ter and white matter, which contains the axons, the long nerve projections that carry electrical messages between neurons.

But other imaging studies indicate that brain shrinkage might be due almost exclusively to loss of white matter. In an MRI study of 70 healthy human brains reported 3 years ago, Marilyn Albert of Harvard Medical School and her colleagues found almost no change in the gray matter, but an 8% drop in white matter, between the ages of 30 and 80. "It used to be thought that we lose neurons every day of our lives. That's just not true—at least for the cortex," says Albert.

Peters and his colleagues go one step further: They have evidence that changes in white matter may account for the cognitive changes in aging rhesus monkeys. They originally got this idea 5 or 6 years ago when they saw a breakdown of the fatty sheaths of myelin that insulate axons in old monkey brains, but not in young ones. Then, over the last 2 years, the researchers followed up on this observation, examining how the change might relate to the animals' cognitive status.

Using preserved tissue from seven old monkey brains from their previous work, they ranked the degree of myelin erosion in one small part of the neocortex. In work not yet published, they discovered that the extent of disorder paralleled the monkeys' degree of cognitive impairment. "We're beginning to think that myelin breakdown might be bringing about most of the changes with age," Peters says. Now the researchers also have indirect clues using MRI that this breakdown occurs throughout the cerebral hemispheres, which include the neocortex, resulting in a 10% shrinkage in white matter in old monkeys. At the same time, they reported at last fall's Society of Neuroscience meeting, they found no age-related differences in the volume of the animals' gray matter.

Myelin loss might contribute to the cognitive deficits of aging because it is necessary for the rapid conduction of impulses along an axon. While the Boston University biologists theorize that myelin breakdown slows neural traffic everywhere in the brain, it may have its greatest impact in the prefrontal cortex because speed may be most critical to the problem-solving tasks performed there. Indeed, it is these types of tasks that elderly people perform less quickly and accurately than do young adults. "Changes in white matter could have significant effects on the brain," says Peter Rapp, a neuroanatomist at the State University of New York, Stony Brook. "But [the Boston group's] observations need to be confirmed in larger numbers of animals" and with quantitative measures of myelin pathology.

Myelin is not the only neuronal component that may atrophy with age, possibly leading to a decline in nerve cell function. In one of the few quantitative, molecular studies of normal brain aging, John Morrison and Adam Gazzaley at Mount Sinai School of Medicine in New York City and their colleagues have discovered evidence for an age-related drop of about 30% in the density of the *N*-methyl-D-aspartate (NMDA) receptor for the neurotransmitter glutamate, which is thought to play a critical role in learning and memory. The receptor loss occurs in nerve cells at the end of the perforant path, a critical neural circuit that funnels information from the neocortex to the hippocampus and is "extremely vulnerable to aging," Morrison says. Moreover, the perforant-path nerve terminals and the neurons to which they connect were both intact, suggesting that the NMDA receptor decrease occurs without associated structural degeneration of nerve cells.

Not only the receptors but the neurotransmitters themselves may decline with age, impairing nerve cell function. Although cells may not die off in large numbers in the cortex, they are lost in other parts of the brain, such as the brainstem, and the loss changes brain chemistry. For example, some brainstem neurons produce dopamine, a neurotransmitter whose levels decline with age in the monkey neocortex, and that drop, Amy Arnsten, Patricia Goldman-Rakic, and their colleagues at Yale University have shown, contributes to the kind of memory deficits seen in normal aging.

But as long as cortical neurons remain alive, there may be ways to boost their function and stave off mental decline in old age. This might be accomplished, for example, by drugs that compensate for neurotransmitter deficiencies, or provide an extra tweak for the remaining receptors, or help prevent myelin loss, if that is indeed contributing to neuronal dysfunction. And those prospects raise hopes that one day, even the subtle memory deficits of old age will become a distant memory.

–Ingrid Wickelgren

Additional Reading

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NEURODEGENERATIVE DISEASE

Searching for Drugs That Combat Alzheimer's

... Last scene of all,
That ends this strange eventful history,
Is second childishness, and mere oblivion,
Sans teeth, sans eyes, sans taste, sans everything.

