BRAIN

In the ultimate mind-body connection, specialized immune molecules

BLOOD-BRAIN BARRIER: This micrograph depicts part of a blood vessel in the brain. The image reveals the crucial arrangement of vessel cells protecting the brain from invasive pathogens and the immune cells that mop them up.

BENDERS

control the wiring of the brain. Now, a trailblazing neuroscientist suggests a way to revamp the circuits of schizophrenia, enhance learning and reverse the ravages of stroke.

BY **KENNETH MILLER**

SOMEWHERE ON THE CAMPUS of Stanford University, among the stately oaks and tile-roofed temples of scientific inquiry, live Carla Shatz's super-mice. They learn complex physical tasks more quickly than their ordinary cousins. If one eye is deprived of sight, they rapidly rewire their brains to compensate, then beat normal one-eyed mice on tests of visual acuity. They recover more readily from some brain injuries, too. What sets these rodents apart is their superior ability to form new neural connections, or strengthen existing ones, in response to experience.

Shatz is proud of her prodigies. But when I ask for permission to visit them, the pioneering neurobiologist turns me down. "They're in a germ-free facility," she says apologetically, glancing away from a video in which a tiny champion powers through a water maze. Only their handlers are allowed into the lab where the animals are kept, Shatz explains, and even they must shower and don sterile garments before entering. That's because the mice have incomplete immune systems. They've been bred to lack proteins — members of a family called MHCI (which stands for major histocompatibility complex class I) — crucial to fighting pathogens. The same mutation that gives them their supremely adaptive brains has left them with extraordinarily vulnerable bodies.

In the body, MHCI proteins are watchdogs, tagging infected cells for immune attack. In the brain, these proteins assume an entirely different role, helping to regulate neuroplasticity — the ability of neural circuits to reshape themselves at every stage of life. Shatz has spent more than a decade probing the latter function. Yet until she proved otherwise, few scientists thought MHCI or other so-called "immune molecules" were even present in a normally functioning brain. In fact, their absence was a basic tenet of neuroscience.

As you may recall from biology class, the brain enjoys what's known as immune privileged status. A dense layer of cells called the blood-brain barrier protects the organ from germs circulating in the body, and from the immune cells

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that combat them. That is why, when you are battling the flu, neither the virus nor the inflammation directed against it spreads into your delicate cerebral neurons. Unless the barrier is breached by injury, autoimmune disease or catastrophic infection, rarely does a T cell, a B cell or any of the immune system's other shock troops get through. It was long believed that the immune system's molecular sentinels — molecules like MHCI — were missing from within the brain, too.

Then, 15 years ago, Shatz stumbled across genes coding for MHCI in fetal cat brains. She soon unearthed MHCI proteins and receptors (molecules that bind with MHCI) in the unimpaired noggins of mice. They eventually turned up in healthy monkey and human brains as well. And as Shatz devoted herself to investigating MHCI and its entourage in those surprising places, she sparked a neuroscientific revolution.

Today, a growing number of researchers are examining the complex ways in which immune molecules affect the brain and nervous system. Manipulating such molecules, these scientists believe, may be key to treating many devastating neurological ailments, from autism and schizophrenia to Alzheimer's and ALS. Shatz even dreams of a "plasticity pill" to restore the neural suppleness of stroke victims — and her latest experiments offer hope that it could someday come to pass.

THIS ETCHING BY Carla Shatz's mother, artist Shanah Shatz, was inspired by an electron-microscope photo of the junction connecting a nerve cell and a muscle.

"People thought we were crazy when we made this discovery," says Shatz, a slender 66-year-old with high cheekbones and a halo of dark curls. "But my parents gave me some advice when I was young. They said, 'Don't worry about what other people think of you.' " She gazes pensively at the computer screen. "I probably generalized it too much."

