role). Fortunately, the neurons of humans are similar to the neurons of other animals—like zebrafish—making it easier for us to learn about ourselves by studying others.

Neurons can have many shapes, but are typically highly branched. Their branches allow them to connect with other neurons. One part of the cell has a bushy, dense set of branches called dendrites. Dendrites are signal collectors: when stimulated by input from other neurons, their cell membranes change in a way that causes an electrical wave to travel over the cell's surface. That signal will travel down the longer, less branched part of the cell called the axon.

The connections between neurons, called synapses, actually tiny spaces where neurons' electrical signals become a chemical ones, and move from one cell to another. The axon of the signaling cell releases a chemical (called a neurotransmitter) that travels to the dendrites of the receiving cell. The result may be either that the receiving cell is excited, and fires its own signal, or that it's inhibited from doing so. A neuron usually gets input from many synapses at the same time, and the combination of those signals determines whether it will produce a signal or not. Interestingly, both the connections between neurons and their responses to signals change as we learn new things.



Drawing of a rat neuron. The cell body houses the nucleus, the branching structures are dendrites. This cell probably had 1000 connections to other cells--relatively simple in contrast to a human neuron, which may have up to 20,000 connections.

Dr. Peter Ohara, UCSF and Dr. Leif Havton, UCLA

Navigating a maze of cells

It's easy to imagine how the vast number of neuronal connections inside a brain creates a network of almost unfathomable complexity. Brain function relies on this intricate maze; it's what creates the processing power that allows a brain to accomplish complicated tasks like responding to a visual cue, moving a body part, or (in humans) recognizing a familiar face and saying "hello."

But how exactly does the brain get wired up? The way this impressive network grows and organizes itself during development (and, sometimes, during adulthood) is a puzzle many researchers are trying to decipher.

So far, scientists know that the number of neurons in an individual's brain is determined by genetics—but how those neurons are wired together, and the number of synapses amongst them, is influenced by environment. By making movies of zebrafish neurons as they develop, scientists in neurobiologist Stephen Smith's Stanford laboratory are getting glimpses of how this process unfolds.

Santiago Ramon y Cajal and the Neuron Doctrine

Imagine looking closely at an object and noticing something previously unseen, a feature that demands a rethinking of what that object is. This is what happened when Santiago Ramon y Cajal, a Spanish doctor who'd dreamed of being an artist, first saw neurons under a microscope in the 1880s. The cells he saw had been prepared with a then-new staining technique that revealed the intricate branches and individuality of a few select neurons in the sample.

At the time, most scientists thought of the nervous system as a mass of connected, mesh-like tissue. What Cajal saw confirmed his alternative idea that the nervous system is comprised of singular, discrete cells. Putting his artistic skills to work, Cajal painstakingly sketched what he saw, looking at sample after sample, creating stunning sketches and exposing details that led him to what has become the foundational ideas in neurobiology—the neuron doctrine. In 1906, he won the Nobel Prize for his contributions.

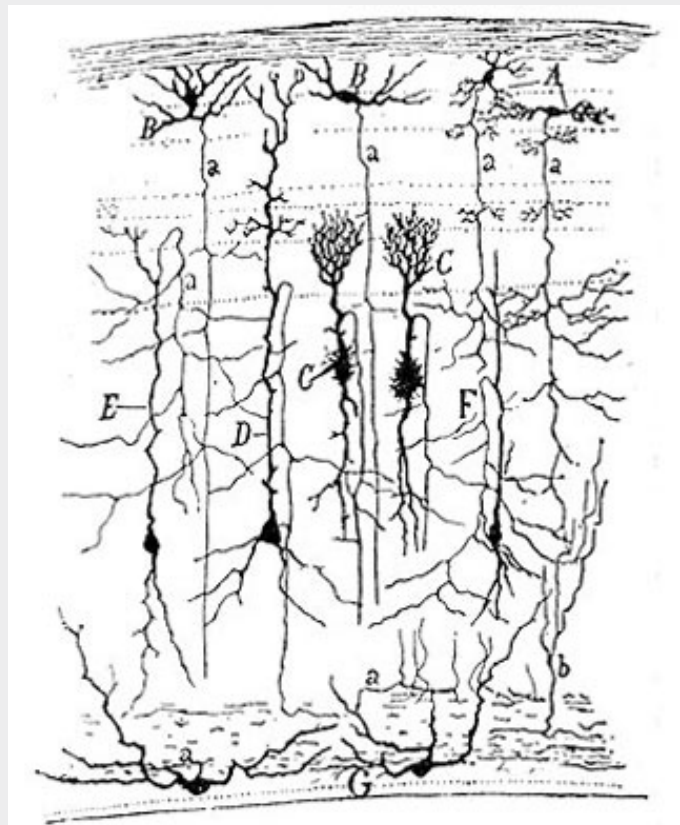


Illustration: Drawing of a section of the sparrow optic tectum, Santiago Ramon y Cajal, 1900

The neuron doctrine is defined by four tenets:

- neurons are discrete cells, not physically connected to each other;
- these cells exist as individual units, metabolizing and growing separately from each other;
- each neuron has three parts: an axon at one end, a cell body, and dendrites at the other end; and
- neural signals travel only in one direction, from dendrites toward axons

Cajal deduced all this by simply observing cells through the microscope and recording what he saw. Many modern biologists do the same thing, using much more highly powered microscopes and sophisticated computer rendering. Cajal's work is a testament to the importance of images and visualization in biology. Though research in the last few decades has revealed that relationships between neurons are more complicated than Cajal could have known, his observations and ideas have withstood a century of scientific advancement—an amazing feat considering the technology and knowledge available to him at the time.

