

Breaking the

During one of his famous staining experiments of the late 1800s—the kind that would eventually lead to a cure for syphilis and a Nobel Prize for Medicine—Paul Ehrlich stumbled on a conundrum that would haunt medicine down to the present day. When he injected dye into the bloodstream of mice, it penetrated every organ except the brain. Kidneys, livers and hearts turned a dark purplish-blue, clear and stark under his microscope, but the brain remained a pale whitish-yellow. When a student of his injected that same dye directly into the brain, the opposite happened: the brain itself turned blue, whereas the

other organs did not. Clearly, the student thought, a barrier—in German, *Blut-Hirn-Schranke*—must exist between brain and blood.

It would take half a century and a microscope roughly 5,000 times more powerful than Ehrlich's for anyone to actually locate this barrier, hidden as it was inside the brain's blood vessels. The average human brain houses roughly 400 miles of such vessels. They bend and twist in an endless array of tangled loops, ultimately ensnaring every single one of the human brain's 100 billion or so neurons. The walls of all these vessels are lined with endothelial cells. To be sure, endothelial cells line the interior of all of the body's vasculature, but they are much more tightly packed in the brain's vessels than they are anywhere else in the body, which explains why neither Ehrlich's dyes nor most of the medications in existence could cross from the bloodstream into the brain.

But long before they could visualize the barrier, doctors had come to both revere and avoid it. "For ages we saw it as like a

A new understanding of the blood-brain barrier as a living, mutable organ may revolutionize the treatment of diseases such as cancer and Alzheimer's

By Jeneen Interlandi

Brain Barrier



brick wall,” says Lester Drewes, a vascular biologist and blood-brain barrier specialist at the University of Minnesota. “And the consensus was that it’s there for a reason, and we shouldn’t mess with it.”

That consensus has shifted. As scientists now know, that brick wall is actually thrumming with activity. Cells on both sides—blood and brain—are constantly communicating with and influencing one another. Not only that, but a wide array of molecular passageways embedded in the endothelial membrane regulate traffic, blocking some substances while shepherding others across. Even white blood cells, long thought to be too large to penetrate the barrier, slip across regularly to patrol for invaders.

Scientists have adopted the term “neurovascular unit” to better describe what they see: not just a wall made up of endothelial cells but a vital organ of sorts, one that consists of many different cell types, including those surrounding the vessels, and plays its own crucial role in development, aging and illness. Thanks to another revolution in microscopy, they are seeing this organ more closely and clearly than ever before.

BROKEN BARRIERS

AT THE UNIVERSITY of Rochester, the view through Maiken Nedergaard’s “two photon” microscope is infinitely more dazzling than even Ehrlich could have imagined. Of course, unlike him, she is looking at a brain that is still inside a living, breathing animal (a mouse, to be precise). She has removed a bit of the creature’s skull and injected dye into its circulation, and now she is watching the blood-brain barrier in real-time: individual cells are crossing out of the bloodstream across capillary walls, which consist of a single layer of endothelial cells. The march is stunning to behold, especially given how inaccessible the barrier was when Nedergaard started her career, some 20 years ago.

Before the advent of two-photon microscopy—an advanced form of imaging that can penetrate the top 300 microns of cortex—researchers could not do much better than Ehrlich; they studied dead tissue fixed to traditional microscope slides. Those kinds of experiments, Nedergaard says, told biologists very little about how the blood-brain barrier actually operates. That is because blood flow is essential to the proper functioning of both brain and barrier—just how essential has surprised and excited scientists who study the barrier.

For example, in a string of recent experiments Nedergaard and her colleagues have shown that when a given cluster of neurons is stimulated, the surrounding blood vessels increase in diameter, thus delivering more blood and nutrients to those neurons at the exact moment that the neurons start firing. If you slow down that stimulation, the vessels contract, and nutrient delivery diminishes. “It’s incredibly dynamic,” Drewes says.

It is also incredibly complex. The capillaries are looped

Jeneen Interlandi is a freelance science journalist based in New York City. She has spent the past year as a Nieman Fellow at Harvard University studying the history of science and medicine.



by astrocytes and pericytes—cells that envelop the entire vascular system and appear to facilitate communication between blood, endothelia and neurons. These cells are in turn orbited by other cells.

Of these, Nedergaard is most intrigued by microglial cells, the central nervous system’s resident macrophages, or defensive cells; microglia patrol the brain and spinal cord for damaged cells and infectious agents, which they then devour. Malfunctioning microglia have already been implicated in a wide range of neurodegenerative diseases—from Alzheimer’s to Parkinson’s—and Nedergaard suspects that their role in those disorders may have something to do with falling down on the job of protecting the blood-brain barrier.

