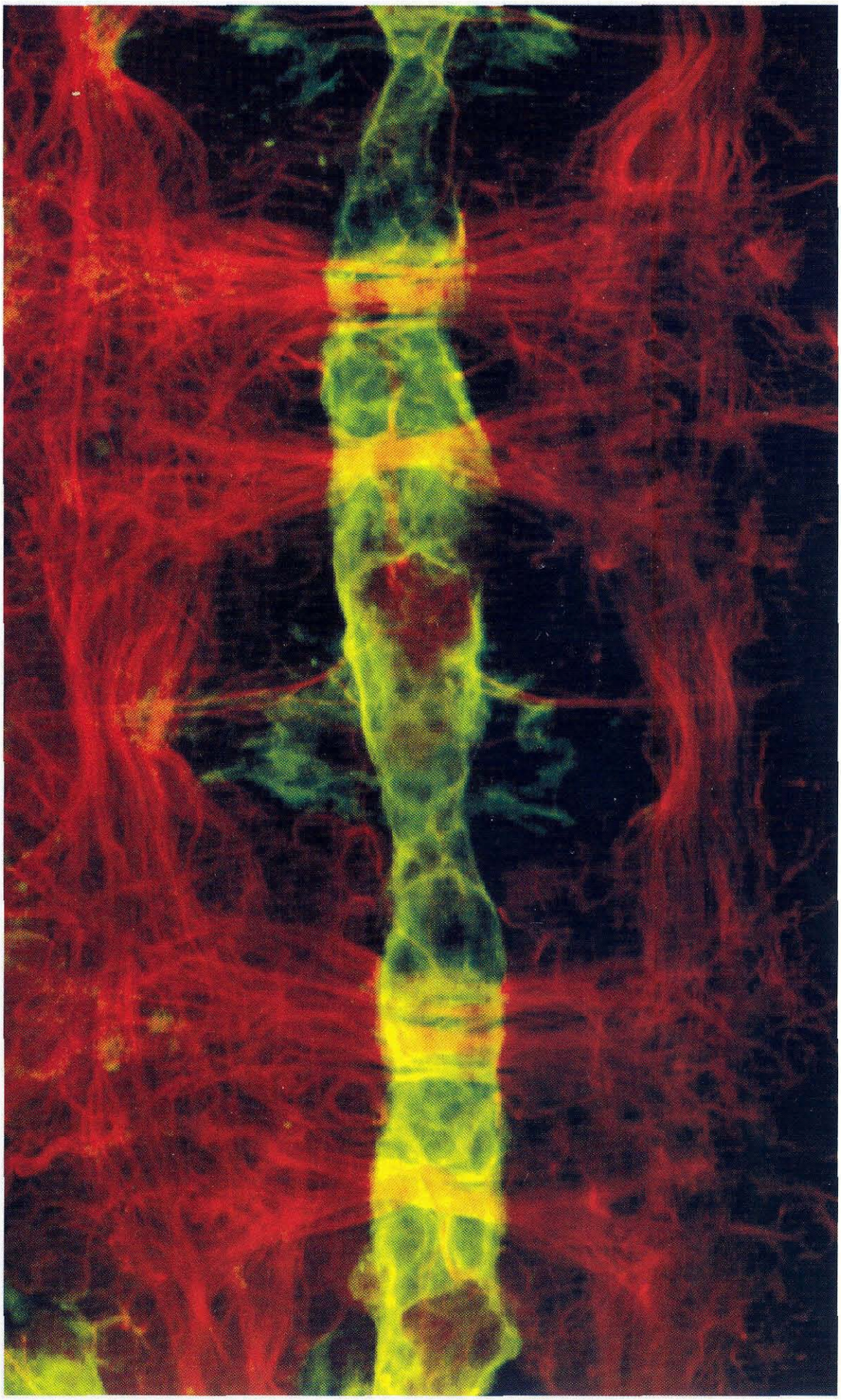


Building an Insect Brain

A single nerve cell can have 10,000 to 100,000 input and output connections.

by Kai Zinn

The fluorescent red wires in this electrician's nightmare is the central nervous system in a single segment of a grasshopper embryo. The two vertical trunks are called the longitudinal connectives, and they run the length of the embryo. The horizontal interconnections are called commissures, and there are two per segment. (This photo actually shows one-and-a-half segments.) Each red line is a bundle of 10 or so axons—the wires of the nervous system—and every connective and commissure has 15 to 20 bundles. The yellow-green band down the center is the midline glial cells that wrap up the neurons' cell bodies and processes.

Neuroscience is the study of the structure and function of the brain, and of the other parts of the nervous system that it controls. There are two basic questions that neurobiologists study: how does the brain work? and, how is it put together during development? These two questions are closely related, since the brain is like a giant electrical circuit and the structure of that circuit partially determines how it works.

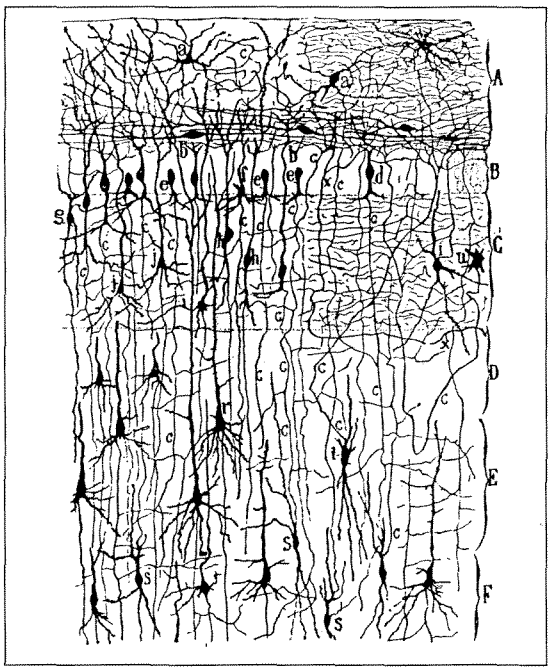
The circuit's building blocks are nerve cells, or neurons, and they have certain characteristics in common. Every nerve cell has a nucleus, which contains the genetic material, surrounded by a cell body in which the proteins that make up the cell's machinery are produced. Extending out from the cell body are branching processes—wirelike growths that make connections with other cells. Most neurons have an array of input processes, called dendrites, which receive signals from other cells, and an output process called an axon, which sends signals to other cells. The actual connections between cells are made at junctions called synapses, and each process usually contains many synapses. A single nerve cell can have 10,000 to 100,000 input and output connections.

