

In Tiny Worm, Unlocking Secrets of the Brain

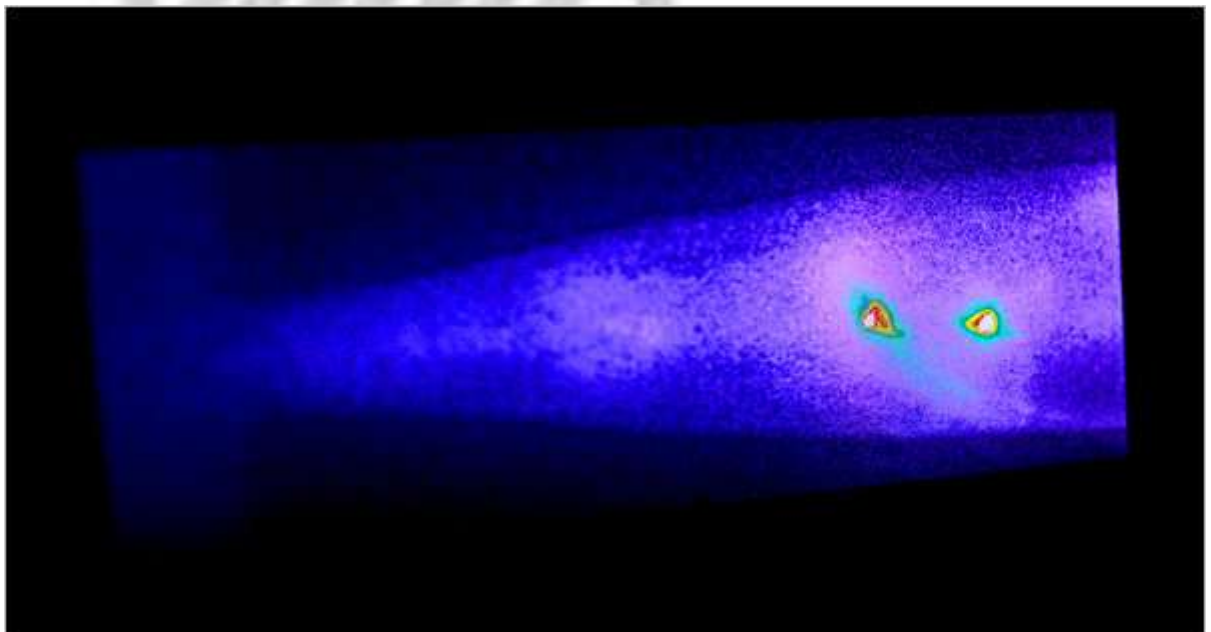
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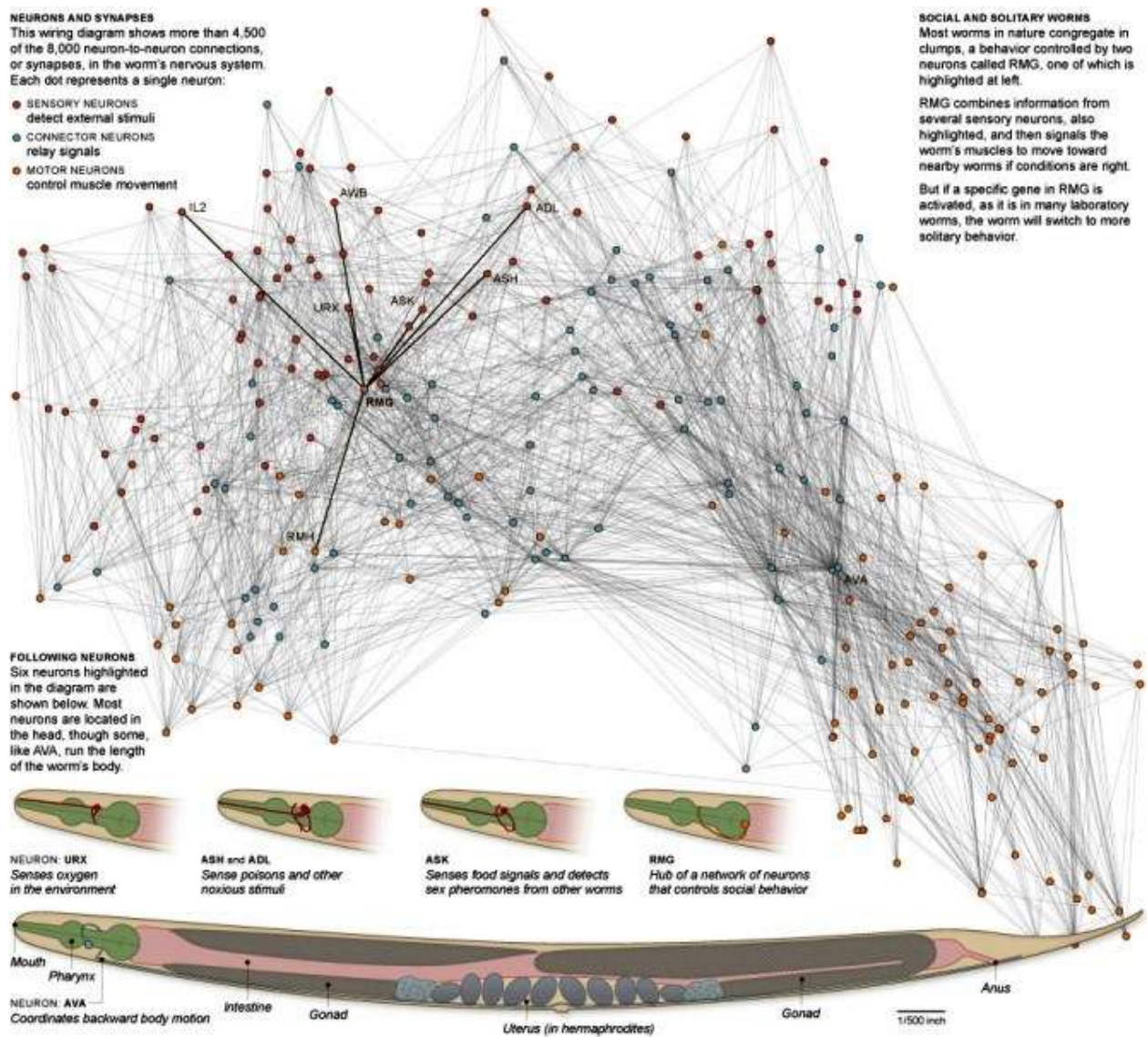