Learning from a fish

Why study fish brains? Zebrafish have several qualities that make them good models for studying neuron development. First, they're small, they're inexpensive, and they grow and reproduce quickly. They also have enough interesting behaviors to make them useful to observe, but not so many that they are confusing.

Another advantage is that their brains develop rapidly: A zebrafish grows from single cell to swimming larvae with a functional brain in under 3 days. In humans, this same feat takes months. This compressed time scale allows scientists to track the entire wiring process of the fish's brain. An extra bonus is that zebrafish are transparent to certain wavelengths of light, so researchers can easily see what's happening inside them as they grow.



A three-day-old zebrafish embryo next to a human hair.

Scientists are also able to engineer zebrafish with specific genetic characteristics. In Smith's lab, for example, they've modified the fish's genome to actually illuminate particular neurons in a living fish's brain. The fish's transparency allows the researchers to bombard it with a powerful laser (affectionately known as the "death-ray"). This light would burn opaque organisms, but shines right through the zebrafish. Using this technique, scientists can watch and measure the growth, development, and activity of specific neurons without harming the living fish.

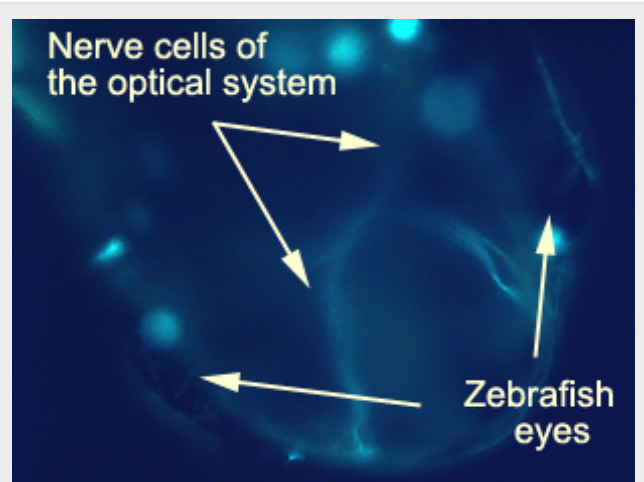
Researchers in the Smith lab study neurons developing in the optic system of just-hatched zebrafish. When a fish is 3 days old, it begins to see. Then researchers put it in a warm bath, under a microscope, and show the tiny fish patterns on a miniature LCD screen. Their measurements have shown that the fish's brain is most active when the screen's moving spots are the same size as its favorite food. These results imply that neurons in the zebrafish's visual system are programmed to detect certain movements more readily than others—a specific brain activity that may help the young fish find its food from its early in life.

Speed-dating neurons make connections

Researchers in the Smith lab have also been able to watch the development of single neurons as they grow and connect to each other to complete the optical system. What they've seen helps explain how the enormous complexity of the brain arises.

It now appears that brain organization occurs in stages. As a developing neuron grows, it sends out tiny exploratory branches called filopodia. These wriggling projections are searching for partners in an effort to make connections that will result in synapses. (It's a bit like neural "speed dating.") Each branch makes tens or hundreds of contacts with other neurons in the densely packed tissue surrounding it, but only a small percentage of these "dates" result in cellular "marriages."

Once formed, the synapse becomes a hot spot; the now-connected neuron sends out new filopodia, thus increasing the number of connections it will have with other neurons. In this way, the neuron is called upon more often, causing it to send out more filopodia and make more connections. Researchers have found that as a zebrafish begins to see, only certain connections and pathways are useful, and the cell begins to lose unnecessary connections. This radical pruning adds precision to the circuit.



A microscope image showing the optic nerve as it's developing in a zebrafish embryo. The fish has been engineered with a protein that allows researchers to see inside it under certain conditions.

The wiring-up process happens quickly: one filopodium will explore for about 5 minutes, retracting if it doesn't make any connections. The bulk of this massive connection-making effort occurs for only a short time during development; the basic wiring of the zebrafish visual system happens between the third and the eighth day of the fish's life. Though its brain continues to grow, and the number of neurons it contains may increase a hundredfold, the primary connections between neurons are laid out in these early days. In humans, such intense brain development happens in stages over the first few years of life—but it's now thought that our brain wiring continues to change and grow throughout our lives.

Scientists used to believe that once an animal's brain was wired up, the resulting network was final and no new connections were possible. But new research is showing

that when our brains are challenged to learn new things, old neurons can be "dating" again, making new connections. In one experiment, adult owls re-learned to see and hunt after having their vision altered by perspective-shifting glasses. Another researcher showed that after practicing for just one week, people learning to play piano showed an expansion in the brain area devoted to finger movement. Contrary to popular belief, old dogs may well be able to learn new tricks—because old brains may continue to grow and change.