There are thousands of different kinds of nerve cells, each with a distinctive shape. These shapes reflect the shape and organization of the nerve cell's processes, which are tailored to its specific functions. For instance, a retinal bipolar cell's dendrites receive input from the eye's photoreceptors, and its relatively short axon sends output to cells that form the optic nerve. A motor neuron's cell body resides in the spinal cord, and its dendrites receive direct inputs from brain neurons in the form of motor commands. The neuron transmits these commands via an axon that extends all the way from the spinal cord to a muscle in, for example, the forearm.

The structure of a nerve cell's processes is largely determined by the pattern of genes activated, or expressed, within it and within the target cells that it connects to. There are probably about 100,000 genes in the human genetic blueprint, but only a fraction of them are active in any given neuron at a particular time. An initial pattern of gene expression is built into each neuron at its birth, but the chemical and electrical inputs that the cell receives change this pattern. Thus, there is a synergy between gene expression and communication among nerve cells. Gene expression can determine the initial connections between cells. Feedback through these connections then regulates gene expression, which in turn modifies the connections.

A nerve cell does not suddenly spring into being with a full-blown array of processes. Rather, it begins life as a cell body from which the processes must grow out toward their eventual targets. The leading edge of each process is a specialized structure called a growth cone, which navigates through the surrounding tissue to the target. Every process has a mission—a set of cells it is driven to seek out and connect to.

My research group's goal is to identify and understand the functions of the genes that control the shape of a neuron and the connections it makes to other cells. Which genes have to be turned on for a cell to have a certain shape, and



This cross section of a rabbit cortex was drawn by the Spanish neuroanatomist Santiago Ramón y Cajal, who shared the Nobel Prize for physiology or medicine with Camillo Golgi in 1906. Golgi invented a method of staining individual nerve cells so that they could be easily seen under a microscope. Ramón y Cajal adapted the method to the brain, and established that the neuron was the fundamental unit of the nervous system.

how do they control the cell's development and growth? We try to answer these questions not by studying the human brain, which is far too complicated, but by looking at the nervous systems of insects, which are much simpler. Some circuits in the insect equivalent of the spinal cord have only a few hundred neurons, and the way in which they connect to one another is completely controlled by genetics. These circuits could be described as hard-wired. The human brain, in contrast, has a vast number of neurons, and most of its connections are not hard-wired.

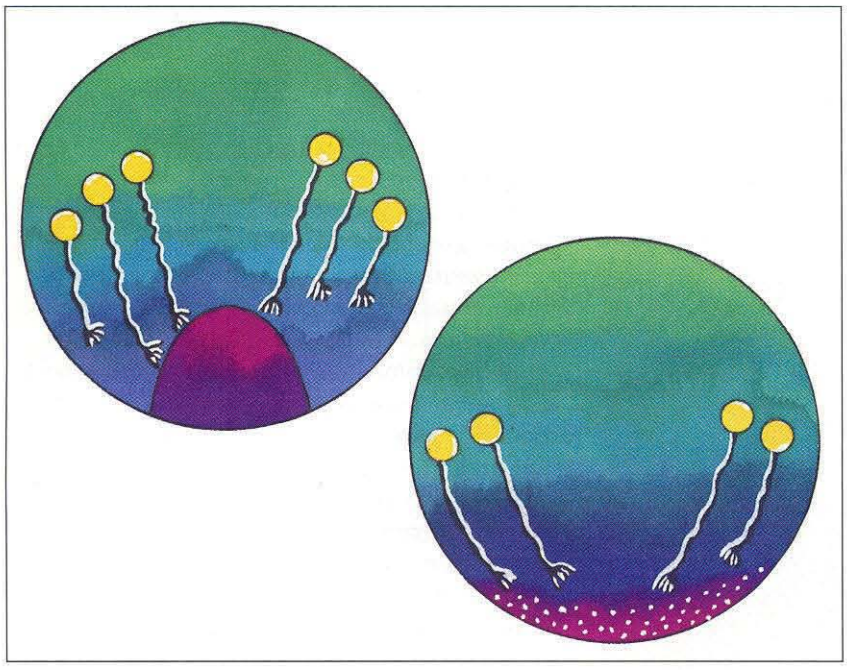
Most of the outer surface of the human brain is composed of a deeply folded, layered sheet called the cortex. Underneath this sheet is a massive amount of wiring—processes like those I mentioned above—that connects the different parts of the brain to each other. Above is a view of a cortex's six layers and a few of the cells within those layers. (The cells are actually much more densely packed.) If you were to unfold it and spread it out, the human cortex would be about the size of a large pizza-box lid. The volume underneath one square millimeter of cortical area contains about 60,000 neurons, about four and a half kilometers of wiring, and some 600 million to 2.5 billion individual synapses. The entire cortex has perhaps 720,000 kilometers of wiring, which is very nearly enough to stretch from the earth to the moon and back again, at least 10 billion neurons, and perhaps 10^{15} synapses. These numbers are so large that it's hard to even begin to think about how to devise a plan to study what directs them to form.

You can actually hand-dissect a grasshopper or fruit-fly embryo, if you don't drink too much coffee that morning.

The problem of understanding how this structure gets built, and where its blueprint information comes from, is further complicated by the fact that it's not hard-wired, as I mentioned earlier. The detailed pattern of connections is different in every human—that's part of what makes you a unique individual, with your own memories and skills—in fact, it's different in every individual mammal. Thus, we can't always extrapolate from one brain to another. The patterns vary because the connections in the mammalian brain at birth are very different from what they will be in the mature animal. As the baby mammal interacts with its environment, these acts of exploration essentially rewire its nervous system. A human infant, for example, can't make coordinated motions, can't focus its eyes, and doesn't know what it's seeing. It can't really interpret the world around it. A five-year-old child, however, can do complex physical tasks, such as assembling Lego blocks into an intergalactic battle cruiser, can communicate what it sees and feels, and basically has a mature pattern of connections within its brain.