VISION QUEST

There are no conventional family memorabilia in Shatz's office, a few steps from her cavernous lab. The space is sleekly functional, with louvered glass walls, and the photos on the shelves are of her professional kin: mentors, colleagues, former students. One framed artwork, however, connects Shatz to her childhood, and the earliest influences on her own brain circuitry. It is an etching by her late mother, an artist who loved science. The image, a mysterious, gray blob surrounding a spot of intense red, was based on an electron-microscope photo of a neuromuscular junction (the connection between a nerve cell and a muscle) clipped from a magazine sometime in the 1980s.

"She was a very serious painter," Shatz says, "and she really understood biology. I think she would have made a great MD. But she stayed home and brought us up — it was that generation." Shatz's father was an aeronautical engineer who helped design the guidance system for the Apollo 13 lunar module. Growing up in West Hartford, Conn., Shatz absorbed her mother's passion for the visual arts. But she was also, as she puts it, a "science nerd" — a gender-radical stance for a girl who entered her teens just before Eisenhower left office. Her parents encouraged her to follow her interests, no matter how unorthodox or disparate.

Shatz followed them to Radcliffe College, where she majored in chemistry but also took classes in design. A course with biochemist George Wald, who had won a Nobel Prize for his work on how the eye perceives color, helped spark her interest in studying the brain mechanisms behind vision. "I realized that I could have my cake and eat it too," recalls Shatz. After she told her adviser she wanted to write her honors thesis on how people see, he sent her across the river to Harvard Medical School, where a pair of ambitious scientists, David Hubel and Torsten Wiesel, were investigating the plasticity of the visual system.

She spent a year studying with the duo, whose work eventually won them their own Nobel. Hubel and Wiesel were already renowned for having charted the architecture of the primary visual cortex (the area of the cerebral cortex, at the back of the head, that receives input from the retinas). As part of this work, they found the region has a pattern of zebralike stripes — dubbed "ocular dominance columns" — made up of neurons that process information from the right eye, the left eye or from both. In the aftermath of the discovery, their focus was finding whether that structured pattern was determined entirely by genes, or whether experience also played a role.

Hubel and Wiesel wanted to learn, among other things, why a child with a cataract — unlike an adult — may lose sight permanently in the affected eye if the obstruction isn't removed promptly. To find out, they deprived cats and kittens of sight in one eye for prolonged periods. In kittens with one eye sutured shut, they discovered, the ocular dominance columns changed radically. The stripes devoted to the sighted eye expanded, while those devoted to the obstructed eye shriveled. These changes occurred only during a "critical period," the scientists observed, when the young animal's neural circuitry was still developing. In adult cats, as in humans older than 6, such plasticity was greatly reduced.

Shatz witnessed some of these landmark experiments and

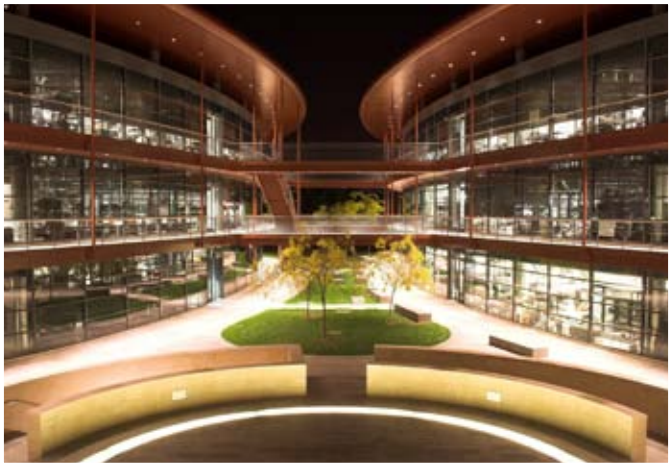
COURTESY CARLA SHATZ

Neurobiologist Carla Schatz

GREGORY COWLEY

they intrigued her. When she graduated in 1969, she knew she wanted to explore the eye-brain connection further. But how? Her two doctor uncles urged her to go on to medical school, but Shatz wasn't sure. A few years earlier, her paternal grandmother — a brilliant woman, the first in her family to go to college — had been crippled and made mute by a stroke. "Here were my uncles, both of them neurologists, and they couldn't do a damn thing for her," Shatz recalls thinking. "There wasn't enough research." She decided to become a neurobiologist.

