Alzheimer's Disease: Causes and Effects

I think we have the genetic tools and the instrumental tools to understand the fundamental basis of Alzheimer's disease.

After two weeks in culture, the single isolated progenitor cell shown on page 27 had differentiated here into dozens of neurons (stained green, with long fibrous processes) and hundreds of glial cells (stained orange). Grafts of such cells hold great promise for replacing nerves that have died because of Alzheimer's disease or other neurodegenerative diseases.

The following article is condensed and adapted from a forum sponsored by Caltech and the Pasadena Star-News, in cooperation with the Los Angeles chapter of the Alzheimer's Association. Held on May 31 in a packed Beckman Auditorium, the forum sought to explore the current status of research on this mind-crippling disease and the therapeutic promise that that research holds for the future. In his welcoming remarks Caltech President Thomas E. Everhart noted that "the more we learn about Alzheimer's disease, the better we can fight it." He also expressed his delight in the full house. "It demonstrates the fact that people are interested not only in this disease but in scientific research and how it relates to their lives. At a time when basic research is facing extraordinary pressures, it is heartening to see so many people attending a program based on science and its consequences for human health."

The program was moderated by physician and journalist Winnie King, and, in addition to the five scientists whose talks are published here, also featured TV actress Shelley Fabares, who spoke on family and social issues surrounding Alzheimer's disease. Fabares spoke of her personal experience as a caregiver for her mother, discussing the progression of the disease, the caregiver's feeling of impotence in the face of its advance, and the overload and isolation that strain the families of Alzheimer's victims. Fabares found help in the Alzheimer's Association and has since become a spokesperson and a member of its national board of directors. She decried the "crippling ignorance" that infects our society with regard to a disease that she said is recognized as the fourth leading cause of death in this country. The number of people with Alzheimer's disease in the United States is expected to reach 9 million by early in the next century. Fabares also spoke out in

favor of a long-term health-care policy and "meaningful health-care reform." She hoped that "this forum will enlighten the world about the nature and extent of Alzheimer's disease."

A videotape of the entire forum proceedings (1 hour, 51 minutes) is available from Caltech's Office of Public Events (Mail Code 332-92, Pasadena, CA 91125) for $39.95.

Caleb Finch
ARCO and William F. Kieschnick Professor in the Neurobiology of Aging, and University Professor, University of Southern California

Alzheimer's disease is a very specific kind of dementia that is linked in still mysterious ways to the aging process. Its most widely known characteristic is an impairment of recent memory and a progressive, slow, inexorable course that deprives the brain of its resources of wisdom and capacity for reasoning. Alzheimer's is not to be confused with vascular conditions that can cause another kind of dementia through strokes. In the dementias common in aging, certain types of neurons shrink and die. Even so, many neurons in the brain of someone with deep dementia still seem to function quite normally. A major feature of these dementias is that the incidence increases progressively during aging, particularly after 65 years of age.

Senile dementias, including Alzheimer's, are

relatively rare before the age of 50. Parkinsonism is another disease whose incidence increases steeply with age; there seems to be no safe age at which one is protected if one hasn't already developed the condition so far. In contrast, two other diseases that affect the nervous system do have safe ages where you are secure from the threat of the disease. One is Huntington's disease, which is the result of a single gene with a dominant effect; the other is the set of diseases called schizophrenias, which may also have a genetic origin, but with strong effects from interaction with the environment. Part of the mystery of these diseases is how one can have inherited a gene at birth that waits so long before it's expressed in its damaging forms. In the case of Alzheimer's disease, there are multiple genetic influences that increase the risk—some of them inevitably, some of them only as risk factors.

One of the major pathological characteristics of the brains of Alzheimer's patients is the senile plaque, a microscopic assemblage of proteins about a hundredth of an inch in diameter. It's found in the vicinity of dying neurons, but there are often other cells in the neighborhood that are quite healthy and active. These are called glial cells, from the Greek word for glue, and they include two types: astrocytes and microglia. The distribution of senile plaques is heaviest in the parts of the brain that are concerned with learning and memory, and the selectivity with which these regions seem to be targeted must in some way be related to the gene activity that determines the particular characteristics of the nerve and glial cells in those areas.

The first protein to be identified with the senile plaque is one called beta amyloid. Now, here is a paradox: each of us has, from birth onwards, in all of our body fluids, traces of this beta-amyloid protein that seem to do us no harm. But somehow in the disease process it aggregates to form these masses that appear to be toxic to neurons. Although it has become the best known of these proteins, amyloid isn't alone in the disease process; there's a long list of other proteins and factors in the brain that are aggregated along with the amyloid in the senile plaques. Some of these are molecules that act like hormones or growth factors that can influence the survival of neurons; others are components of the inflammatory system that troubles our joints with age. A great deal of effort is now under way to understand how these molecules get together and how they influence the toxicity of amyloid.

We can make amyloid fibrils aggregate in a test tube with the same effect as found in the senile plaque. My laboratory is studying another
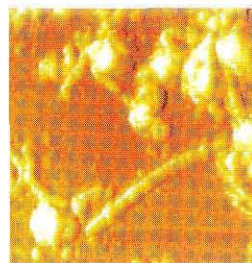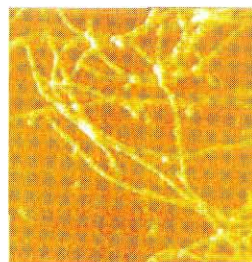
Atomic force microscopy images show two proteins found in the senile plaques of Alzheimer's disease brains. In this experiment, chemically synthesized, pure beta amyloid aggregated by itself (top) or was aggregated in the presence of the protein clusterin (bottom). Clusterin considerably modifies the extended filaments of beta amyloid, increasing its harmful effects. From Oda et al., *Exp. Neurol.*, in press.

one of the molecules in the plaque, clusterin, and we have found that mixing the two together in a test tube drastically changes the organization of the amyloid, and, moreover, increases its harmful effects. The focus on amyloid is now leading investigators to consider other molecules that interact with it that may modify its toxicity and may suggest an approach to treatment.