With those pessimistic words, William Shakespeare, astute observer that he was, described the final stage of life. Unfortunately, his description is still depressingly apt for many people. As medical science has prolonged human life, it has also brought a sharp increase in the numbers of people suffering from one of the most calamitous afflictions of old age: Alzheimer's disease, which steals a person's mind, ultimately making him or her as helpless and needy of care as any newborn infant.

Alzheimer's also exacts a crushing social toll. According to figures compiled by the Alzheimer's Association, some 4 million people currently have the disease in the United States alone, at a total cost of up to $100 billion per year, much of it for long-term custodial care in nursing homes. And with the population aging, "the size of the problem is going to grow extremely over the next decades," says Denis Evans, an epidemiologist at the Rush Center for Aging in Chicago. Evans's gloomy forecast is based in part on a prospective study of 3809 elderly in Boston that he directed. Called the East Boston Survey, it found that nearly half of people over age 85 have Alzheimer's—and that age group is growing more rapidly than any other. Based on these trends, the Alzheimer's Association estimates that there could be up to 14 million cases by the middle of the next century.

But in spite of these dismal forecasts, scientists studying Alzheimer's disease are beginning to see the first glimmerings of hope that this devastating disease might be checked. For years, Alzheimer's was considered a more or less inevitable consequence of aging. But growing evidence suggests that it's not. Normal brains do not seem to suffer the widespread neuronal loss typical of Alzheimer's (see p. 48). What's more, Alzheimer's devel-

opment, like that of other chronic conditions—heart disease, for example—can be influenced by both genetic and environmental factors. Some of these, such as the possession of certain gene variants, can greatly accelerate the onset of the disease, whereas others, such as the female hormone estrogen and certain anti-inflammatory or antioxidant drugs, may offer some protection.

And even though researchers do not yet have a full cellular and molecular understanding of what causes Alzheimer's neurodegeneration, what they have learned about the basic biology of the disease has "really changed the way people think about Alzheimer's," says Neil Buckholtz, who oversees Alzheimer's research for the National Institute on Aging (NIA). Indeed, they have several ideas, which are not mutually exclusive, about what might be at fault—and each of them suggests possible therapeutic approaches. Among the possible culprits: brain inflammatory changes; deposition in the brain of a small neurotoxic protein called β-amyloid; excess phosphate addition to another brain neuron protein called tau; possession of a particular variant of a cholesterol-carrying protein called apoE4 that may inhibit the growth of the projections neurons use to communicate with one another; and mutations in two recently discovered proteins, called presenilins, whose functions are unknown.

Although only one drug, which is called Cognex and is modestly effective at best, has been approved for treating Alzheimer's, researchers in academia, government, and the pharmaceutical industry are now devising a host of new drug strategies that might improve patients' symptoms—or better yet, slow down or prevent the neuronal degeneration. "There are a number of drugs in trials that might alter the course of Alzheimer's. I feel much better about this [disease] than I have in a long time," says psychiatrist Kenneth Davis of Mount Sinai School of Medicine in New York City, who has been involved in the search for Alzheimer's therapies for several years.

Among the drugs now moving into clinical trials are the anti-inflammatory steroid prednisone, antioxidants such as vitamin E, and estrogen supplements. And with several other drugs in preclinical testing, the list will likely grow.

And even if none of these turns out to prevent the disease entirely, they could still have an enormous impact. Because Alzheimer's symptoms don't usually become apparent until late in life, simply delaying their onset by 5 years could reduce the number of cases by as much as 50%. Says neurologist Richard Mayeux of Columbia University College of Physicians and Surgeons in New York City, "With this kind of disease, if you delay it 5 or 10 years, you've done the world a great service."

In the clinic. Although Alzheimer's prevention is the ultimate goal, the drugs that have moved farthest along the road to clinical use attempt to treat the memory loss and other symptoms, such as agitation and emotional outbursts, wrought by the disease. The strategy is to replace the functions of the neurons that have already degenerated, much as the drug L-dopa is used to counteract the nerve cell loss that causes Parkinson's disease.