There's experimental evidence for this rewiring. David Hubel, Torsten Wiesel, and their colleagues at Harvard Medical School have shown that if a monkey or a cat is kept from seeing out of one eye during a critical period following birth, the animal will never be able to see out of that eye again. The researchers placed an opaque patch over the eye—or even a translucent one that admitted light but blurred out forms—and then removed the patch after a couple of weeks. Brain-activity measurements showed that no inputs from the just-uncovered eye were reaching the brain, even though the eye was perfectly functional. This is because the newborn starts out with an unorganized pattern of connections between the eyes and the brain. As the animal looks around, feedback from the brain tells the nerve cells in the eyes which connections are providing useful information and should be maintained, and which aren't and should be changed or eliminated. If one eye is covered, the animal receives useful information solely from the other eye, whose connections proceed to take over the entire part of the brain that should be driven by both eyes. The connections get locked in during the first several months of an animal's life, and the displaced eye can never again provide input.

Given such dependence on the outside environment, why should we think that anything we learn about insect brains would be relevant to understanding the human brain? A variety of experiments over the last 10 years have shown that many of the molecules that are used to wire



Below: A scanning electron micrograph of a grasshopper embryo, magnified some 25 times. Its antennae are labeled "A," and its legs are labeled "L." The handbag it's clutching in its lowest set of legs is actually a fruit-fly embryo.

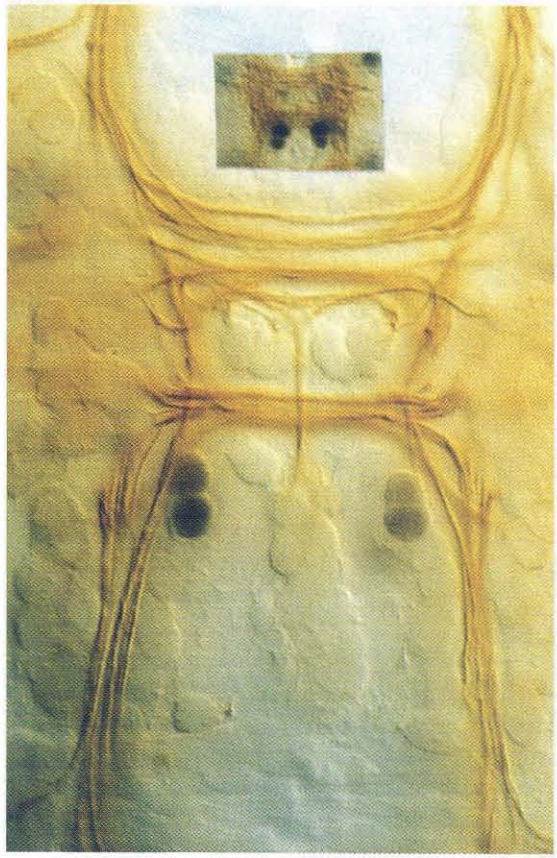
the nervous system are the same in all organisms, or largely the same. The existence of these so-called "chemoaffinity molecules" was proposed by the late Roger Sperry, Caltech's Board of Trustees Professor of Psychobiology, Emeritus, who won the Nobel Prize together with Hubel and Wiesel. (Sperry studied optic-nerve regeneration in fish. [For more on Sperry's theory of chemoaffinity, see the memorial on page 31 of this issue.]) One example of these omnipresent chemoaffinity molecules is shown above. The upper drawing is a cross section of the spinal cord of a vertebrate embryo. A set of cells known as commissural neurons (yellow) grows processes down toward a structure called the floor plate (red), which lies along the midline of the spinal cord's ventral, or belly, side. The growing processes turn at the floor plate and extend along the length of the spinal cord to form their final connections. A molecule made in the floor plate tells these processes which way to grow. The lower drawing is a cross section through an embryonic soil roundworm called a nematode, which has about 300 nerve cells in its whole body. Neurons in the body wall similarly extend their axons down to the ventral midline (also in red), and they then turn and grow along the axis of the body. The floor-plate molecule was recently discovered by Marc Tessier-Lavigne and his colleagues at UC San Francisco, and it turns out that essentially the same molecule is found in the roundworm. In fact, you can take the molecule from a nematode and put it into a spinal cord culture, and the neurons will do the same things that they would do if the vertebrate molecule were there.

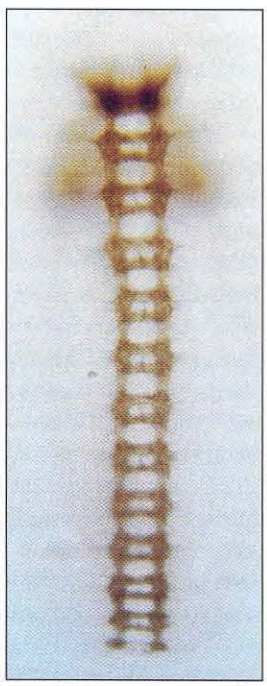
Such discoveries furnished the motivation behind our work. We presume that in a genetically hard-wired circuit, a set of molecules instructs the neurons to form the correct connections. In a system such as the mammalian brain, interactions with the environment may redeploy these same molecules (or others like them) and use them in new ways to rewire the system based on what the animal experiences. If we study a nervous system that is not extensively rewired, we may be able to figure out in detail the genetic rules that control the construction of its circuit. These rules may also prove applicable to the experience-driven rewiring of the mammalian brain.

My research group works with grasshopper and fruit-fly embryos. The two organisms have very similar central nervous systems, and many of the individual connections between nerve cells are the same in both. Back in the early 1980s, Corey Goodman's laboratory (then at Stanford, now at UC Berkeley) performed many of the classic experiments that defined the broad rules for the assembly of the organisms' central nervous systems, and showed that their circuits are highly conserved between insect species. Each kind of embryo has particular advantages. A grasshopper embryo measures several millimeters long compared to a fly's one millimeter, and grasshopper cells are much larger and easier to work with, but fly genetics have been studied in great detail.