The accumulation of amyloid in aggregated form is not unique to humans; it's a biological phenomenon of aging that can be seen in other primates and prosimians. The top figure on the opposite page indicates the part of the lifespan when amyloid begins to accumulate in each of several species—humans, chimpanzees, rhesus monkeys, and lemurs, the latter with a lifespan of only 10 years. It's clear that even short-lived prosimians, which diverged from the rest of our lines more than 40 million years ago, also accumulate beta amyloid in their brains with aging. This may be the molecular equivalent of original sin. The scientific puzzle is to understand why it aggregates, forms senile plaques, and damages nerve cells.
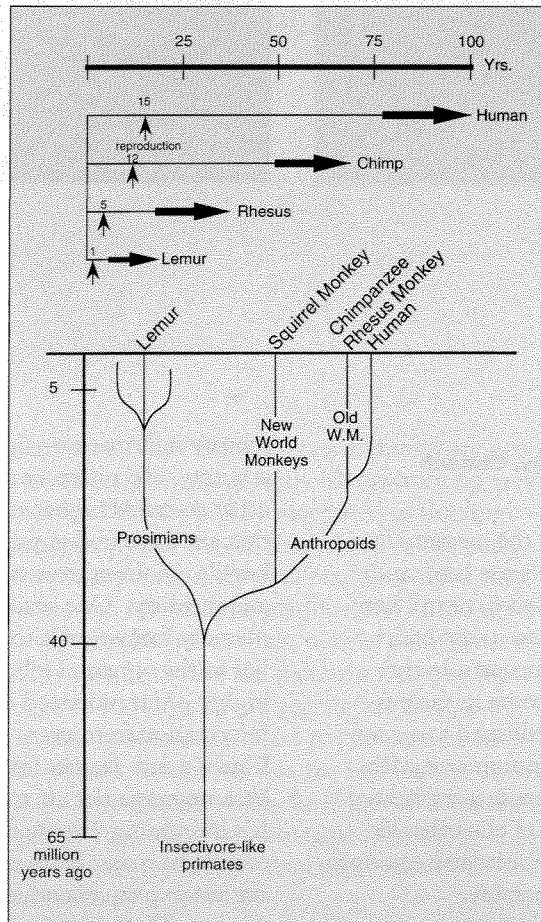
Besides amyloid aggregation, another change found very broadly in the brain during aging is the activation of the glial cells. It appears that virtually everyone on the face of the earth over the age of 50 has astrocytes and microglia that are beginning to wake up and look highly active. This is a basic part of the aging process that may be interrelated with the onset of Alzheimer's, Parkinson's, and other neurodegenerative diseases. These kinds of changes are common to all mammals in corresponding portions of the life-

Right: Beta amyloid accumulates during aging in the brains of humans and other primates. The graph shows the age when beta amyloid is first detected (thick arrow) in species with different life spans, according to their positions on hte phylogenetic tree.

Below: The horizontal bars indicate the occurrance of changes during normal aging in four mammals with different life spans: rat (3 years), dog (15 years) rhesus monkey (30 years), and human (80 years). Note that rats do not accumulate beta amyloid but do show most other changes.

span. The graph at left below shows that the same general processes of neuron atrophy, deposits of amyloid, and increased activity of the glial cells is a broad phenomenon during brain aging in mammals that may precede clinical signs of disease.
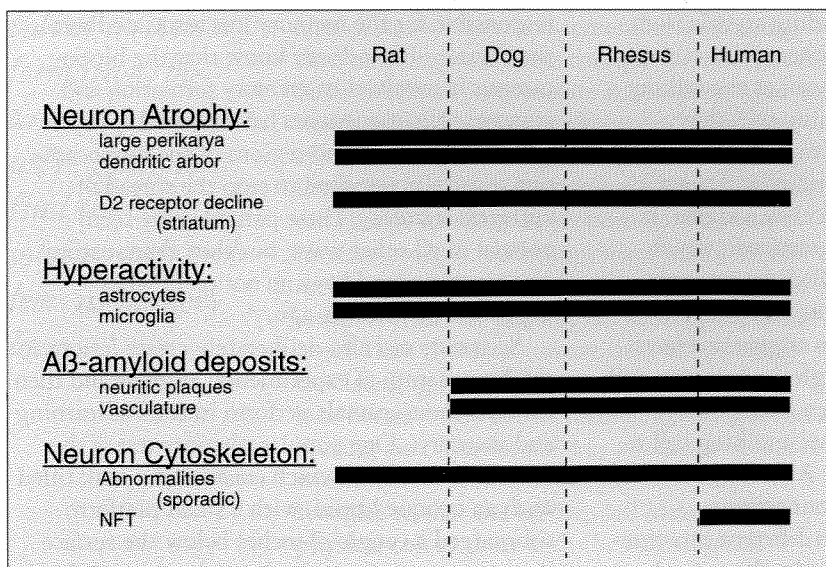
Studies of the genetics of Alzheimer's disease have located genes on at least four different chromosomes. This genetic diversity might ultimately serve as a basis for recognizing which individuals are at risk for the onset of Alzheimer's at a certain age, and which individuals might be treated preventively. As we learn more about the disease, researchers may discover pharmaceutical approaches to Alzheimer's based on the particular mutations and genes that one carries. Other therapeutic targets include devising drugs to influence amyloid metabolism, to modify senile plaque, and to replace neurotransmitters that are lost as nerve cells degenerate. It's a broad field, and the problems are very complicated. We don't expect it to be resolved in a few years. I think that Alzheimer's research is in the same position that cancer research was in about 40 years ago, when one or two forms of cancer could be treated.

I'll close with an optimistic statement: I think we have the genetic tools and the instrumental tools to understand the fundamental basis of Alzheimer's disease. If a person born with a gene for Alzheimer's lives 70 years without any symptoms, it should be possible to suppress that gene's bad effects for the rest of his normal life span. And in the foreseeable future, assuming that reasonable funding is available, much progress will be made.

Caleb Finch received his BS (1961) from Yale and PhD in cell biology from Rockefeller University (1969). He has been a member of the faculty at the University of Southern California since 1972, as professor of gerontology and biological sciences since 1978, as the ARCO and William F. Kieschnick Professor in the Neurobiology of Aging since 1985, and as University Professor since 1989.
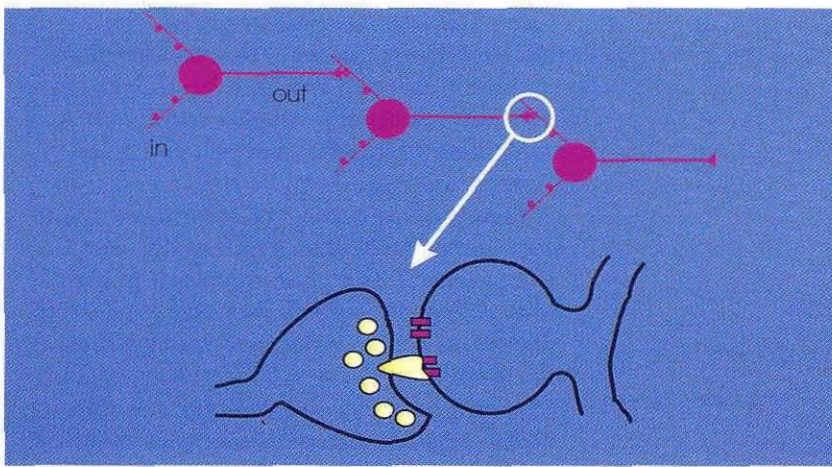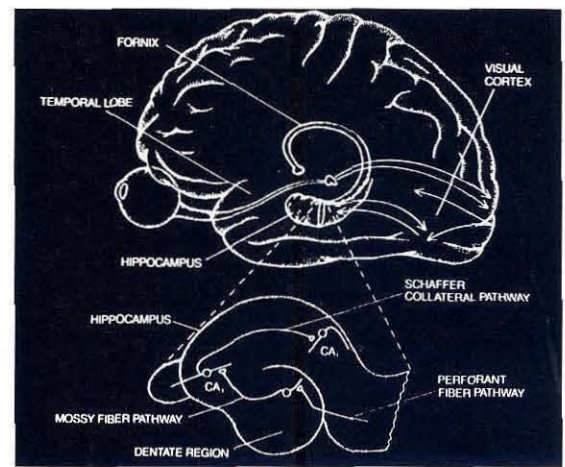
Above left: Neurons, which can be thought of as having a talking end (left) and a listening end (right), communicate at junctions called synapses. There the talking cell releases a chemical signal (yellow) across the synaptic cleft, which stimulates an electrical signal (pink) in the listening cell, which then "talks" to the next neuron down the line.
Above right: One of the brain's memory-storage sites is the hippocampus, named for its seahorse shape and located in the temporal lobe at the base of the brain.