Nedergaard reasons that every time endothelial cells die, as they do both naturally and in response to injury, their loss must leave a transient opening in the barrier, one that surviving endothelial cells would be too slow to close, given that they are sewn together by links known as tight junctions. The presence of these linkages would mean that in healthy brains, some other cell type must act to close those gaps. In one set of experiments, Nedergaard wielded a laser to breach brain capillaries in live mice. Within 10 to 20 minutes, she says, microglial cells had completely surrounded the damaged area. “They ensheathed the capillary with amazing speed,” she says. “It was actually quite beautiful.”

Her team is now trying to find out if microglial cells are in fact the first line of defense—the emergency crew that comes out and temporarily closes the barrier until damaged endothelial cells can be repaired or replaced. “You could imagine,” Nedergaard says, “that if the microglial cells are not functioning properly, then small leaks aren’t repaired as fast, and you get neurodegeneration.” Her hypothesis is just one of many that scientists are testing as they work to understand what role the blood-brain barrier plays in disease.

Take, for example, multiple sclerosis, a disorder that is characterized by episodes of debilitating muscle pain, numbness and vision trouble. Doctors have known for ages that MS is caused by the breakdown of myelin, a rubbery sheath that

IN BRIEF

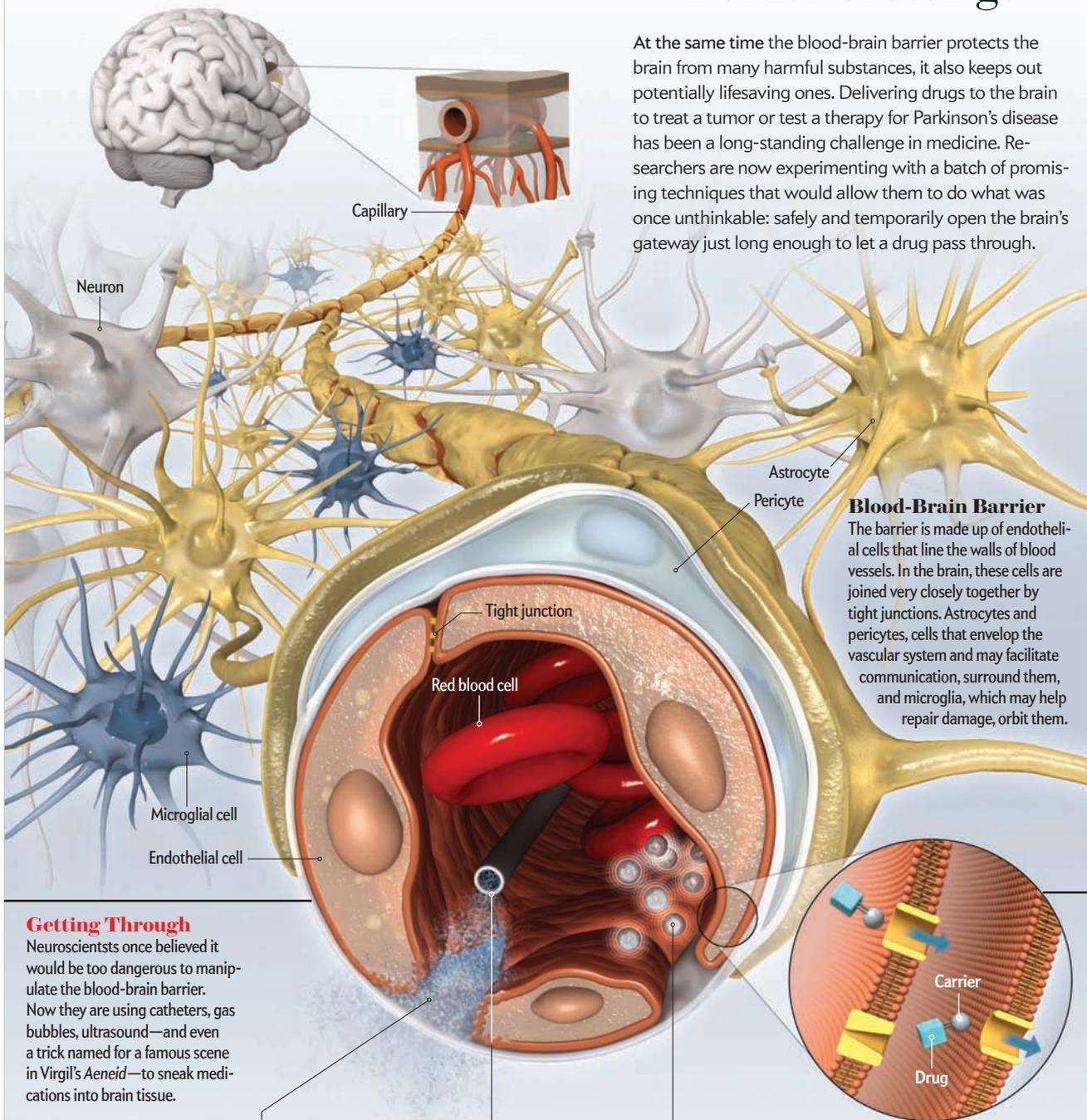
For more than a century, scientists believed that the blood-brain barrier was a sacred, impermeable wall. In fact, it is made up of ordinary blood vessels with one extraordinary property: the cells that make up their lining are packed together so tightly that they