There are several techniques for making the neural hardware and its wiring patterns clearly visible. We can inject individual cells with fluorescent dyes, and track where their processes go. (You can actually hand-dissect a grasshopper or fruit-fly embryo, if you don't drink too much coffee that morning.) We also use antibodies that recognize specific neuronal structures and bind to them, staining them an easily visible brown. We can even tag different antibodies with assorted brightly colored tails, so that we can see several different structures at once. Once we've used one of these methods, we flatten the segment out and photograph it under a microscope at a magnification of about 500. We use special types of microscopes, such as one called a confocal microscope, to focus on a very thin layer of tissue. Ordinary microscopes collect light reflected from the entire thickness of the sample, so the details in any particular layer are blurred by the layers above and below it. A confocal microscope, however, has a pinhole above the sample through which the reflected light must pass. The pinhole's distance from the sample determines the layer whose reflected light is allowed to pass. The pinhole is scanned across the sample, and the transmitted

The ladder of bundles defines the axons' possible routes in the way that a grid of city streets defines municipal bus routes. And, like a downtown bus route, an axon may follow one street for a while, then turn at an intersection onto a cross street, and so on until it reaches its destination.





Above: Identical segments of a grasshopper and a fly embryo (inset), identically prepared, identically stained, and shown to the identical scale. The axons, stained brown, clearly reveal the double-runged ladder pattern. Since the embryo is symmetrical around its midline, each cell on the left has a twin on the right. The black-stained cell bodies are the neurons named aCC (top cell in each pair) and pCC (bottom cell in each pair) in the diagram on the opposite page.

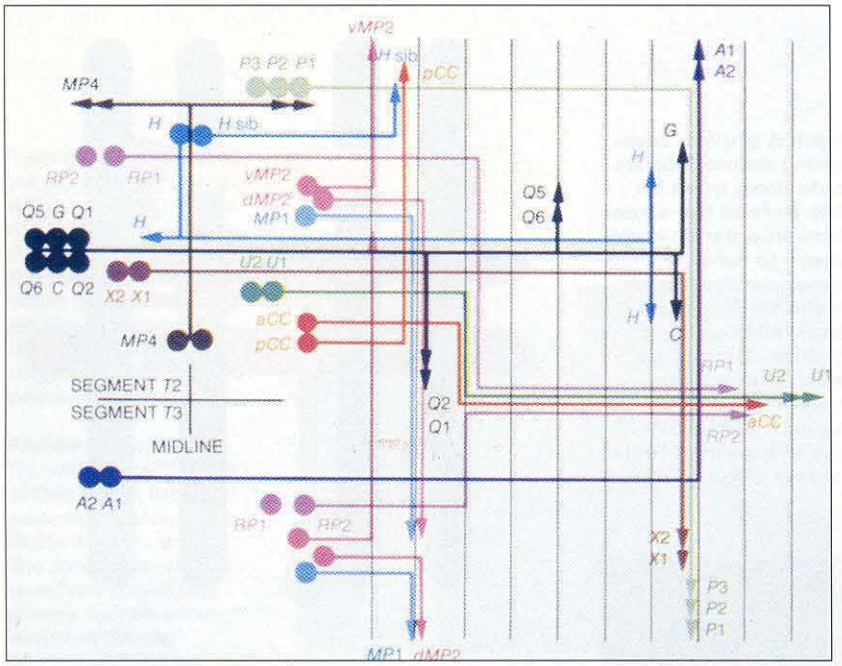
Left: A whole fly embryo's central nervous system. The developing brain is the out-of-focus blurs at the top and to either side of the ladder. The double-runged ladder is the insect equivalent of the vertebrate spinal cord. Some of the motor neurons that lead away from it are also visible.

light is recorded by a CCD camera.

In the fly, the wiring is compressed into a much smaller area, so it's more difficult to distinguish individual axon bundles—individual wires, as it were. However, fruit-fly genetics have been intensively studied for 75 years, so we have a tremendous amount of information about the contents of the fly's genetic blueprints. We can create mutants in particular genes and see how those mutations affect the structure of the nervous system. That is, when we find a gene we think does something important, we can "knock out" the gene—mutating it to render it nonfunctional. If something abnormal happens as a consequence, this provides clues to what the gene does in a normal animal. (These kinds of studies on the structure and function of the fly nervous system were pioneered at Caltech in the early 1970s by Seymour Benzer, Boswell Professor of Neuroscience, Emeritus, and his colleagues.)

Both species' embryos are divided up into about 15 segments that, at early stages of development, are very similar to one another. There are subtle differences in the details of the neural wiring from segment to segment within an embryo, but the wiring in any segment in one embryo is identical to that in the corresponding segment in any other embryo at the same stage of development. Thus our experiments are reproducible, which is often not possible with higher organisms. Since the basic circuit is almost identical in each segment, if we can understand one set of blueprints, we will understand them all.

Each segment contains a section of the central nervous system and a set of peripheral nerves. The entire central nervous system (left) looks like a ladder. The longitudinal connectives—the sides of the ladder—extend the length of the embryo. The rungs are called commissures, and they are bundles of axons that cross from one connective to the other. (The first few rungs become the brain.) Each segment contains two commissures. Like the mammalian spinal cord, the insect nerve cord contains motor neurons that send axons to specific muscles in the body wall. These motor connections are identical in every embryo, as I mentioned above, and their development has been studied in detail by many people. The embryonic peripheral nervous system consists of sensory neurons, whose shape and arrangement are also invariant. The sensory neurons carry input from the environment. Their cell bodies lie near sensory organs, and they extend processes to the nerve cord and make specific connections. When the embryo hatches, these neurons will receive pressure and chemosensory input that will tell the larva such things as which



Left: A partial wiring diagram of a segment. The circles are cell bodies; the arrows are axons. The 10 vertical lines on the diagram's right-hand side are individual axon bundles in the longitudinal connective, and the bundle going off horizontally to the right is the intersegmental nerve. Cells of the same color come from a common ancestor. Neurons aCC and pCC, stained black on the opposite page, are colored red here.