Erin Schuman
Assistant Professor of Biology, Caltech

One strategy to ameliorate the memory loss associated with Alzheimer's disease is to study the fundamental mechanisms that control how and where memories are formed in the brain. We have approximately 10^{11} neurons in the brain, and even though they come in various sizes and shapes, they're all dedicated to a common function, which is to communicate with one another. Networks of these interconnected neurons underlie every aspect of our behavior, from the simplest reflex to complicated emotions, thoughts, and learning and memory.

We can think of an individual neuron as a factory with a receiving end and a shipping end. Neurons communicate by sending signals from one cell's shipping end to another cell's receiving end. Or you can think of one cell as the talking cell and the other as the listening cell; they connect in the synapse. The language of these cells is a combination of electricity and chemistry. When the talking cell receives a signal, it releases a chemical, a neurotransmitter, which travels across a small space called the synaptic cleft to interact with specialized receptors in the listening cell. These receptors trigger an electrical impulse that travels through the listening cell to its shipping end, which then releases a neurotransmitter and thus becomes a talking cell to the next neuron in the chain. An average neuron forms about 1,000 synaptic connections.

The brain is organized into different functional units, beginning with the units that encode sensory information—including the visual, auditory, and olfactory areas. Then there are other so-called higher-order areas of the brain that are dedicated to processing information and handle emotions, reasoning, language, learning, and memory. One brain area important for memory formation is the hippocampus, which lies in the temporal lobe and receives a lot of higher-order processed information, including information from every primary sensory unit. This is a nice feature for a memory-storage site because memories are multi-sensory experiences. It's interesting to note that the hippocampus is one of the major brain areas that's damaged by the senile plaques and tangles of Alzheimer's disease. Thus, it's reasonable to work with the hypothesis that damage to the hippocampus is responsible for the memory loss associated with the disease. How do we know that the hippocampus is involved in memory formation and storage? Neuroscientists have studied epileptic patients who have had regions of the hippocampus surgically removed to stop the spread of epileptic seizures. These patients may seem normal in all other ways, but they cannot store any memories and have no notion of what happened 10 minutes ago.

Scientists can also deliberately create lesions in the hippocampi of experimental animals and then see how these animals perform in tests of learning and memory. One popular memory test is the Morris water maze, which consists of a tank filled with an opaque liquid, with a small platform submerged a couple of inches below the surface. A rat or mouse placed in the tank can't see the

Memories are formed by changing the strength or the number of synapses. The strength of the synapse can be increased when the talking cell sends more neurotransmitter or when the listening cell adds more receptors.

STRENGTH   NUMBER
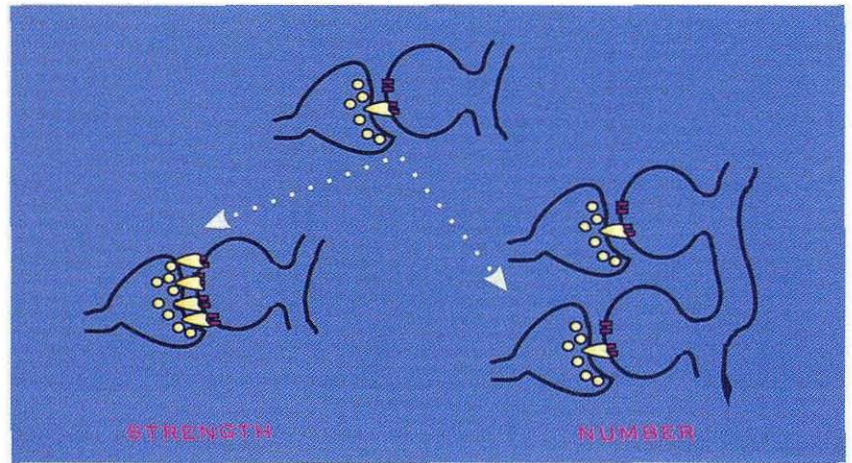
Our ultimate goal is to achieve an understanding of all the molecules, proteins, and genes that are involved in memory formation, because that's where we're going to find the clues to solving such problems as memory loss.

platform. Now, although rats and mice are good swimmers, they don't really like swimming very much, so they're highly motivated to find high ground. When they're first placed in the tank, they swim around the pool randomly until they happen to run into the platform and climb up on it. Over subsequent trials, normal animals learn fairly quickly where the platform is and swim right to it; they still can't see it but they've learned its location relative to cues in the room. In contrast, an animal that has had lesions created in its hippocampus takes far longer to learn the task and spends a lot more time swimming around because it can't remember where the platform is.

What is actually changed about the properties of neurons in the hippocampus or other brain areas when we learn and remember something? And how can we address this question in the lab? Since neurons communicate at synapses, what we want to do is change the properties of the synapses or change the way that information flows in the brain. There are two basic ways the brain can change its synaptic properties. One way to increase synaptic communication would be to increase the strength of transmission at a single synapse, either by making the talking cell shout (send more chemical transmitter) or by having the listening cell add more receptors so it can hear better—sort of like turning up the gain on a hearing aid. The second way to change synapses with memory would be simply to add new synapses so that the talking cell makes more physical connections with the listening cell.