allow very few substances to cross into brain tissue. **The barrier** is a vital organ in its own right, thrumming with activity as cells communicate with one another to decide which molecules to block and which ones to let through. Many more cells pass

through the barrier than scientists previously realized. **To reflect this new understanding**, scientists now call the blood-brain barrier the neurovascular unit. **Many believe** that learning how to open and close it may be the key to curing a host of diseases.

Border Crossings

At the same time the blood-brain barrier protects the brain from many harmful substances, it also keeps out potentially lifesaving ones. Delivering drugs to the brain to treat a tumor or test a therapy for Parkinson's disease has been a long-standing challenge in medicine. Researchers are now experimenting with a batch of promising techniques that would allow them to do what was once unthinkable: safely and temporarily open the brain's gateway just long enough to let a drug pass through.



Blood-Brain Barrier
The barrier is made up of endothelial cells that line the walls of blood vessels. In the brain, these cells are joined very closely together by tight junctions. Astrocytes and pericytes, cells that envelop the vascular system and may facilitate communication, surround them, and microglia, which may help repair damage, orbit them.

Getting Through
Neuroscientists once believed it would be too dangerous to manipulate the blood-brain barrier. Now they are using catheters, gas bubbles, ultrasound—and even a trick named for a famous scene in Virgil's *Aeneid*—to sneak medications into brain tissue.

HYPEROSMOTIC SOLUTION
Some solutions, like mannitol, have the capacity to suck moisture out of surrounding tissues. When doctors inject mannitol into an artery leading to the brain, it absorbs water from the brain's endothelial cells, causing them to shrivel up. The tight junctions then open, and drugs can slip through.

MICROCATHETERIZATION
Doctors thread a tiny catheter through the blood vessels up to the brain and use mannitol to open a small part of the barrier near the site they wish to treat. Next they inject drugs via the same catheter. This method is already used to administer anticlotting agents following a stroke.

MICROBUBBLES
Club soda in the bloodstream? A physician injects the patient with a saline solution containing microscopic gas bubbles. Once they reach the brain, a beam of focused ultrasound makes them vibrate in a specific location, causing the blood-brain barrier to open and allow drugs to pass through.

TROJAN HORSES
The name suggests a drug hidden inside something else, but these drugs actually come attached, like a wagon, to the end of a compound that slips naturally across the blood-brain barrier. Drug company Genentech has shown that these work in mice, but human trials are several years away.

encases and insulates the axons (the signal-emitting “wires”) of neurons just as rubber encases telephone wire. But why these attacks occur in episodes and what triggers those episodes have remained a bit of mystery. A growing roster of magnetic resonance imaging studies suggests that breaches in the blood-brain barrier precipitate MS attacks. These aberrant openings allow too many white blood cells to cross from the capillaries into the brain and attack the myelin. Based on a few new studies, scientists now think that highly reactive oxygen molecules may attack and thereby weaken the barrier, essentially rusting it, and that antioxidants, which block the effects of the reactive molecules, may thus make for good barrier stabilizers. “We have always thought of MS as a disease of the immune system,” Drewes remarks. “Now we’re starting to think of it as a disease of the blood-brain barrier.”

The same seems to go for epilepsy. Doctors and scientists have known for some time that seizures correspond to transient disruptions of the blood-brain barrier, but until recently most have assumed that those breaches are a consequence of the seizures, not a cause. That thinking has begun to change. Epilepsy researchers at the University of Amsterdam have found that artificially disrupting the blood-brain barrier of rats is a reliable way to increase the number of seizures they suffer and that the more a rat’s barrier has been disrupted, the more likely the animal will develop temporal lobe epilepsy. Studies conducted at the Cleveland Clinic in both pigs (experimental) and humans (observational) also found that seizures occurred after—not before—the barrier was disrupted.