In 1976, after returning to Hubel and Wiesel's lab for doctoral studies, she became the first woman to receive a Ph.D. in neurobiology from Harvard. In 1978, she was hired by Stanford, soon becoming the first woman to become a tenured professor there in basic science. Commandeering a lab of her own, she set out to expand on her mentors' work, and she wound up wandering into utterly new terrain.



CURVING WINGS define Stanford's James H. Clark Center, home of the interdisciplinary Bio-X, which Shatz directs.

FIRE AND WIRE

Hubel and Wiesel had uncovered the principles of how neuroplasticity works in the brain: Basic neural architecture is hardwired. For instance, the eye is genetically programmed to connect with the visual, not the auditory, part of the brain. Fine-tuning that circuitry — connecting the eye to a specific part of the visual cortex — is shaped by experience.

To understand how this works, it helps to remember a bit more basic biology. When neurons are stimulated, they spike with enough electric current to send neurotransmitters across a tiny gap called a synapse, where other neurons, in turn, are similarly stimulated and provoked. As the signal passes down the line, entire circuits are put in play. Neurons that handle lots of robust, well-synchronized signals sprout more neurotransmitter-generated terminals, and their connections with other neurons along the signaling pathway grow stronger. (Neuroscientists have a slogan for this: "Cells that fire together wire together.") Synapses that transmit few, weak or out-of-sync signals are pruned away. In this way, brain circuits are remodeled with use.

By the late 1970s, researchers had learned much about how the process plays out in the mammalian visual system. Genetically programmed molecules guide embryonic nerve fibers from the light-sensitive retina toward the visual cortex, where images are perceived. At first, the connections are highly approximate; the stripes of the ocular dominance columns are partially formed in humans and other mammals at birth. Then, input from the eyes helps refine the neural pathways until, over a period of months to years depending on the species, the columns mature and stabilize.

But major questions remained. Where, exactly, did the influence of genes leave off and experience begin? What biochemical processes allowed sensory input to change the brain's wiring? Why was neuroplasticity greater in juveniles than in adults, not only in the visual system but other areas as well?

Searching for answers, Shatz decided to focus on the lateral geniculate nucleus (LGN), a clump of tissue shaped like a piece of elbow macaroni, set behind each eyeball, that serves as a relay station shunting visual signals to the ocular dominance columns. Layers of the LGN start forming around the 47th day of gestation in fetal cats. The layers are almost completely formed by the time the animal is born, around day 60. It had long been assumed that this process was hardwired until the animal opened its eyes, at which point experience finished the job. Shatz, however, suspected that the layering might be guided by spontaneous nerve impulses from the retina when the animal was still in utero.

In 1988, she tested that hypothesis on cat fetuses using a poison called tetrodotoxin, which prevents nerve impulses from firing. On the 42nd day of gestation, she surgically removed the fetuses, fitted them with tiny tanks that pumped the neurotoxin into their brains, then returned them to the uterus. ("Carla has good hands in the lab," recalls Stanford neurobiologist Sue McConnell, then one of Shatz's postdocs.) Outwardly, the fetuses continued to develop normally. But when their brains were examined two weeks later, Shatz's hunch proved correct: The LGN's layers had failed to develop.