To understand how these changes occur and

how memory occurs, we need to understand the process at a molecular level. We can do this by teaching an experimental animal something and then looking in its brain and observing what has changed about the synapses. When scientists have done that, they've seen that usually both of these kinds of changes—increasing transmission strength and adding synapses—occur. We can also take the brain out of the animal and make the changes ourselves, to try to mimic what happens when the animal learns something in its real environment. In my lab, and in many others, researchers remove the hippocampus from rats and then slice it into sections about 0.5 mm thick. Even when we slice the hippocampus up, it maintains its usual properties and the synapses behave normally. We can keep the tissue alive and record the synaptic activity by stimulating the talking cells and recording the size of the listening cell's response, which is always the same over time if stimulated in the same way. If we then stimulate the hippocampus in the right way, by applying strong stimulation to the talking cells, we can dramatically increase the size of the response of the listening cell. This enhanced response, which is called long-term potentiation, can last for long periods of time. Thus, using this technique we can study memory formation in a reduced situation where we have molecular control over the individual cells and synapses.

Our ultimate goal, however, is to achieve an understanding of all the molecules, proteins, and genes that are involved in memory formation, because that's where we're going to find the clues to solving such problems as memory loss. We
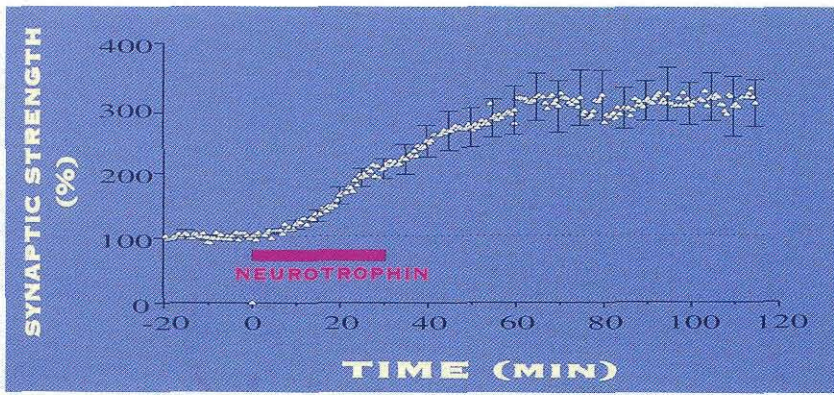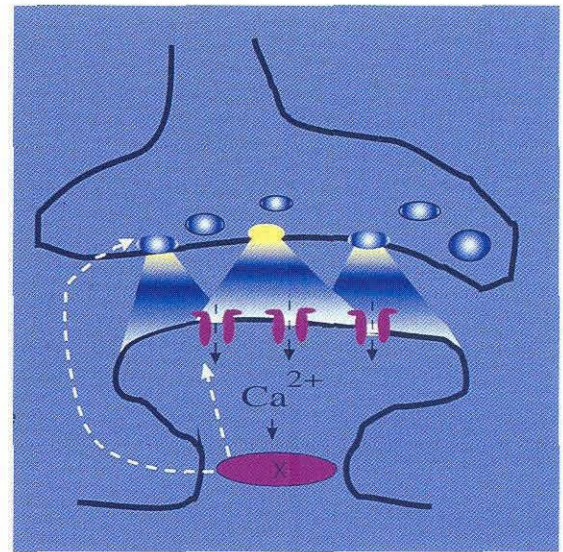
Above left: Neurotrophic factors, which promote the growth and survival of developing neurons in the embryo, also appear to increase synaptic strength in the adult brain.
Above right: When memories are formed, the talking cell (here on top) releases its neurotransmitter, which activates a calcium receptor in the listening cell. The calcium then activates a bunch of enzymes that can make the listening cell's receptors more sensitive, as well as prompting the release of more neurotransmitter.

know a fair amount about the molecular mechanisms of long-term potentiation. When long-term potentiation is initiated, a certain receptor is activated that allows calcium to enter the listening cells. Calcium is a very important intracellular messenger that can activate a whole variety of enzymes. These calcium-activated enzymes can do two things: either change the properties of the receptors in the listening cell to make them more sensitive to the same amount of neurotransmitter, or generate a signal that travels back to the talking cell telling it to release more neurotransmitter. We know that these kinds of cellular events underlie short-term memory. It's our guess, and recent work suggests, that for long-term memories signals are actually transmitted up to the nucleus to influence gene expression and change the amount and/or kind of proteins expressed.

The above mechanisms describe changes in synaptic strength. Unfortunately, we don't have a similar molecular understanding of how structural changes—that is, changes in synaptic number, the other way memory could be formed—might occur in the adult brain. We can, however, take advantage of a great deal of work that's been done on structural changes during brain development in the embryo. We know that proteins called neurotrophic factors promote the growth and survival of neurons when the nervous system is developing. In this case, the talking cell releases the neurotrophic factor, which then binds, just like the neurotransmitter, to receptors on the listening cell, and then travels to the nucleus, where it influences gene expression. We hope that the adult nervous system might em-

ploy similar mechanisms to change the structure of synapses.

It's quite possible that the same proteins that change structure also change synaptic strength and vice versa. In my laboratory we have determined that these neurotrophic factors that promote neuron growth and survival can also change synaptic strength in the adult nervous system. In the same experiment with the hippocampus slice that I described earlier, when we added the growth-promoting factor we saw an increase in synaptic transmission. This means that the brain may actually use the same molecules to increase synaptic transmission and to promote growth, and shows that the nervous system can use the same sets of molecules over and over again to form these different kinds of memories.

Finally, it is our hope, and it's the hope of basic science, that if we can achieve an understanding of the essentials of synaptic transmission and how memories are formed normally at the molecular level, we will be able to design treatment strategies that may be effective in reversing the memory loss associated with Alzheimer's and other diseases.

Erin Schuman joined the Caltech faculty as assistant professor of biology in 1990. She earned her BA from the University of Southern California in 1985 and her PhD from Princeton in 1990. She's currently a John Merck Scholar and an Alfred P. Sloan Research Fellow.

Robert Katzman, MD
Research Professor of Neurosciences,
University of California, San Diego

Right: Plotting the log of the prevalence of Alzheimer's disease against age produces a straight line. Approximately 5 percent of the population will have the disease at 75; that increases to 10 percent at age 80. If the onset of Alzheimer's could be delayed by only five years (dashed line) the prevalence of the disease would be cut in half.
Below: Alzheimer's is similar to other chronic diseases, with initiating factors that occur well before onset, a latent phase with no symptoms, then a period in which subtle changes are occurring but are difficult to observe. When symptoms do become obvious, it can still be another year or two before an accurate diagnosis can be made.