In an eighth-floor laboratory overlooking the East River, Cornelia I. Bargmann watches two colleagues manipulate a microscopic roundworm. They have trapped it in a tiny groove on a clear plastic chip, with just its nose sticking into a channel. Pheromones — signaling chemicals produced by other worms — are being pumped through the channel, and the researchers have genetically engineered two neurons in the worm's head to glow bright green if a neuron responds.

These ingenious techniques for exploring a tiny animal's behavior are the fruit of many years' work by Dr. Bargmann's and other labs. Despite the roundworm's lowliness on the scale of intellectual achievement, the study of its nervous system offers one of the most promising approaches for understanding the human brain, since it uses much the same working parts but is around a million times less complex.



Scientists have engineered two worm neurons to glow bright green if a neuron responds when the worm is exposed to certain chemicals



[To view this picture enlarged, please click on it]

Caenorhabditis elegans, as the roundworm is properly known, is a tiny, transparent animal just a millimeter long. In nature, it feeds on the bacteria that thrive in rotting plants and animals. It is a favorite laboratory organism for several reasons, including the comparative simplicity of its brain, which has just 302 neurons and 8,000 synapses, or neuron-to-neuron connections. These connections are pretty much the same from one individual to another, meaning that in all worms the brain is wired up in essentially the same way. Such a system should be considerably easier to understand than the human brain, a structure with billions of neurons, 100,000 miles of biological wiring and 100 trillion synapses.

The biologist Sydney Brenner chose the roundworm as an experimental animal in 1974 with this goal in mind. He figured that once someone provided him with the wiring diagram of how 302 neurons were connected, he could then compute the worm's behavior.

The task of reconstructing the worm's wiring system fell on John G. White, now at the University of Wisconsin. After more than a decade's labor, which required examining 20,000 electron microscope cross sections of the worm's anatomy, Dr. White worked out exactly how the 302 neurons were interconnected.

But the wiring diagram of even the worm's brain proved too complex for Dr. Brenner's computational approach to work. Dr. Bargmann was one of the first biologists to take Dr. White's wiring diagram and see if it could be understood in other ways.

Cori Bargmann grew up in Athens, Ga., a small college town in the Deep South where her father taught statistics at the University of Georgia. Both her parents had been translators and met while Rolf Bargmann was working at the Nuremberg trials. Her mother, Ilse, would read to her in German the works of the Austrian animal behaviorists Konrad Lorenz and Karl von Frisch, planting the seeds of an interest in neuroscience.

"I went into science because I loved the labs," Dr. Bargmann says. She liked the machines and instruments, the fun of building things with one's own hands, of learning what no one else knew. An outstanding student, she chose for her Ph.D. degree to work in the M.I.T. lab of Robert A. Weinberg, a leading cancer biologist. The first mutated genes capable of causing cancer were being isolated. "It was an incredibly exciting time," she says.