Shatz now had indirect evidence that the layers were formed in response to retinal signaling, coded for not by experience but by genes. But it wasn't until three years later that she was able to observe the signaling in action. Working with her colleague Denis Baylor, she harvested retinas from fetal cats and ferrets, putting each piece in a dish of nutrient medium to keep it alive. Then she placed each piece of retina over a grid of electrodes wired to sense collective neural activity. The electrodes detected waves of synchronized nerve impulses sweeping through the retinal tissue, in patches of up to 100 cells at a time. "It was like neighborhoods of nerve cells in the eye were placing phone calls to the brain to check connections," Shatz recalls.

In a developing fetus, she surmised, those phone calls would reach groups of nerve cells in the LGN; as their synaptic connections with the retinal neurons strengthened, the LGN neurons would begin forming "area codes" of their

own. Meanwhile, synapses with poor connections would be trimmed away. After birth, visual input would further sort out the LGN's wiring, until layers associated with right and left eyes were complete. It would take much more research, however, to determine exactly how the process worked.

In 1992, Shatz moved on to the University of California, Berkeley, with her then husband, a fellow neuroscientist. The marriage was in trouble — undermined by the pressures of academic life and a failed course of fertility treatments — and it ended soon afterward. Despite the turmoil, Shatz continued to plumb the mysteries of LGN development. And in 1994 she launched the series of experiments that would overturn long-held assumptions about the brain's splendid isolation from the immune system.

Shatz wanted to know what genes were active in the LGN when the retina sent its prenatal signaling waves. So she and her postdocs used tetrodotoxin to halt the retinal signals in fetal cats. Then they compared the expression of thousands of genes in two samples by measuring levels of messenger RNA. (The DNA in genes code for mRNA, which, in turn, codes for proteins.) This test determined that only a few genes switched off when signaling was blocked — and one of them was the gene known to code for MHCI.

Shatz and her team were nonplussed. They knew that MHCI wasn't supposed to be expressed in a healthy brain — only in an injured or diseased one, when the blood-brain barrier had broken down. Yet because none of them was a specialist in neuroimmunology, they didn't quite grasp the outrageousness of their finding.

"We were blissfully ignorant," Shatz says, "of the fact that we had run into a dogma."

"People thought we were crazy when we made this discovery."

GLUE FOR THE BRAIN

There are several reasons why nobody before Shatz had found genes coding for MHCI in normal neurons. In part, her advantages were technical: a more comprehensive screening test than brain researchers usually employed, and a more sensitive type of follow-up testing. But perhaps most important, other scientists hadn't been looking in the right place at the right time.

Outside the brain, MHCI proteins and the genes coding for them are hard to miss. MHCI molecules perch on the surface of most of the body's cells, displaying peptides — snippets of proteins — that reveal what's going on inside. They present this evidence to killer T cells, immune-system agents that hunt for byproducts of viral infections, cancerous mutations or anything else that doesn't register as "self."

Each killer T cell carries thousands of receptors programmed to recognize a particular foreign peptide. If it encounters such a snippet, the T cell uses chemical weapons called cytotoxins to destroy the host cell — and the invading pathogen, as well.

Inside the brain, MHCI is far more elusive. It appears at lower levels, and only in certain areas at any given moment. In fact, Shatz observed no actual MHCI proteins when she first examined those fetal cat LGNs; what she found were biochemical signs indicating that MHCI genes were active. A less intrepid scientist might have chalked up the initial "hit" as a false positive. But Shatz pressed further.

First, she and her postdocs tested for MHCI gene expression at different stages of LGN development. They found that levels peaked as the right-eye-left-eye layers finished segregating, and fell sharply afterward. Then the team searched elsewhere. They detected little or no MHCI expression in some areas of the brain, but they found it in several other places, including the visual cortex while the ocular dominance columns were forming, and in the hippocampus — an area of the brain associated with learning and memory — at all ages.

Next, the team used tetrodotoxin to block nerve impulses in a kitten's eye when it was 6 weeks old, during the critical period of development. When nerve impulses fell, MHCI expression decreased as well.