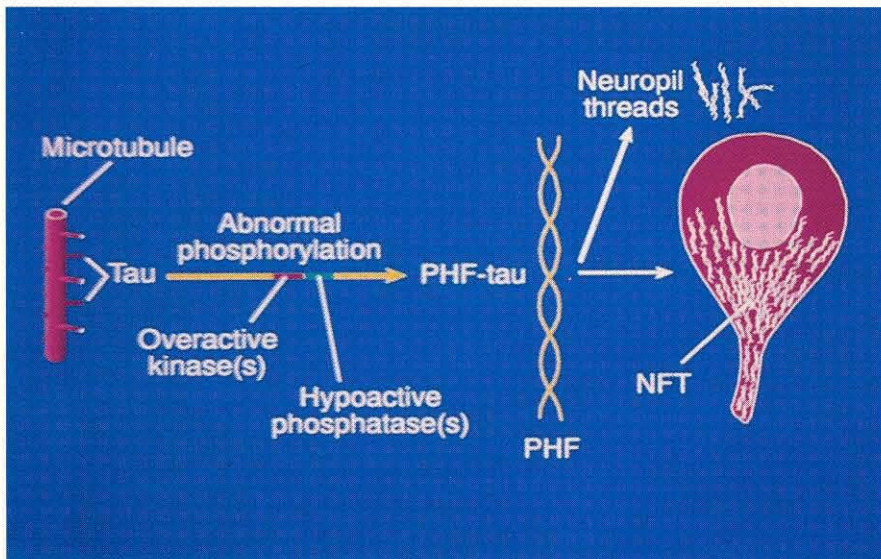## Alzheimer's Disease as a Chronic Disease
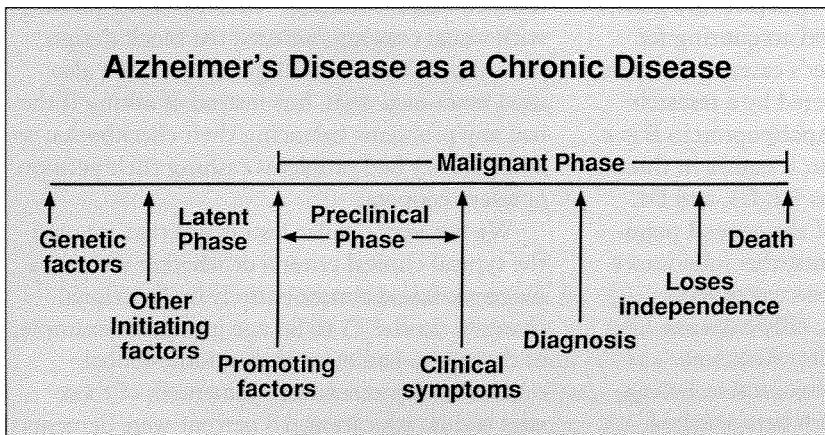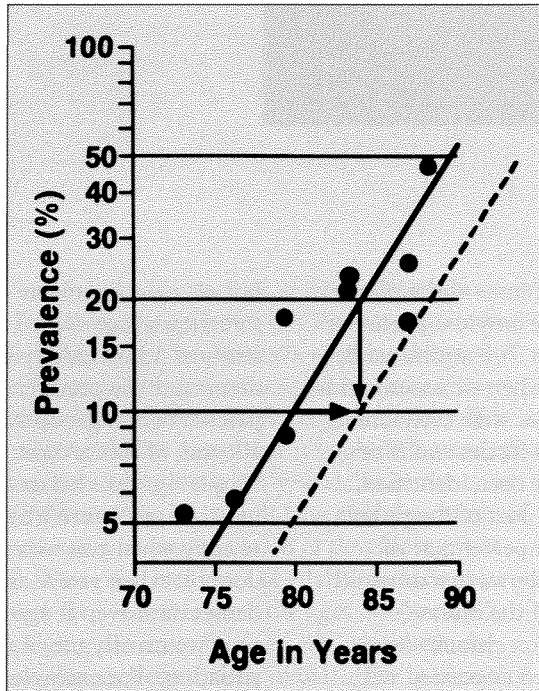
Although dementia is a complex of symptoms that can be caused by more than 70 diseases, Alzheimer's disease accounts for between two-thirds and three-fourths of all cases of dementia. Criteria published in 1980 by the American Psychiatric Association define dementia as characterized by loss of intellectual ability of sufficient severity to interfere with social or occupational function, impairment of memory, and impairment of at least one other area of cognition in individuals who were alert and awake. This definition has enabled us to diagnose dementia very well—in the case of Alzheimer's with 85 percent accuracy, which is remarkable considering that there is no specific biochemical test for it. Unfortunately, this definition does not pick up the early stages of the insidious onset of Alzheimer's disease. Family members will often disagree by years about when the disease began.

I believe, as do many others, that Alzheimer's can be viewed much like other chronic diseases, such as cancer, where there are initiating factors that occur well before the actual onset of the disease. Recent data suggest that the latent phase may last about 15 years, a phase in which changes could perhaps be picked up with neuropsychological tests but can't be observed clinically. We can't make an accurate diagnosis until a year or two after clinical symptoms appear, which is pretty late in the game.

When Alois Alzheimer first outlined the pathology of this disease in 1907, he described the neuritic plaque and the neurofibrillary tangles—abnormal nerve cells that are full of abnormal fibrils. Although most of the investigators today are working on amyloid and the plaque, many of the brain cells in Alzheimer's disease do have these neurofibrillary tangles, which probably contribute to cell death, and we now know quite a bit about their chemistry. A protein called tau, which normally helps stabilize the microtubules that are needed for the transport of proteins in nerve cells, in Alzheimer's somehow gains many phosphorous groups attached to each single molecule. It then separates from the microtubule, forms perihelical filaments and then neurofibrillary tangles. But we don't know whether this tau process is a secondary event or a primary one.

We know quite a bit more about the neuritic

Above: Implicated in the neurofibrillary tangles characteristic of the brains of Alzheimer's patients is a protein called tau, which normally stabilizes microtubules that transport proteins in nerve cells. In Alzheimer's disease, the tau protein acquires phosphorous groups, breaks off, and forms parahelical filaments (PHF), which accumulate, forming neurofibrillary tangles (NFT) that cause the cell to die. Below: The gene for apolipoprotein E4 increases the risk of Alzheimer's dramatically, particularly if the E4 allele is inherited from both parents. With E4 from only one parent, the risk falls off appreciably. Data after Corder, et al., *Science*, vol. 261, p. 922, 1993.

Probability of Developing AD by the Age of 85 (Familial Cases)	
Allele	Percentage
4/4	91.3
4/3	47.8
4/2	20.0
3/3	20.8
3/2	18.8
2/2	< 15

plaque. The amyloid in the center of the plaque is a breakdown product of the amyloid precursor protein, which contains about 700 amino acids. The beta amyloid is formed when 42 amino acids break off. In studying families with early-onset Alzheimer's (patients in their forties and fifties), nine different mutations have been identified, which bracket this particular part of the protein. This doesn't represent a large percentage of Alzheimer's patients, but it shows that amyloid is, in fact, one of the causes of the disease.