Scientists elsewhere have identified two barrier proteins whose malfunctioning might play a role in Alzheimer’s disease. One protein (known as RAGE) shepherds the molecule beta-amyloid into the brain from the bloodstream; the other (called LRP1) shoos it out. When the balance between these two is disturbed—when too much beta-amyloid is let in or too little is expelled—the brain plaques associated with Alzheimer’s arise. Although clinical applications are a long way off, the finding has given at least some cause for hope: in mouse experiments, researchers were able to prevent the buildup of beta-amyloid by blocking the functioning of the gene that gives rise to the RAGE protein in endothelial cells; it is at least possible that RAGE-inhibiting drugs (which are now being developed) might accomplish the same feat in humans.

Of course, repairing leaks in the barrier is only half the challenge. The other half is creating deliberate openings so that needed drugs can get across. So far doctors have found at least

one proven way to do this, and the race is on to find as many more as possible.

OPEN SESAME

FOR ALL ITS COMPLEXITY, traffic between the bloodstream and brain is governed by an exceedingly simple set of rules. To cross, a compound must either be smaller than 500 kilodaltons (like most antidepressants, antipsychotics and sleep aids), able to use one of the natural gateways embedded in the barrier itself (like the Parkinson’s drug L-dopa), or be lipophilic, meaning it has an affinity for lipids and can thus bind to and slip across the lipid cell membrane (like alcohol, cocaine and heroin). By most estimates, 98 percent of all medications fail to meet any of these criteria, which means they cross the barrier in quantities so minuscule as to be medically useless—or they do not cross at all.

Past efforts to exploit these rules have been fraught. Making drugs more lipid-soluble, for example, enabled them to penetrate the blood-brain barrier with relative ease, but as scientists soon discovered, this strategy had several downsides. Some drugs crossed over, only to be kicked out rather quickly; others got stuck in the membrane itself and so could not do their jobs. Meanwhile all of them penetrated the body’s other organs with an alarming lack of discrimination.

As a medical resident some 30 years ago, Edward A. Neuwelt lit out on a different path. A neurosurgeon and director of Oregon Health and Science University’s Blood Brain Barrier Program, Neuwelt developed the first surgical procedure for breaching the barrier. First, he injects a solution called mannitol into an artery leading to the brain. Because mannitol is hyperosmotic, meaning that it contains significantly more sol-

ute than the brain’s endothelial cells do, the solution sucks the water out of the cells, causing them to shrivel up like fingertips soaked in water too long. The shrinking pulls apart the tight junctions, leaving gaps large enough for drugs (delivered through the same artery) to slip through. Somewhere between 40 minutes and two hours later, the endothelial cells will swell back to their normal size, re-forming tight junctions and sealing the barrier once again.

For nearly two decades Neuwelt has been using this technique to pry open the blood-brain barrier of a very specific type of patient: those with brain tumors that might be expected to respond to chemotherapy, if only the drugs could get across.

One of those patients was Joanie Lafferty, a 57-year-old mother of three who, back in 2007, was diagnosed with central nervous system lymphoma (a cancer that starts in the lymphatic

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system and spreads to the brain). Doctors gave her roughly one month to live. When she first arrived at O.H.S.U.—two weeks after the initial brain biopsy—the right side of her body was paralyzed. Her insurance company had cautioned her against the procedure, which they said was still experimental and could trigger a stroke or cause permanent epilepsy, or worse. But as far as Lafferty was concerned, she had nothing to lose. “This was the only option on the table,” she said. “And I wanted to live.”

Thus, just a few weeks after being diagnosed, Lafferty let Neuwelt and his team thread a catheter through her groin, up into her left carotid artery, then use it to deliver two solutions: the hyperosmotic mannitol, followed quickly by the chemotherapeutic methotrexate. The next day they repeated the procedure, using her right carotid artery instead. A month later, and every month after that for a year, Neuwelt and his team repeated the protocol: first through the left artery, then the right, mannitol pried open her blood-brain barrier so that methotrexate could be shot across and attack her tumor. By the end of her second treatment, she was able to walk out of the hospital without a wheelchair. Two months later she was in full remission. Five years later she still is.