Above: The brown, blotty, spiky thing in the top center of this photo is the growth cone of an axon on the move, sniffing its way through the jungle of tissue cells in search of its target. The neuron's cell body is about 14 inches away from the growth cone at this scale, down somewhere to the lower right.

side is up, and where to find food. Much of the work on the development of the fly peripheral nervous system has been done in the laboratory of Yuh Nung Jan (MS '70, PhD '75) and Lily Jan (MS '70, PhD '74), at UC San Francisco.

Within each commissure and connective there are 15 or 20 different axon bundles, each of which might contain 10 axons. The ladder of bundles defines the axons' possible routes in the way that a grid of city streets defines municipal bus routes. And, like a downtown bus route, an axon may follow one street for a while, then turn at an intersection onto a cross street, and so on until it reaches its destination.

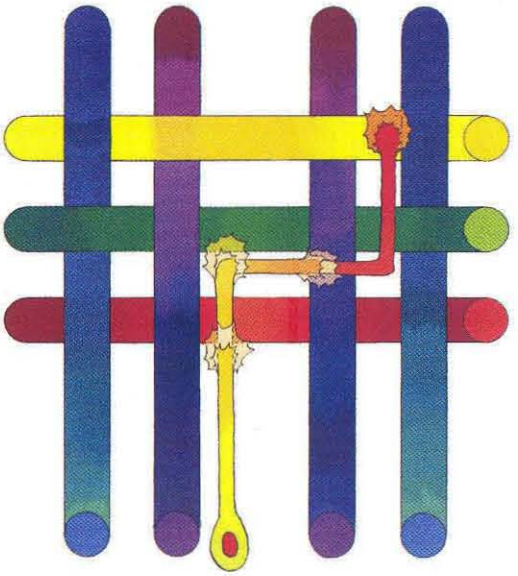
Part of the wiring diagram for a segment at an early stage of development is shown above. At this point, each segment has about 250 nerve cells, each of which can be individually identified. So, for instance, in every grasshopper embryo you examine, you'll find sister cells called aCC and pCC. The aCC cell always sends its axon out along the intersegmental nerve and on to a dorsal muscle, while pCC's axon takes a different route along a longitudinal connective. Cell aCC is a motor neuron, while pCC is an interneuron—a type of cell that makes connections only between other neurons. Thus, even though they came from the same parent cell, the two neurons end up doing very different things.

Every cell within the array knows its identity, and which bundle it should extend its axon along. The axon's growth cone (at left) senses the environment and determines where the process should go. The growth cone probably recognizes markers on the surfaces of the bundles it encounters that tell it "this bundle is different from that bundle, and this is where I'm supposed to turn." This is called the labeled-pathways hypothesis. So in the example above, the one cell is expressing the pattern of genes that makes it cell aCC, and its growth cone is looking for a cue that says "aCC turn here." This cue will allow it to follow the correct pathway.

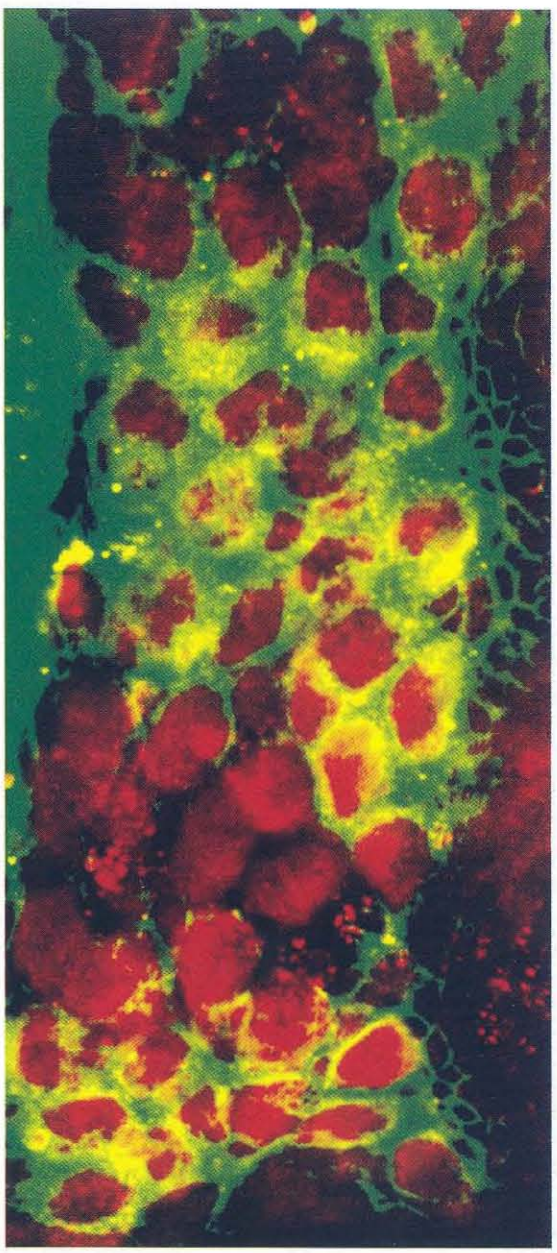
Experiments done in Goodman's laboratory, in which the growth cone's target was removed, have provided evidence for such labeled pathways. Consider a neuron that's supposed to find a pathway defined by the axon of another neuron. If you kill the second neuron with a laser, the first neuron's growth cone will grow to where the pathway should be, and then stop or wander randomly. It can't continue on its prescribed route, because its pathway is missing.

We'd like to understand how the cell makes the sequence of decisions that ultimately sends an axon along a predetermined route to make a particular connection. We could ask two basic questions about this process. First, how does a cell know it's supposed to be a nerve cell at all? Why didn't it become something else? What molecules made it choose a career as a neuron? Second, how does a nerve cell choose the route along which it should extend its growth cone? This question has three parts: the initial decision to choose a particular pathway; subsequent decisions to turn off that pathway onto others; and finally, the decision to stop when the target is reached. For instance, in the watercolor sketch on the next page, we can ask what molecular information tells the axon to go straight across

The growth cone has to communicate back to the cell body and say, "This is the wrong pathway," or "We're up here, and we should make a turn to the left."