This approach was first suggested in the late 1970s and early 1980s, when researchers learned that the cortical regions of the brains of Alzheimer's patients show massive destruction of cholinergic neurons, so called because they communicate with other nerve cells by releasing a chemical called acetylcholine. That discovery prompted efforts to try to make up for those losses with drugs that beef up the brain's acetylcholine content. Cognex is the first example.

The drug, which is made by the Parke-Davis division of Warner-Lambert, works by inhibiting acetylcholinesterase, the enzyme that breaks down acetylcholine. Clinical studies have shown that Alzheimer's patients who can tolerate the drug, especially the higher doses, show some improvement in their cognitive functions, roughly equivalent to turning back the clock on their symptoms by a few months to a year. Cognex's side effects, which include liver toxicity and gastrointestinal disturbances such as nausea and diarrhea, can be so debilitating, however, that many people can't tolerate the most effective doses.

But even this limited success has touched off a massive search for other acetylcholine boosters with fewer side effects, and several candidates are now moving through the drug development pipeline. "Everybody is now interested in the general category of cognition enhancers," says Axel Unterbeck, who heads up Bayer's Alzheimer's drug development effort.

Currently up for approval by the U.S. Food and Drug Administration, for example, is a cholinesterase inhibitor called Aricept manufactured by the Japanese pharmaceutical company Eisai and licensed to Pfizer in the United States. Other cholinesterase inhibitors, from companies such as Bayer, Sandoz, and Janssen, may soon follow. Still other drug companies, including Lilly, Parke-Davis, and Bayer, are developing chemicals that mimic acetylcholine's effects directly.

But acetylcholine boosters can only compensate for part of the neuronal loss in Alzheimer's. Unlike Parkinson's, where the damage is focused primarily on a particular set of dopamine-producing neurons, Alzheimer's destroys not just cholinergic neurons but also those using other neurotransmitters, including glutamate, dopamine, and serotonin. Some of these other deficits have also become targets for potential therapies.

One such effort comes from Gary Lynch of the University of California, Irvine, Gary Rogers of Cortex Pharmaceuticals, also in Irvine, and their colleagues. They have identified a class of chemicals they call Ampakines that facilitate transmission of signals through the AMPA receptor (hence the name Ampakines), a type of glutamate receptor thought to be needed for "long-term potentiation," a boosting of neuronal responses

SOME POSSIBLE DRUGS FOR PREVENTING OR TREATING ALZHEIMER'S

Drug	Activity	Proposed Mechanism of Action
Cognex, Aricept	Acetylcholinesterase inhibitor	Compensate loss of cholinergic neurons
Ampakines	Enhance activity of AMPA receptor	Improve memory by enhancing long-term potentiation
Prednisone, ibuprofen, other NSAIDS	Anti-inflammatory	Prevent inflammatory damage to neurons
Vitamin E	Antioxidant	Protects against free-radical damage
Premarin	Female hormone	Promotes neuronal survival
Nerve growth factor	Maintain cholinergic neurons in brain	Promotes neuronal survival
Calcium channel blockers	Inhibit calcium ion entry into neurons	Reduce calcium toxicity
Cholesterol-lowering drugs	Lower apoE4 concentrations	Prevent apoE4 toxicity to neurons
Protease inhibitors	Block β-amyloid production	Prevent neuronal loss to β-amyloid toxicity

that has been linked to memory formation. Ampakines have already been found to reverse age-related memory loss in rats, and early clinical trials, largely directed at determining whether the drugs can be safely taken by humans, have begun in Europe.

An ounce of prevention. Cognex and the other drugs aimed at replacing lost neurotransmitters "were never designed to be anything but palliative," Davis says. They treat symptoms, but cannot reverse the underlying neuronal degradation. Keeping the degradation from happening in the first place is many researchers' ultimate goal. And there is growing evidence, largely from human epidemiological studies and studies of neurons in lab culture, that such commonly used drugs as estrogen, anti-inflammatories, and antioxidants might help stave off Alzheimer's. "There are indications that we will be able to delay onset of the disease through neuroprotective agents. My general attitude is that things look pretty positive," says Zaven Khachaturian, who was Buckholtz's predecessor at NIA and is now director of the Alzheimer's Association's Ronald and Nancy Reagan Research Institute.