Her task was to clone a rat gene called neu. When mutated, the gene causes a tumor, but one that the rat's immune system can attack and destroy. Several years later, the human version of neu, called HER-2, was found to be amplified in breast cancer, and its receptor protein product is the target of the artificial antibody known as Herceptin, a leading breast cancer drug.

For her postdoctoral work, Dr. Bargmann decided to work on animal behavior. The mouse is a standard organism for such studies, but she did not like hurting furry animals. "In Weinberg's lab I would start to cry every time I had to do anything with a mouse," she says. A nonfurry alternative was the fruit fly. She interviewed with a leading laboratory in California, but her husband at the time did not wish to move there.

That left the roundworm. There are now several hundred worm labs around the world, of which perhaps 30 or so, like Dr. Bargmann's, focus on the worm's nervous system. In 1987, "worms weren't entirely respectable," Dr. Bargmann says. But right there at M.I.T., H. Robert Horvitz had established one of the first serious worm labs in the United States. She joined his lab and read everything written on the worm, including all the back copies of the little field's informal journal, *The Worm Breeder's Gazette*.

She noticed that a particular behavior of *C. elegans* had been described but not well explored: it can taste waterborne chemicals and move toward those it finds attractive. Dr. White's wiring diagram had been published the year before, in 1986. With this in hand, she told Dr. Horvitz she planned to identify which of the worm's 302 neurons controlled its chemical-tracking behavior.

He thought the project was too ambitious, but said she could spend six months on the attempt. Each neuron in the worm's brain is known, and is assigned a three letter name. Specific neurons can be identified under a microscope and zapped with a laser beam, allowing the neuron's role to be deduced from whatever function the worm may seem to have lost.

Dr. Bargmann slogged her way through the task of killing each neuron one by one. Telling one neuron from another under the microscope is not easy. "It's like knowing each grape in a bunch is different, but not quite being able to see it," Dr. Horvitz said. "The first thing she had to do was learn the worm's neuroanatomy, and she did so in a

way only one other person has ever done.” (He was referring to John E. Sulston, who traced the lineage from the egg of all 959 cells in the adult worm’s body).

She discovered, by accident, the neurons that control the worm’s switch into hibernation, a survival strategy for when food is scarce or neighbors too many. Finally, she found the neurons that control taste, showing that without them the worm could not track chemicals, and that it retained this ability even if she killed all the other neurons in the worm’s body.

She also discovered that the worms have a sense of smell — the ability to detect airborne chemicals — as well as a sense of taste. Since worms eat bacteria that feed on decaying plants and carcasses, she figured they should be able to detect and home in on the aromas of putrefaction. The redolent draft from these experiments caused a certain degree of complaint in Dr. Horvitz’s lab. After she succeeded, she says, “Horvitz told me that my great strength as a scientist was that I could think like a worm.”

“Cori is talented beyond thinking like a worm,” Dr. Horvitz now says. “She can think like very few other people in a rigorous and creative way, and so has repeatedly developed new kinds of approaches.”

Dr. Bargmann moved in 1991 to the University of California, San Francisco, to start her own lab. She began by following up her finding that worms have a sense of smell. In 1991, Richard Axel and Linda Buck discovered the molecular basis for the sense of smell: there are about a thousand genes, at least in rats, that make odorant receptors, proteins that stud the olfactory nerves’ endings in the nose and respond to specific odors.

The *C. elegans* genome had just been decoded, and Dr. Bargmann was able to identify the worm’s odorant receptor genes. In fact, they have 2,000 of them, twice as many as the rat.

“This is what they do,” Dr. Bargmann says. The worm cannot see. Its world is one of smells, not sights. It needs to scent the soil bacteria that are its prey, while avoiding those that are poisonous to it. Ten percent of its genes are dedicated to making it a champion connoisseur of odors, mostly unpleasant.

With the odorant genes in hand, Dr. Bargmann could apply genetics to figuring out how the worm’s sense of smell worked. By working with mutant worms, she showed that a specific odor receptor recognizes a specific odor, a finding that was implied by the Axel-Buck discovery but that no one had managed to nail down.

She found that worms with a mutation in a gene called *odr-10* could not smell diacetyl, a chemical that gives butter its odor and is also produced by a bacterium that is a favorite worm food. The *odr-10* gene, which makes the odor receptor protein that detects diacetyl, is active in neurons that guide the worm toward a scent.