Right: A growth cone, having memorized its route map, must be able to read the street signs in order to know where to turn. It somehow distinguishes the various pathways, although not by their color. This sketch shows the growth cone interacting with each pathway in turn as it feels its way along.



Right: The right-hand half of the sheet of neuron-progenitor cells in a grasshopper embryo segment. Neuroblast 1-1 is the red cell in the upper left corner. This is a double-stained micrograph—the red-flourescing antibody binds to neurons, while the green one binds to mesectoderm cells, which define the midline. Where both markers bind to the same cell, their superposition comes out yellow, marking the sheath glial cells that surround the neuroblasts. The green strip along the photo's left edge is the segment's midline.

the red pathway, then turn right on the green pathway, and finally turn left on the blue pathway and make a connection near the yellow pathway. As mentioned above, these decision mechanisms involve surface contact between the growth cone and the pathways. They also involve signaling events, because the growth cone has to communicate back to the cell body and say, "This is the wrong pathway," or "We're up here, and we should make a turn to the left." The cell body then has to make new proteins that will cause the growth cone to change its direction of growth.

To consider the first question, of neuronal career choice, we have to understand how the cells that will give rise to neurons are organized. The neural zone of an insect embryo starts out as a gridlike sheet of neuron-progenitor cells called neuroblasts, which we identify by their row and column number. The biography and genealogy of each of these cells is genetically predetermined, and has been exhaustively studied on the cellular level. We know what each cell, and its descendants, is going to do before it does. For example, one red cell in the illustration at left is neuroblast 1-1. This will divide to produce a set of daughter cells, and the first daughter cell will in turn divide to produce the aCC and pCC neurons. The other neuroblasts produce other neurons that also have their own unique identities.

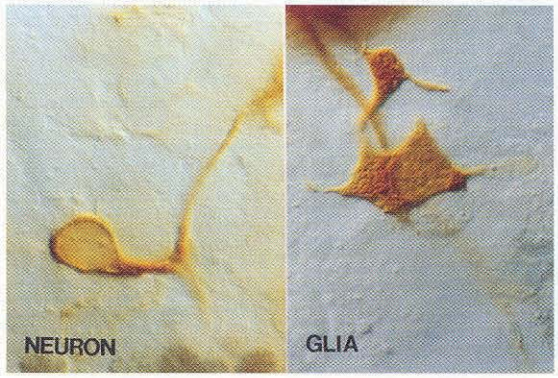
Some neuroblasts also generate glial cells, which are support cells that wrap up an axon and insulate it. Nerve activity is fundamentally an electrical phenomenon, and without the glial cells for insulation, the neurons wouldn't be able to function. The two kinds of cells are easy to

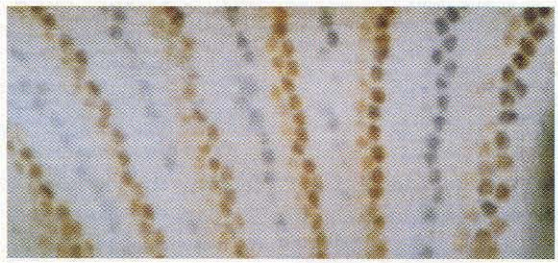
Top: By their shapes ye shall know them: a neuron (left) has a round cell body with a long process extending from it, while a glial cell has an irregular shape—this one has scalloped edges and a bulbous process on top.

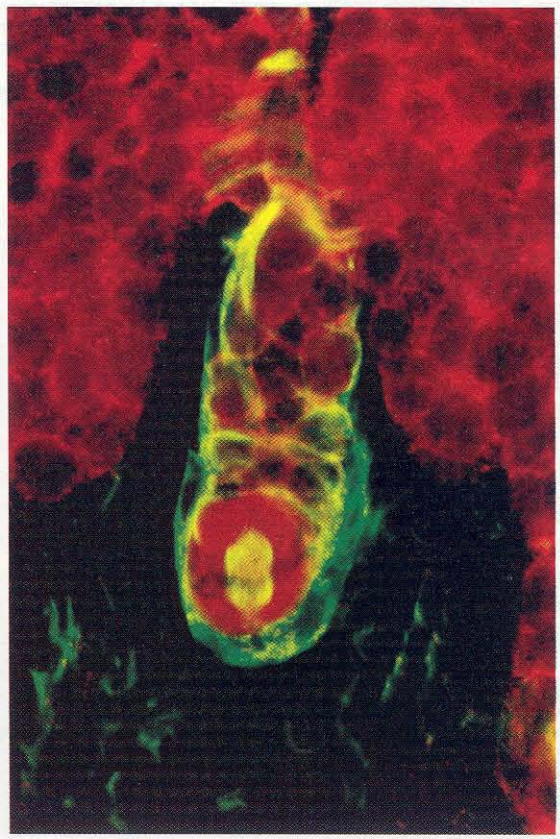
Middle: This fruit-fly embryo will soon divide itself into segments along the dotted lines, under the direction of two master-regulatory genes called *even-skipped* (being expressed in the brown-stained cells) and *engrailed* (in the black-stained cells).

Bottom: The red cell at the tip of this column of cells is called the MNB, or median neuroblast, and it has been caught in the act of dividing. (The MNB's yellow filling is its chromosomes moving apart.) Previous divisions of the MNB gave rise to the pillar of red nerve cells as well as the green glial cells surrounding them. The other yellow regions are a superposition effect.

Below: This posterior midline glial cell has been nicknamed "Batman" for its winglike processes. To see why, turn the magazine upside down.