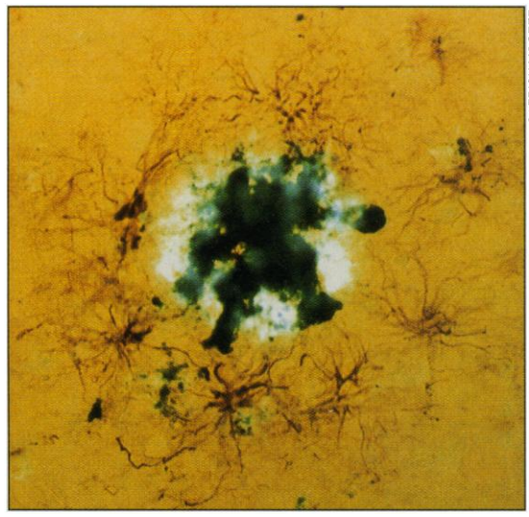
Indeed, the NIA's Alzheimer's Disease Cooperative Study (ADCS) has already begun clinical trials of all three types of drugs. In addition, the Women's Health Initiative (WHI), a large study—it will ultimately include 164,500 women between the ages of 50 and 79—sponsored by the National Institutes of Health and aimed at identifying ways of preventing older women's health problems, will examine whether estrogen replacement therapy can prevent Alzheimer's.

The furthest along is an ADCS trial designed to test whether vitamin E, an antioxidant, and selegeline—an antioxidant as well as an enhancer of brain dopamine concentrations often given to Parkinson's patients—can slow Alzheimer's progression in people who already have mild to moderate disease. The study, which began in 1992 and included 342 people with moderately advanced Alzheimer's, has concluded and the data are currently being analyzed, says neurologist Leon Thal of the University of California, San Diego, who oversees the ADCS.

One rationale for studying antioxidants is the long-standing belief that reactive oxygen free radicals given off as byproducts of the cell's metabolic activities might underlie aging-associated degeneration (see Article by Sohal and Weindruch on p. 59 and Perspective by Lithgow and Kirkwood on p. 77). Another comes from work linking free-radical production and β-amyloid, which is present, surrounded by degenerating nerve terminals, in the abnormal plaques that stud Alzheimer's brains. Dave Schubert's group at the Salk Institute has evidence that, at least in cell culture, β-amyloid's neurotoxicity may be due to its ability to increase production by nerve cells of hydrogen peroxide, a chemical that releases hydroxyl radicals, which can in turn damage cell membrane lipids and other cell components. The Salk workers also found that they could block β-amyloid's toxic effects on the neurons with antioxidants, including vitamin E.

What's more, as Columbia's Mayeux notes, vitamin E and selegeline are both widely used and have shown little toxicity. "If you have a drug that has even a marginal effect, if it's safe, it's worth doing [the trial]," he says.

The same reasoning is sparking interest in nonsteroidal anti-inflammatory drugs (NSAIDs). A series of epidemiological studies over the past few years has indicated that these drugs, which include aspirin, ibuprofen (sold as Advil and Motrin), and naproxen sodium (Aleve), protect against Alzheimer's development. Patrick McGeer, of the University of British Columbia in Vancouver, has recently completed a meta-analysis of those studies, and he says 16 of the 18 analyzed have shown protective effects. The minimum reduction in Alzheimer's cases among people taking NSAIDs was about 50%, and in some studies it was as much as 75%. "If you combine the statistics, the effects of NSAIDs are simply overwhelming," McGeer says.



PATRICK MCGEER

Surrounded. This Alzheimer's plaque shows two types of glial cells, the brain's version of inflammatory cells. Stained purple in the plaque itself are microglial cells, and encircling it are star-shaped astrocytes *(brown stain)*.

McGeer isn't surprised by those results. A great deal of work, much of it from McGeer and his longtime collaborator Joe Rogers of the Sun City Health Research Center in Sun City, Arizona, has picked up signs of inflammation, such as inflammatory cells and proteins in plaques, in Alzheimer's brains (*Science*, 18 June 1993, p. 1719). Still, as McGeer is the first to concede, the epidemiological studies are not without problems. One major concern is that the poor memories of Alzheimer's patients' often mean that researchers have to get the information about NSAID and other drug use from family members or other caregivers who don't always know what medications the patient might have taken in the past. That could lead to underreporting of NSAID use by the patients.