Besides the amyloid gene on chromosome 21, early-onset cases have been associated with another gene, not yet identified, on chromosome 14. But the most important susceptibility gene, located on chromosome 19 and accounting for about 30 percent of Alzheimer's cases, is the gene for apolipoprotein E4, discovered by a research group at Duke University. Apolipoprotein E is a low-density cholesterol carrier; it comes in three varieties, commonly known as E2, E3, and E4. Although only two percent of the general population has E4 from both parents, this inheritance accounts for about 15 to 16 percent of Alzheimer's patients. And if you only have one E4, you're twice as likely to develop Alzheimer's as the general population. Apolipoprotein E4 can form complex compounds with beta amyloid, converting it from a soluble peptide to an insoluble fiber very quickly.

Besides discovering the genetic risk factors for Alzheimer's disease, recent studies in a variety of institutions have turned up some apparent protective factors against the disease. Anyone, no matter how intelligent, can develop the disorder,

but over the aggregate education appears to be somehow protective. I was involved in a 1987 study of 5,000 elderly individuals in Shanghai to determine the prevalence of dementia. Since 27 percent of this group had never been to school at all, and, of those, most couldn't read or copy simple figures, this created some testing problems. If you haven't been to school and learned to copy when you were a kid, you don't start doing it when you're asked to do it during a dementia survey at age 70. So we adapted the tests we usually use in the United States to this situation. For example, our memory test used actual physical objects that were identified and touched and memorized. Because of the problem with visual copying, we used the block design from the children's intelligence test. We also used functional tests, but instead of asking if they had any problems balancing their checkbooks, we asked if they had problems cashing their pension or salary coupons.

We got very similar results whether we used the typical clinical criteria or whether we used a diagnosis based almost entirely on functional changes. In the 75 to 84 age group, for example, of those who had more than middle-school education, 4 percent were demented; of those who had no education, 18 percent were demented; those with elementary education were in between, at 12 percent.

A number of studies in such places as Bordeaux, France, and in North Manhattan have found the same kind of phenomena. The North Manhattan study found that not only does lack of education, defined here as less than eight years

In the process of embryological development, many neurons (blue) send out their nerve fibers (green) to contact their target cells (yellow). But in this normal competitive process, many neurons don't make it, and they die off.
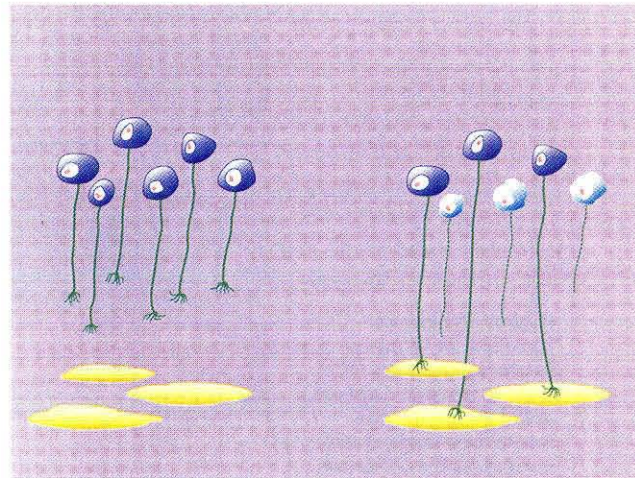
of school, double the risk of developing Alzheimer's-type dementia, but low occupation had the same effect. Individuals with both low occupation and little education had an almost three-fold chance of developing dementia. We interpret this to mean that if you're educated, you may have a five- to seven-year delay in the onset of Alzheimer's, and you'll have half the chance of someone with no education of developing it at all. Since the incidence of Alzheimer's doubles every five years between the ages of 65 and 85, delaying the onset by five years would cut the prevalence of the disease in half. The question of course arises whether late-life education will help. We don't know yet, but it's something worth looking at.
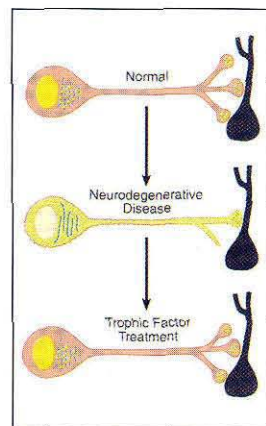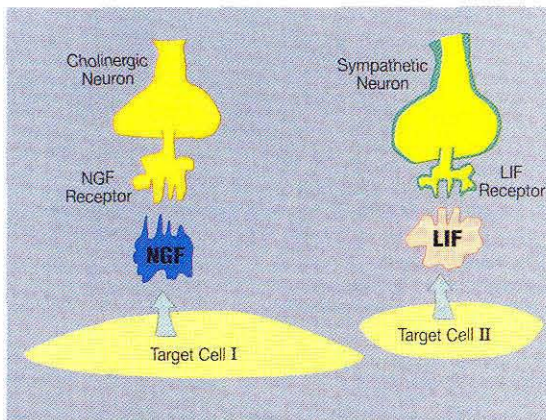
Robert Katzman earned his BS (1949) and MS (1951) from the University of Chicago and his MD from Harvard Medical School (1953). Much of his career was spent at Albert Einstein College of Medicine, where he was chair of neurology and professor of neuroscience. In 1984 he came to San Diego as professor of neurosciences and department chair at UCSD. Since 1994 he has been research professor of neurosciences. He has also held appointments as attending neurologist at the UCSD Medical Center and at the San Diego Veteran's Administration Medical Center.

Paul Patterson
Professor of Biology, Caltech

The bottom line on Alzheimer's disease is the death of nerve cells. Neuronal death occurs in other pathological conditions—Parkinson's disease, stroke, and so on—and, in fact, it is a part of normal embryological development. All of us have undergone a massive amount of neuronal death as embryos. Many neurons are born and send out their nerve fibers, or processes, to contact target cells, but only a fraction of those nerve cells actually successfully make contact and live. The rest of the neurons, from one half to two-thirds of them, will die as part of normal development. It is as if the neurons are competing with one another in a kind of Darwinian fight for survival. What they're competing for, in part, are proteins called trophic factors—from the Greek word for nourishment—which are secreted by the target cells. These trophic factors keep the neurons alive.

One family of trophic factors consists of proteins related to nerve growth factor (NGF). NGF is secreted by particular kinds of target cells, and it has a certain molecular shape that allows it to bind very specifically to receptor proteins on the surface of neurons that require it for survival. There are many other families of trophic factors, each produced by different target cells and each acting on different neuronal populations. One type of neuron that responds to and requires NGF is the cholinergic neuron, which is

Cholinergic Neuron

Sympathetic Neuron

NGF Receptor

LIF Receptor

NGF

LIF

Target Cell I

Target Cell II

**Right: Different families of trophic factors, such as NGF (nerve growth factor) and LIF (leukemia inhibitory factor) are secreted by different target cells. Each of the trophic factors has a particular molecular shape that allows it to bind specifically to receptors on the surface of the neurons that need it to survive.
Below: After intervention with trophic factors, neurons that have started to shrivel up and lose contact with their target cells (center) are restored and their contacts reconnected (bottom).**

Normal

Neurodegenerative Disease

Trophic Factor Treatment

rescues these neurons from death but promotes the ability of these rats to learn and remember new tasks.