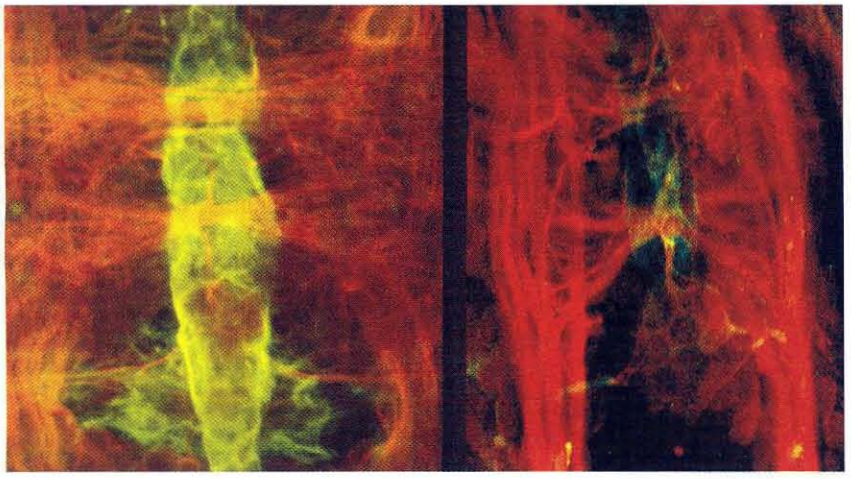




distinguish—neurons are round and have processes, and glial cells are irregularly shaped.

We want to discover what information instructs a particular neuroblast to generate the specific set of daughter cells that it produces. But, as I have just shown, that sequence of events actually encompasses several decisions. We have studied the molecular basis for one of them: how does a neuroblast daughter decide whether to become a neuron or a glial cell? Two of the molecules we've looked at that may be involved in cell-fate decisions are called the engrailed and even-skipped proteins. (The term "engrailed" comes from heraldry, and refers to a wavy line that resembles a row of fish scales. It looks a bit like the normal pattern of bristles on a fly's wing—a pattern that changes in the mutant.) The engrailed and even-skipped proteins bind to DNA, the genetic material, and switch genes on or off. Gene regulation is like a pyramid. At the pyramid's apex are the master regulators, which control a battery of secondary regulators, which control the final products at the pyramid's base. That is, some genes cause the synthesis of protein molecules that control the expression of other genes, which then cause the synthesis of the protein molecules that actually make up the cell. So once a developing cell makes the decision to switch on a master regulator, that decision determines everything that happens subsequently. The engrailed and even-skipped proteins are master regulators, and are found in both insects and humans. In the early fruit-fly embryo, these two molecules are expressed in alternating stripes, and they determine segmentation. There is one engrailed stripe per segment. Later on, these stripes control the identities of the neuroblasts that arise from them. So, for instance, neuroblast 1-2, which makes the engrailed protein, will give rise to a different set of cells than will 1-1, which does not. Similarly, the first daughter of 1-1 (but not of 1-2) makes the even-skipped protein, and this may control the identities of the cells that it produces.

One lineage that Barry Condron, a postdoctoral fellow in my lab, has studied in detail is shown at left. The sequence begins with the MNB, or median neuroblast, an unpaired cell at the segment's midline. It gives rise to a certain set of neurons, which, along with the axons they produce, are labeled red. In addition, it generates all those green cells, which are glial cells that wrap up the bundle of red axons. Thus, the MNB is multipotent—it can generate both neurons and glia. Since the cells in the MNB lineage produce the master-regulator engrailed protein, we can ask if its presence affects whether



Opposite, A: The now-familiar central-nervous-system segment, stained black with an antibody that recognizes all neurons. "A com" is the anterior commissure and "P com" the posterior one, "con" is one of the longitudinal connectives, and "ISN" is the intersegmental neuron.
B: An antibody specific for fasciclin I binds only to the ladder's rungs.
C: A fasciclin II antibody binds exclusively to the ladder's sides.

Above, left: The normal product of the MNB's divisions is a grapelike bunch of nerve-cell bodies hanging from a stem of axons (both in red), almost invisible within their sheath of green glial cells that fit tighter than control-top pantyhose. Right: Sabotage the *engrailed* gene, and the glial cells never appear, but are replaced by extra nerve cells.

a given cycle of cell division produces a neuron or a support cell.

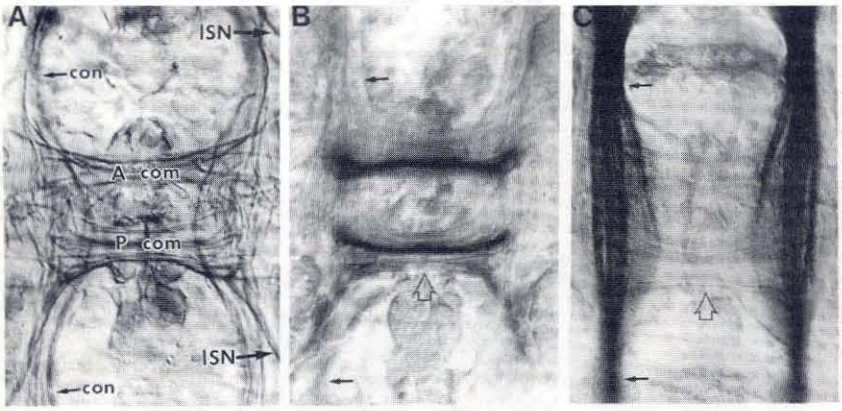
If MNB doesn't activate the *engrailed* gene, something quite interesting happens. If we microinject the neuroblast with DNA that prevents the engrailed protein from being expressed by its offspring, the result is shown above right. There are no green cells at all—that is, the glial cells that normally enclose the axons didn't form. Instead, there's a larger than normal number of red cells. (A normal embryo is shown at left for comparison.) The red cells are irregularly shaped, because they're not held in by the sheath of green glial cells. Unconfined, the extra neurons burst out of the side of the bundle and form something like a little tumor, which has a very characteristic shape. So, without the *engrailed* gene, all the progeny become red cells. This molecule apparently determines whether a neuroblast's daughters become neurons or glia.