One recent study has minimized that problem, however, and still shows protective effects of NSAIDs. The Baltimore Longitudinal Study of Aging (BLSA), which is supported by the NIA and began in 1958 (although women weren't admitted until 1978), takes in people while they are still healthy and then brings them in every 2 years for thorough exams to assess both their physical and mental health. The participants write down all the drugs they are taking, thus giving the study a continuous record of their drug use.

At the annual meeting of the American Academy of Neurology, which was held in San Francisco in March, Claudia Kawas of Johns Hopkins University School of Medicine reported that taking NSAIDs other than aspirin for two or more years reduced BLSA participants' risk of getting Alzheimer's by up to 60%. Aspirin, a milder anti-inflammatory, did not show a significant risk reduction, although it has in other studies.

The next step is a prospective clinical trial, and the NIA's ADCS has begun testing the anti-inflammatory steroid prednisone in 150 patients with mild to moderate Alzheimer's. Some of the people working on the anti-inflammatories disagree with that choice of drug, however. For one thing, prednisone is not an NSAID. And for another, steroids can have serious side effects, such as kidney damage and brittle bones, already a problem for many older people, especially women. "Why monkey around with steroids when the others [NSAIDs] are safer and much better established?" asks McGeer. John Breitner of Duke University Medical Center, whose team is among those finding evidence that anti-inflammatories protect against Alzheimer's, agrees: "We may be subjecting people to a cure that's worse than the disease."

The trial's organizers maintain that they can minimize the steroids' side effects by choosing appropriate doses for the trial, although they concede that the drugs are unlikely to be widely used as Alzheimer's preventives. They also argue that prednisone is the best choice for this early trial because it is both more potent than the NSAIDs

and better at crossing from the blood into the brain. "We know it's effective for suppressing central nervous system inflammation. It hits the appropriate target," says the trial leader, Paul Aisen of Mount Sinai School of Medicine. As a result, he maintains, it will provide a better test of the hypothesis that anti-inflammatories can slow the course of the disease. "The point is that if prednisone works, it will tell us about the inflammatory component," Kawas says. "I think it will be good scientific information."

Good scientific information is also being sought about estrogen. The ADCS trial will look at whether the estrogen Premarin, chosen because it's the hormone normally used for estrogen replacement therapy during and after menopause, can slow disease progression in 120 women with mild to moderate Alzheimer's. The WHI will look at whether Premarin can prevent the disease in women who don't yet have it. Ultimately, some 8000 women will be eligible for the WHI Alzheimer's study, making it "the largest randomized study ever done on Alzheimer's prevention," says Sally Shumaker of Bowman-Gray School of Medicine in Winston-Salem, North Carolina, who is running that segment of the WHI.

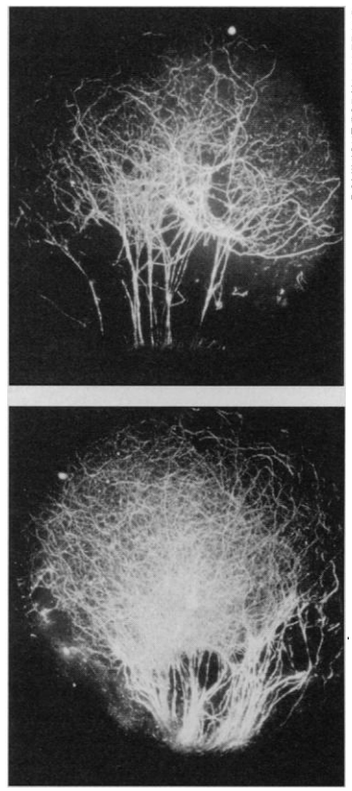
Like the other drugs now being tested, estrogen has shown hints of efficacy in both epidemiological and lab studies. The BLSA study, for example, looked at estrogen as well as NSAIDs and found a lower incidence of Alzheimer's in women who took the hormone than in those who didn't. Other epidemiological studies, including one conducted by Victor Henderson at the University of Southern California in Los Angeles, have come up with similar results.