To see what would happen when nerve cell impulses were heightened, Shatz and postdocs injected rats with a drug that causes seizures. MHCI expression in the hippocampus and cerebral cortex soared. When Shatz and her team tested for actual MHCI proteins (as distinct from switched-on genes) in slices of rat brain from those same regions, the results were positive.

Pondering these findings, Shatz developed a tentative theory about what MHCI was doing beyond the blood-brain barrier. Under normal circumstances, it clearly wasn't mediating immune function. Instead, it seemed to be associated with remodeling the brain.

MHCI levels rose in areas undergoing changes and fell when remodeling was complete or when a shot of tetrodotoxin interrupted it. Shatz hypothesized that MHCI molecules might function as a kind of "synaptic glue," stabilizing contacts between neurons once appropriate connections had been established.

Shatz expected these controversial notions to meet with some resistance once she made them public. Still, she was stunned when the prestigious journal *Nature* rejected the paper in which she reported her team's findings. "They said we must have done something wrong," she recalls, "and we should go back and figure out what it was."

"KNOCKOUT" DISCOVERY

In college, Shatz had been a member of the Radcliffe ski team. The experience taught her a lesson that she later applied to her scientific career: "When you're on a really steep slope, and you can't see the bottom, you have to just push off and trust your own abilities." So instead of packing up her metaphorical skis and going home, she sent the MHCI paper to *Neuron*, whose editors could find no flaws in the methodology. It was published in September 1998.

Many of Shatz's colleagues thought the results were crazy, but others offered her equipment and materials to pursue this daring new line of inquiry. If MHCI proteins — so crucial to immune function — also helped shape neural circuitry, the implications were profound. To begin with, what would happen to developing brains if these molecules went awry? Shatz got a hint when she looked up ailments associated with mutations in genes coding for the human version of MHCI. The list included schizophrenia and autism. Both disorders were also associated with maternal infections during pregnancy.

In genetically vulnerable individuals, Shatz wondered, could a mother's flu — and her immune reaction to it — disrupt MHCI's action in the fetal brain?

To answer such questions, Shatz would first need to investigate how the brain might function without any MHCI at all. Fortunately, an immunologist at Berkeley, David Raulet, had bred a line of mice incapable of expressing functional MHCI proteins. Raulet donated some of these so-called "knockout" mice to Shatz, who found two striking anomalies.

The first anomaly confirmed her hunch that MHCI helped fine-tune circuitry: In the absence of the immune molecule, the animals' LGNs never developed layers.

The second abnormality was, literally, a shocker: The knockout's neurons responded differently to electrical stimulation than neurons in mice that were unimpaired. In ordinary mice, persistent low-frequency stimulation to the hippocampus weakens synaptic connections between neurons; high-frequency stimulation strengthens them. In mice lacking MHCI, both kinds of stimulation made connections stronger.

So maybe MHCI didn't really provide glue for neural synapses. Maybe it did the opposite, telling neurons in the adult brain when to let those synaptic connections weaken — and, ultimately, be pruned away. That would allow circuits (like the LGN's layers) to stabilize, instead of perpetually change. With the ability to put the brakes on plasticity and halt its own runaway growth, the brain could organize itself efficiently.

But faulty MHCI expression could wreak havoc. Before birth, it might lead to imprecise synaptic pruning, leaving too many connections and setting the stage for autism or schizophrenia. Later in life, overactive MHCI expression might cause excess pruning, leading to the loss of connectivity seen in neurodegenerative diseases such as Alzheimer's, Parkinson's, ALS and multiple sclerosis. Overactive MHCI expression might also drive the harmful synaptic pruning that follows injury to neural tissue after stroke or spinal cord damage.

Many neurological ailments involve both the elimination of synapses and the inability to form new ones, resulting in a devastating loss of plasticity, as illustrated by the futile struggle of Shatz's grandmother to regain her language and motor skills after her stroke. "I hope maybe someday we can make a pill that will enhance plasticity. Maybe we can learn to manipulate these MHC Class I molecules or their receptors at will."