For patients younger than 60, Neuwelt’s team boasts a median survival of 13 to 14 years, with significantly better cognitive outcomes, compared with standard whole-brain radiation treatment. Of course, not all cancer drugs can be delivered across the barrier, and not all brain tumors can be treated in this way. So far only a handful of drugs have been tested and proved safe for delivery. Because the procedure sends mannitol from the carotid through the entire brain and thus opens much of the barrier, it carries risks of tissue swelling, infection and toxicity.

Even as Neuwelt and his team work to refine their procedure and expand its application, doctors elsewhere are developing alternatives. One of the most promising is direct microcatheterization. Like Neuwelt’s barrier-disruption technique, this method also involves threading a catheter into the blood vessels and using mannitol to pry the barrier open. Rather than stopping at the carotid artery, however, the microcatheter reaches all the way up into the brain itself and opens just a tiny portion of the barrier, near the site of pathology. “It’s a very targeted procedure,” says John Boockvar, who is the New York Presbyterian Weill Cornell neurosurgeon leading the clinical trial.

Whether that will be an advantage or disadvantage remains to be seen. On one hand, opening less of the barrier reduces the risk of tissue swelling and seizures, not to mention the amount of brain tissue that is exposed to toxic chemotherapy. On the other hand, as Neuwelt points out, site specificity is a disadvantage when it comes to treating whole-brain diseases such as cancer or even advanced Alzheimer’s. “With microcatheter, you’re only attacking the tumor you can see,” he says. “But with brain tumors especially, it’s the spots you can’t see that end up killing you.”

Microcatheterization is already routinely used to deliver anticlotting agents to the brain in stroke victims; Boockvar and his team are testing its efficacy in delivering several antitumor drugs. Eventually, they say, the technique could be used to treat Alzheimer’s, Parkinson’s or, theoretically, any brain disease for which medications exist but need help crossing the barrier.

Another strategy for breaching the barrier involves focused ultrasound and microbubbles (microscopic gas bubbles). Researchers inject saline solution containing microbubbles into

the bloodstream. They then apply a focused ultrasound beam, which causes the bubbles to vibrate rapidly, prying open the tight junctions at a precise location. Drugs—also injected into the bloodstream—can then slip into the brain. Some time later the tight junctions re-form, closing the barrier once again. Researchers at Harvard University, Columbia University and other institutions are developing microbubbles and focused ultrasound. The technique has proved safe in monkeys and is progressing rapidly toward human trials.

Of course, breaking the barrier open is not the only way to get drugs across; another way is to sneak them through the barrier’s existing portals by attaching them to compounds that use those portals naturally. Scientists working to develop such drugs refer to them as Trojan horses, which is a bit of a misnomer. The drug is not hidden inside the familiar compound but rather is attached to the end, like a wagon. In some cases, however, the method works. A Trojan horse developed by Genentech was able to reduce brain plaques by 47 percent in rodent experiments. This particular drug enters the brain through the same receptors that transport iron across the barrier. Similar drugs (for not only Alzheimer’s but other neurodegenerative diseases) are being developed at the University of California, Los Angeles, and elsewhere, each progressing slowly toward the same goal: drugs ready for human trials.

ALPHA AND OMEGA

IN THE MEANTIME, evidence of the barrier’s significance is quickly seeping past the study of diseases into the fundamental processes of development and aging—the beginning and end of life itself. Experiments in the 1920s suggested that the barrier was immature in newborns, a perception that persists among developmental biologists and barrier researchers today. But recent studies have shown that tight junctions form almost as soon as blood vessels begin to penetrate the embryonic brain. In fact, investigators have begun to suspect that the barrier plays a crucial role during development by providing the brain with a specialized internal environment without which neurons might not be able to grow and connect.

Then, as we grow old, that specialized environment may start to come apart. Researchers have begun to suspect that subtle changes in the blood-brain barrier—a reordering of cerebral vasculature, perhaps, or small, slow leaks in the barrier itself—are what clear the path for age-related neurodegeneration, in all its malevolent permutations. “It’s the next big thing we have to look into,” says Drewes, who has been studying the barrier for well over two decades. “It seems our biggest lesson yet might be how very little we actually understand.” ■

MORE TO EXPLORE

Development of the Blood-Brain Barrier: A Historical Point of View. Domenico Ribatti, Beatrice Nico, Enrico Crivellato and Marco Artico in *Anatomical Record*, Part B: *New Anatomy*, Vol. 289, No. 1, pages 3–8; 2006.

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Ultrasound Elasticity Imaging Laboratory at Columbia University. <http://orion.bme.columbia.edu/ueil/research.php>

SCIENTIFIC AMERICAN ONLINE

View the blood-brain barrier as seen through a microscope at ScientificAmerican.com/jun2013/bbb