Some recent molecular studies have suggested a possible mechanism for estrogen's apparent protective effect. Several years ago, Dominique Toran-Allerand and her colleagues at Columbia University College of Physicians and Surgeons found that in the developing brain, estrogen promotes the growth of the axons and dendrites, projections that nerve cells send out to communicate with one another. And work by Bruce McEwen's team at Rockefeller University in New York City shows that estrogen has the same effect on adult neurons that have been injured. More recently, Toran-Allerand's team has found that neurons, including some of those that degenerate in Alzheimer's, contain receptors for both estrogens and nerve growth factor (NGF). They think that estrogen may cooperate with NGF and other so-called neurotrophins in helping neurons differentiate and survive.

A role for estrogens in neuronal health might explain why women seem to be more susceptible to Alzheimer's than men. Their neurons may become more vulnerable to damage once estrogen production drops off after menopause. "Whatever the cause of Alzheimer's, [neuronal vulnerability] may be much worse in an estrogen-deprived condition," Toran-Allerand suggests. And conversely, estrogen replacement may stave off the disease.

Future directions. While a verdict on the vitamin E–selegeline trial will be in later this year, it will take years before the outcome of the prednisone and estrogen trials are known. By that time several additional drug strategies may be ready to be tested. One involves efforts to preserve brain neurons with nerve-cell growth factors like NGF, which is needed for the survival of cholinergic brain neurons and improves memory and learning in animals.

NGF itself wouldn't be a useful drug, however. As a protein, it's too big to cross from the bloodstream into the brain and would have to be injected directly into the brain to reach its target cells. "You want a small molecule you could swallow," says neurologist William Mobley of the University of California, San Francisco. Mobley himself is trying to find NGF mimics by determining how NGF binds to its receptor, then designing a small drug that will bind to the receptor in the same way, tweaking it into action. But this approach, he concedes, "is a tough row to hoe," with success not expected anytime soon. He predicts that the pharmaceutical industry, with its ability to perform massive screens for molecules that can mimic NGF's effects, will get there before he does.



DOMINIQUE TORAN-ALLERAND

Hormonal help. Cultured brain neurons treated with high estrogen concentrations *(above)* show much more neurite outgrowth than do neurons in low estrogen concentrations *(top)*.

Other researchers hope to come up with drugs that target the specific proteins implicated in Alzheimer's etiology. One such strategy aims to prevent the formation of β-amyloid by inhibiting the protease enzymes that release it from the larger protein in which it is first synthesized. "Many of us in the pharmaceutical industry are working on this," says Bayer's Unterbeck. Or it might be possible to prevent β-amyloid from aggregating into its most toxic form. Researchers will also want to find out whether they can lower brain concentrations of apoE-4, perhaps with cholesterol-lowering drugs, or reduce the chemical changes in the tau protein that have been linked to Alzheimer's.

They are also trying to find ways to interfere with the event that actually kills the neurons, whatever started them down their path to destruction: an influx of calcium into the cells. In work described in the 17 May issue of *Science* (p. 1017), for example, Philip Landfield and his colleagues at the University of Kentucky College of Medicine in Lexington described evidence suggesting that increased calcium influx might contribute to Alzheimer's development.

They found that the density of the channels that let calcium ions into cells was approximately three times greater in neurons from old rats than in those from young animals. What's more, their results suggested that the increased calcium-ion channel density may have contributed to the poorer performance of older animals in a learning task. "Our working hypothesis is that the increase in calcium channels in nerve cells in aging is a cause of the heightened vulnerability [to neurotoxic damage]," says Landfield. As a result, says Khachaturian of the Alzheimer's Association, "I think calcium will eventually become a major target for a large number of neurodegenerative diseases."

Which, if any, of these approaches will bear fruit remains to be seen. But the sheer number of them is inspiring optimism. "It's absolutely remarkable what's going on," says Craig Smith, CEO of Guilford Pharmaceuticals in Baltimore, whose company is among those developing small neurotrophic molecules. "We're at the halfway point of this 'Decade of the Brain,' and by the end we may be seeing some interesting things." He and other Alzheimer's researchers would be only too happy to prove Shakespeare wrong about the final stage of life.

–Jean Marx

Additional Reading

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