There are, as you might imagine, some potential problems with implanting tubes in people's heads. Over months or years it might cause an infection in the brain, or the tube might move slightly, sending the trophic factor to the wrong place, and so on. Therefore, considerable work is focusing on alternative methods of delivery. One on which we are working is a biological method, that is, implanting living cells that secrete large amounts of NGF directly into the brain. Skin cells are isolated from rats, grown in culture in large numbers, and then injected with the gene for NGF (or another trophic factor on which we are working called LIF, for leukemia inhibitory factor). Cells that are producing high levels of the trophic factor are selected for use as a living graft of cells to be implanted into the part of the brain where the neuronal loss is occurring.

In addition to aged animals, other models involve surgical lesions placed in very discrete locations in the brain. In one surgical model, nerve fibers of cholinergic neurons are cut on one side of the brain, leaving the other, intact, side as a control. In this case, the genetically engineered cells producing NGF are grafted into the lesioned side of the brain and the results assessed days or weeks later. When the brains of these animals are examined histologically, the tissue sections reveal that the lesioned neurons survive far better when they are near a graft of cells secreting NGF —they are large and healthy, with many sprouts. When the same experiment is done and the behavior of the rats is tested using the water-maze test that Erin described, the performance of a rat whose cholinergic neurons have been lesioned is very poor. It displays a very poor ability to remember where the platform is located. In contrast, lesioned rats that have received grafts of NGF-secreting cells display a markedly improved spatial learning and memory performance.

A variety of experiments from a number of different labs around the world have shown that NGF and other trophic factors have the capacity to rescue neurons under many different circumstances. It does not seem to make a difference how the neurons have been made to atrophy— whether by aging, surgical lesions, or various toxins; survival and growth can be improved with trophic factor under many toxic conditions. This, of course, has important implications for diseases for which we do not yet know the causes. In addition, some reports have indicated that administering NGF to normal, healthy rats

among the most prominent cells that die in Alzheimer's disease.

These findings raise the possibility that we could use such trophic factors to interact with the specific populations of neurons that die in various neurodegenerative diseases. If a neuron starts to shrivel up and lose contact with its target cell as the disease progresses, we might potentially be able to intervene with the appropriate trophic factor that could restore this neuron to health, allow it to reattach its connections, and thereby ameliorate the problems (say, memory loss) that its dying was causing.

One of the challenges in such a scheme is delivery: How can we deliver trophic factors to a person's brain? The simplest and most direct way is to implant surgically, in the appropriate part of the brain, a tube connected to a pump of NGF. This is, in fact, actually being tested in humans, and extensive animal research is also being conducted. Rats are one animal model because as they age, they can display deficits at certain learning and memory tasks that are correlated with a loss of cholinergic neurons, as happens in Alzheimer's patients.

When tissue slices from rat brains are stained to reveal cholinergic neurons, the brains of healthy, young adult rats show numerous such neurons, but the brains of aged rats show very few because the neurons have shrunk and disappeared as they do in Alzheimer's brains. But in an aged rat brain that has been injected with NGF, the neurons are larger and more numerous. Corresponding behavioral tests have shown that in many cases the administration of NGF not only

In the Morris water-maze test, a normal rat quickly learns to find its way directly to the platform (blue square) hidden just below the surface of a pool of water, remembering where it is from clues in the surroundings. The pool is represented here by the circle, and the rat's path is shown in orange. A rat whose cholinergic neurons have been cut cannot, however, remember how to find the platform and swims around in circles (center). But such a rat that has had an NGF-containing graft implanted in its brain can find the platform pretty well (bottom)—not as well as the normal rat but a big improvement over the one with the lesioned brain.



Normal

Lesioned

Lesioned with NGF graft
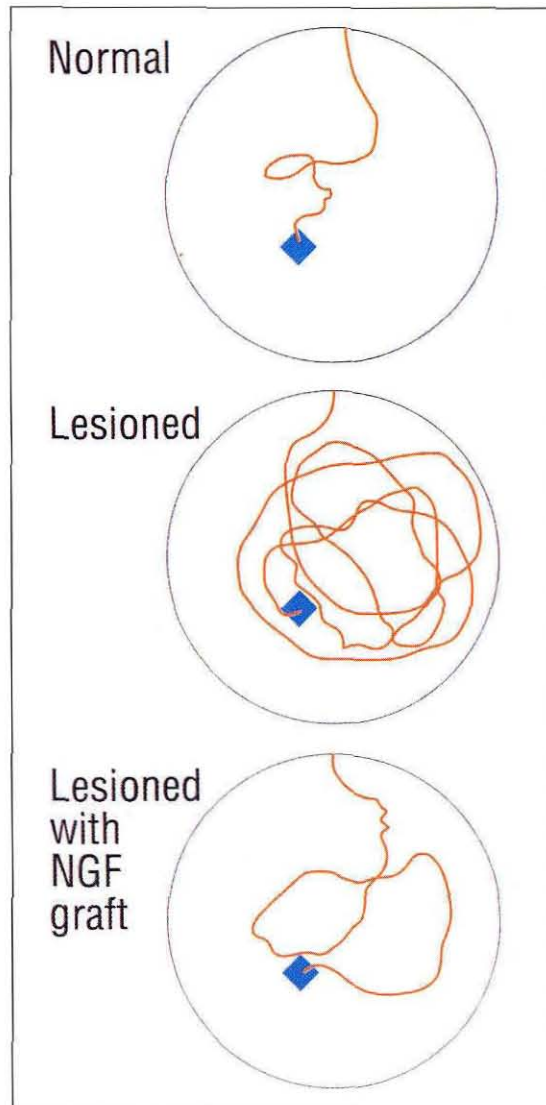
improves their learning and memory capacity. This may give hope to all of us!

A final interesting point concerns another feature of the grafted cells in the brain. An experiment that we've done in collaboration with Fred Gage of UC San Diego involves placing grafts of NGF-secreting cells at some distance from NGF-responsive cells in the brain. In spite of this distance, the NGF-responsive neurons will send processes directly toward the graft, ultimately surrounding and infesting it. The nerve processes do not grow randomly, but directly toward the graft. This means that we can control the direction of nerve outgrowth by where we place the graft. This is experimentally very useful, but it also points out a potential problem in applying this technique in humans. If one misplaces the graft or the tube of NGF even slightly, nerve processes might grow to the wrong location, and the patient could be worse off than before.

Although it is still the early days of this field, one can draw several conclusions at this point: 1) A variety of basic-science experiments on the normal embryological death of cells and the role of trophic factors provide results that have direct applicability to the clinical study of a number of neurodegenerative diseases—not only Alzheimer's disease, but Parkinson's and others. 2) The applicability of such trophic factors to situations in which we do not yet know the cause of the disease is potentially very important. 3) Animal models are essential for the study of these diseases.