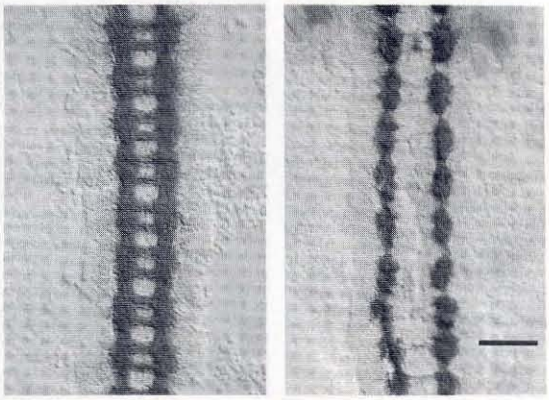
Which leads to the second question we're exploring: what happens once a cell has opted to become a neuron? What cues does it use to know in which direction to extend its growth cone? You might imagine that if the growth cone wanted to turn at the second cross-path, it would be looking for a signpost molecule that was on the second path but not the first. These signposts could be protein molecules on the surfaces of axons and cell bodies along the pathways. Such proteins would have particular shapes that would be recognized by other proteins on the growth cone's surfaces, and would tell the growth cone to turn left or right. To identify these signposts, one might search for proteins that are expressed only on the surfaces of cells along particular pathways. The whole set of such molecules might determine the set of all possible decisions that any one growth cone could make.

Two such proteins, identified by Michael Bastiani in Goodman's laboratory, are shown on the opposite page. Fasciclin I is expressed only on one bundle in each commissure or rung. Fasciclin II, in contrast, appears only on the longitudinal pathways at this stage of development. So, for instance, a nerve cell might know that it has to recognize fasciclin I, and turn left on that pathway. Once it did so, it would know that it should then search for a pathway that has fasciclin II, and turn right when it got there, and so on.

We can test these rules by making mutants. For example, if a fly embryo does not make fasciclin I (and a certain gene controlling something else has also been mutated), then none of the commissures form. The embryo generates a nervous system that lacks crossbars, but still contains the longitudinal pathways. If we could make mutations of the whole set of such genes, and combine these mutants in different combinations, we might understand the set of rules involved in constructing the array.

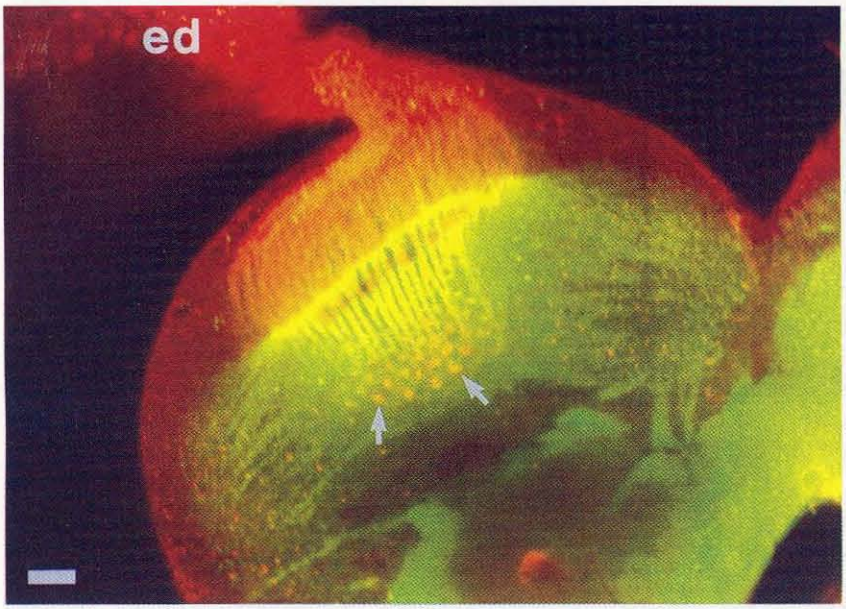
We're also searching for the molecules on the growth cone that could read the signposts and tell the cell whether it's going the right way. One such set of molecules are the protein-tyrosine phosphatases (PTPases). Shin-Shay Tian and Chand Desai, postdoctoral fellows in my lab, have shown that four of these PTPases are found on most or all growth cones. The molecules are then left behind on the axons of the central ner-





Above, left: A normal fly central nervous system. Right: A double mutant that doesn't make fasciclin I, and whose central nervous system is a rungless ladder. The scale bar at bottom right is 20 microns.

Below: Wired for sight—the fly's embryonic eye disk (labeled "ed") is in the upper left corner. The red bundle running from it to the brain's optic lobe (at center) is the optic nerve. The cells bearing PTPases on their surfaces fluoresce green, and the orange dots (arrowed) are where connections are being made between the nerve and the brain. The scale bar at lower left is five microns.



vous system ladder after the growth cone moves on, at which point they may serve a second, as yet unknown, function. They are also expressed in the developing larval brain's optic lobes, as shown below. These are regions that receive input from the neurons in the fly's eye. It is possible that the PTPases have roles in determining the organization or function of the axons arriving from the eye.

These PTPases are proteins that span the cell membrane. The part of the molecule outside the cell probably recognizes proteins on another cell surface, and the part inside the cell catalyzes a chemical reaction that removes a phosphate group from another protein, which in turn sends a signal within the cell. So these molecules could couple pathway recognition to a signal that tells the cell to make a decision. They are highly conserved by evolution—for example, the PTPases called DLAR (found in flies) and LAR (found in humans) have very similar structures. Whenever a molecule is this similar in such different species, it probably means that it does something of fundamental importance. Once evolution finds something that works, it sticks with it.

In summary, I've shown a few examples of molecules that are involved in the construction of the insect axonal array. But even insects are very complex. They may have about 25,000 genes, of which at least half are involved in the construction of the nervous system. It's going to take a long time to understand how all those genes interact. The system is still very much a black box. Our experiments are basically fishing expeditions—we're just searching for genes that have something to do with this process, and then categorizing them by what they do. We're not at the stage yet where we can define an overall hypothesis for the mechanism by which the circuit is put together. Our lab hopes to learn about some aspects of the puzzle, and to extrapolate this knowledge into figuring out something about how vertebrate brains, and thus the human brain, are put together. □

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