A plasticity pill could help heal the brain after stroke.

SHATZ stands outside her Stanford lab, where she wants to reverse brain disease by restoring plasticity.

GREGORY COWLEY

REMOVING THE BRAKES

Shatz, then 52, had just been hired by her alma mater, Harvard Medical School, as the first woman to chair the neurobiology department. Over the next few years, she began to fill in the details of how MHCI works in the brain at the molecular level, and how it affects animal behavior.

One important task was to learn how MHCI proteins deliver their instructions in the brain. The most likely scenario, Shatz believed, was that the molecules sat on a neuron's postsynaptic terminals, where electrochemical signals from other neurons are received. MHCI would send messages back across the synapse — orders like, "Please stop trying to connect." To find evidence for the phenomenon, her team began looking for MHCI receptors in the brain — literally, molecules that would connect with MHCI to help get the message through. And in 2006, they identified a likely candidate: PirB, a molecule found in some immune system cells, which also turned out to be present in neurons.

If PirB partnered with MHCI in neural circuits, Shatz thought, the absence of either molecule should have similar effects on the brain. That theory was confirmed by experiments in which her postdocs removed one eye in mice bred to lack PirB. In adult knockouts as well as juveniles, the ocular dominance columns associated with the missing eye expanded much more markedly than in ordinary mice. Whenever you removed the MHCI receptor, the brakes were lifted and plasticity took off.

To see how those qualities played out behaviorally, Shatz gave her lab mice some challenging tasks. First she deprived juveniles of vision in one eye so that the corresponding brain cells failed to make connections; once the mice reached maturity, they were put in a water maze that required them to recognize a pattern of fine lines to find a floating platform. MHCI knockout mice, with no MHCI and more plastic brains, did far better than their ordinary counterparts. In another experiment, using animals with unimpaired vision, mice unable to produce MHCI were quicker to learn how to keep from falling off a rotating rod; they also remembered the trick for longer time periods. These animals, Shatz said, "performed like Olympians."

A downside, of course, was that the MHCI knockouts were seriously immunocompromised. Mice without MHCI were also more vulnerable to epilepsy, an unfortunate side effect of untrammeled neuroplasticity. Nor could anyone be sure that

their unusual strengths in some cognitive areas weren't balanced by unusual weaknesses elsewhere; in animals that can't speak, such tradeoffs are hard to gauge. What was certain was that Shatz opened a vast field of exploration.

If immune molecules acted as brakes on plasticity, those brakes might be pharmacologically adjusted. For a host of neurological ills, Shatz says, that meant "a new kind of hope."

NEW AVENUE FOR THERAPY

In 2007, Shatz returned to Stanford. She'd been hired to run Bio-X, an interdisciplinary program meant to foster collaboration among life scientists, medical scientists, computer scientists, engineers and physicists. The program's home, the James H. Clark Center, is an aggressively biomorphic structure whose two curving wings could be taken for the hemispheres of a giant brain. In her office (in the left hemisphere), Shatz's face lights up as she describes her latest collaboration. It began when she and Rona Giffard, an anesthesiologist and a longtime stroke researcher, were whispering in the corner at a boring staff meeting.

"I said, 'We're working on these really cool molecules, and if you knock them out, the animals have more brain plasticity,' " Shatz recalls. Giffard was fascinated, and soon the pair designed a series of experiments aimed at advancing the quest for the "plasticity pill" Shatz had long dreamed of.