Dr. Bargmann switched things around so that *odr-10* was expressed only in a neuron that detected scents repulsive to the worm. These worms backed away from the buttery odor, showing that it is not the odor receptors but the wiring of the nervous system itself that determines whether the worm deems an odor delicious or detestable.

This was a surprising result because most people thought that sensory information was perceived as neutral, with the brain deciding later from the context whether it was good or bad. Some scientists said that only worms behave this way, but the same result was later obtained in mice.

Dr. Bargmann sees the arrangement in evolutionary terms. “The more reliable a piece of information is, the more it will be shifted into the genome,” she says. That way, an

organism does not have to risk learning what is good or bad; the genes will dictate the right behavior by wiring it into the nervous system. Worms are wired up to know that diacetyl means good eating.

Having studied the worm by mutating its genes, Dr. Bargmann then looked at natural variation in the genetic basis of worm behavior. Most worms in nature like to congregate in clumps, but the laboratory version of *C. elegans* has developed an unusual liking for being on its own. She linked this difference in behavior to the switch of a single amino acid unit in a protein called npr-1 (for neuropeptide Y receptor-1).

It took several more years to learn how the system worked. It turns out that social behavior in the worm is controlled by a pair of neurons called RMG. The two RMG neurons receive input from various sensory neurons that detect the several environmental cues that make worms aggregate. RMG integrates this information and sends signals to the worm's muscles.

The usual role of the RMG neurons is to promote social behavior, but when the npr-1 gene is active, the RMG neurons cannot receive input from their sensory neurons, and the worms switch to solitary behavior.

While working out the worm's sense of smell, Dr. Bargmann fell in love with another olfactory researcher, Richard Axel. Dr. Axel works at Columbia University, and she was able to join him in New York by finding a place at Rockefeller University. Dr. Axel was helping her clear out her apartment in San Francisco when he heard he had won the Nobel Prize.

Right after that pleasant news, he had to drive to the local Goodwill store to drop off the stuff to be given away. "People think that if you're married to a scientist you talk about science all the time," Dr. Bargmann says. They read each other's papers before publication, but they don't plan experiments together. Dr. Axel works on how olfactory information is handled in the cortex, the highest level of human and mouse brains.

"Probably once or twice a week we are sitting at dinner and Richard says, 'The cortex is hopeless,' and I say, 'That's why I work on the worm.' " Dr. Bargmann said.

After studying the little animal for 24 years, she believes she is closer to understanding how its nervous system works.

Why is the wiring diagram produced by Dr. White so hard to interpret? She pulls down from her shelves a dog-eared copy of the journal in which the wiring was first described. The diagram shows the electrical connections that each of the 302 neurons makes to others in the system. These are the same kind of connections as those made by human neurons. But worms have another kind of connection.

Besides the synapses that mediate electrical signals, there are also so-called gap junctions that allow direct chemical communication between neurons. The wiring diagram for the gap junctions is quite different from that of the synapses.

Not only does the worm's connectome, as Dr. Bargmann calls it, have two separate wiring diagrams superimposed on each other, but there is a third system that keeps rewiring the wiring diagrams. This is based on neuropeptides, hormonelike chemicals that are released by neurons to affect other neurons.

The neuropeptides probably help control the brain's general status, or mood. A strong hint of how they work comes from the npr-1 gene, which makes a protein that responds to neuropeptides. When the npr-1 gene is active, its neuron becomes unavailable to its local circuit.

That may be a reason why the worm's behavior cannot be computed from the wiring diagram: the pattern of connections is changing all the time under the influence of the worm's 250 neuropeptides.

The connectome shows the electrical connections, and hence the quickest paths for information to move through the worm's brain. "But if only a subset of neurons are available at any time, the connectome is ambiguous," she says.

The human brain, too, has neuropeptides that set mood and modify behavior. Neuropeptides are probably at work when the pain pathways are cut off in acute crises, allowing people to function despite serious wounds.

The human brain, though vastly more complex than the worm's, uses many of the same components, from neuropeptides to transmitters. So everything that can be learned about the worm's nervous system is likely to help with the human system.

Though the worm's nervous system is routinely described as simple, that is true only in comparison with the human brain. The worm has 22,000 genes, almost as many as a person, and its brain is a highly complex piece of biological machinery. The work of Dr. Bargmann's and other labs has deconstructed many of its operational mechanisms.

What would be required to say that the worm's nervous system was fully understood? "You would want to understand a behavior all the way through, and then how the behavior can change," Dr. Bargmann says.

"That goal is not unattainable," she adds.

This article has been revised to reflect the following correction:

Correction: June 20, 2011

A previous version of this article misstated the number of neurons in the human brain. The number is up to 100 billion, not 100 million.