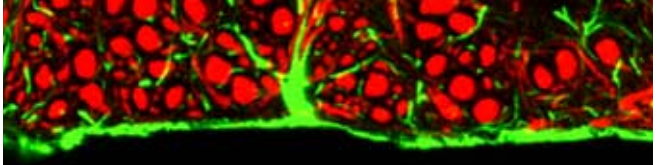
The team took mice lacking MHCI and had them perform athletic feats: balancing on a spinning rod, crossing a horizontal ladder. Then they gave the animals strokes, cutting off blood to the artery supplying motor and sensory areas of the brain. Days later, when these knockout mice were given the same tasks, the animals regained their expertise faster and more fully than ordinary mice with strokes. What's more, lab tests indicated that while the areas of brain damage started off the same for both sets of mice, the damage diminished more for the knockout mice over the following days.

"This opens a new avenue for therapy," says Shatz, whose team published their findings in *Neuron* in March 2012. Right now, the only widely available treatment for preventing brain damage from stroke is tissue plasminogen activator (tPA), which breaks up blood clots; it must be given within a few hours to be effective, and though it limits initial damage, it doesn't help the brain restore lost synapses or form new ones. Much of a stroke's destruction, indeed, occurs after the initial injury, as even healthy synapses within the affected area are pruned away — a process apparently mediated by MHCI proteins. If a pharma company can find a way to temporarily disable those molecules (most likely by blocking their receptor, PirB), that secondary damage might be avoided, and the brain might also do a better job of repairing itself.

Shatz knows it may take many years to develop such a drug, but she's savoring the proof-of-concept moment. Her only regret is that her grandmother can't be here to witness it. "To think that this could be applied clinically …" she pauses, remembering her beginnings.

"It's kind of like a big, wonderful circle." D

Kenneth Miller *is a journalist living in Los Angeles.*



TRACKING THE BODY IN THE BRAIN

In the years since Shatz's discovery of MHCI in normal brain cells, other scientists have been studying the action of immune molecules in the brain, as well. In Europe and China, a series of large-population studies confirmed that mutations in the area of the genome controlling MHCI increase the risk of schizophrenia. At Johns Hopkins University, neuroimmunologist Carlos Pardo found abnormal levels of MHCI and other immune proteins in the brains of autistic patients. At Princeton, neurobiologist Lisa Boulanger — one of Shatz's former postdocs, who now has her own lab — began investigating whether changes in MHCI levels could cause neural signaling problems mimicking autism in mice.

At Stanford, a team led by neurobiologist Ben Barres discovered that synapses in the developing brain produce two other immune proteins, C1q and C3, associated elsewhere in the body with complement proteins, which work in concert with antibodies to destroy invading microbes. Further research showed that fetal mice bred to lack these molecules — like animals lacking MHCI, and like humans with autism or schizophrenia — undergo inadequate synaptic pruning in some parts of their brains. Perhaps not by coincidence, abnormally high levels of C1q and C3 have been found in people with Alzheimer's, glaucoma and other disorders involving excessive pruning. Could these molecules be malfunctioning together with MHC in neurological diseases? "No one knows," says Barres. "I talk to Carla all the time. We're all working on pieces of the same puzzle."

At Caltech, developmental neurobiologist Paul Patterson found he could induce the core symptoms of autism and schizophrenia in mice by giving their mothers the flu during pregnancy, or by arousing their immune systems in utero with an injection of foreign RNA. Patterson, one of the few scientists who began studying the role of immune molecules in neurons before Shatz, has long focused on cytokines, messenger molecules that regulate inflammation in the body and (as Patterson eventually discovered) act as growth factors in the developing brain. Outside of the brain, cytokines are released by immune cells fighting infections, and they can alter MHCI expression in a complicated feedback loop. Could cytokines also change MHCI levels in a fetus's brain during the mother's illness, disrupting normal synapse pruning? Possibly. "The story," says Patterson, "is still developing."

At UC Davis, neurobiologist Kim McAllister is trying to further the narrative by studying how MHCI functions in Patterson's congenitally messed-up mice. "Carla's work has really led to a paradigm shift in how people think about immune molecules in the brain," she says. "But there are 50-plus MHC genes in mice, and we don't know what most of them do. We're really at the tip of the iceberg as far as understanding this." — KM