Finally, current work in our lab and that of many others is aimed in several different directions. It is critical to find the best form of delivery, and to extend the rodent work into primate disease models. We are also looking for new trophic factors that will work on other kinds of neurons in the brain. Many types of neurons die in these neurodegenerative diseases, including Alzheimer's, and we want to find trophic factors for each of them. Past success in these areas provides hope for currently untreatable neurological diseases.

Paul Patterson has been professor of biology at Caltech since 1983 and executive officer for neurobiology since 1990. He received his BA from Grinnell College in 1965 and his PhD from Johns Hopkins University in 1970. Before coming to Caltech he was on the neurobiology faculty of Harvard Medical School.

undifferentiated cell

various kinds of differentiated cells
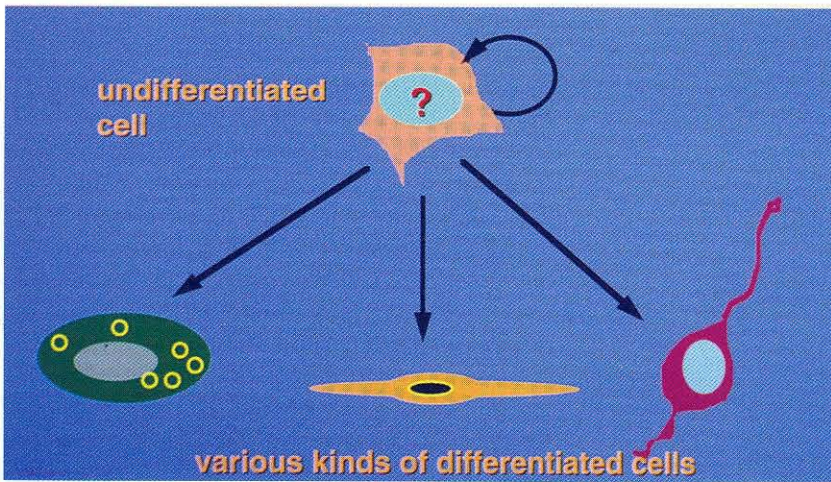
A progenitor cell is one that hasn't yet differentiated—that is, it hasn't yet decided what it wants to become. It divides and generates daughter cells, which do differentiate and assume different forms and functions.

David J. Anderson
Associate Professor of Biology, Caltech

Perhaps the most revolutionary potential kind of therapy for brain diseases is cell-replacement therapy, that is, transplanting cells to replace the function of neurons that have died in diseases such as Alzheimer's disease. In order to develop such a therapy and take it from science fiction to science fact, we need to know, first of all, which type of cell we need to transplant. Since neurons are dying, it would seem to make sense to replace them with more neurons. But another cell, called a progenitor cell, is also being studied as a transplant candidate in my own lab and in others around the world.

A progenitor cell is a cell that we originally thought existed only in the embryonic brain. It's an undifferentiated cell, that is, it doesn't have any particular function except to make more of itself by dividing and to generate other cells— daughter cells—which then differentiate, taking on the specialized form and function that they need, in this case, to make the brain do what it does. Progenitor cells have enormous proliferative capacity; they can divide, which makes them much easier to cultivate in the laboratory than neurons, which can't divide. A particular kind of progenitor cell, called a stem cell, gives rise to the greatest number of daughter cells, and can be thought of as the most primitive of the progenitor cells. Progenitor cells are also easy to grow,

and because they are immature, primitive cells, they can adapt more easily than neurons can to a new environment, such as the one they might encounter if transplanted into a brain. Cells are like people: as they get older, they tend to get more set in their ways.

But before we think about transplanting these cells, we need to know more about them, specifically whether there's a special progenitor cell for each type of neuron, or whether a generic type of progenitor cell can give rise to all the different types of neurons that die in various neurologic diseases and therefore can be used to treat them all. In order to determine how many different cell types a progenitor cell can give rise to, we can study them in the laboratory in two basic ways. One is to study them in culture. We put the progenitor cells in petri dishes, and to some of those we add one kind of hormone—or factor, or signal—and then to other petri dishes containing the same kind of cells, we add a different kind of signal. Then we ask whether the progenitor cells differentiate into different cell types in the different petri dishes. And it turns out that they *do*, and we can define the menu of possible fates available to a progenitor cell by these experiments. We don't know ahead of time what conditions, or factors, to use to get the cells to differentiate, but we can use this as a system to search for such conditions. This has the side benefit that once we know the conditions that will turn a progenitor cell into, say, a cholinergic neuron, or a neuron that makes dopamine, we might be able to use that information down the road to supply those factors along with the progenitor cells when they're transplanted into the brain.

Another way to find out what progenitor cells can give rise to is to transplant the cells into experimental animals such as rats. We can transplant these cells, labeled with a dye, into an embryo (or an adult brain), and then follow them to see where they go and what they become. Such transplant experiments in my lab (in collaboration with Fred Gage at UC San Diego) and in several other labs around the country have indicated that these progenitor cells are quite plastic, that is, they have a wide range of developmental capacities, suggesting that they could be used for more than one type of disease. But we still don't know whether there is one single generic progenitor that we could use to treat all neurodegenerative diseases. This is a goal of much current research.

We have two methods of growing progenitor cells for these experiments. One method manipulates the cells from the outside by supplying

condition "A"   condition "B"
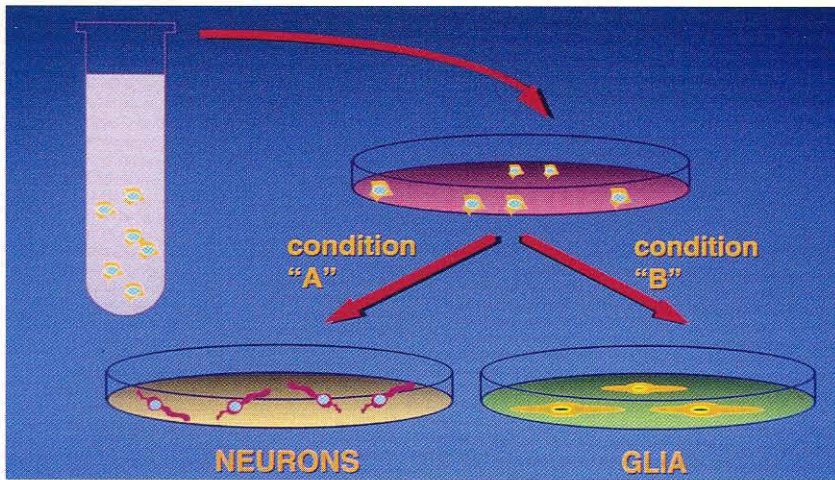
NEURONS   GLIA

**Above: Progenitor cells can be isolated and studied in culture to determine what conditions cause them to differentiate into specific daughter cells—neurons, say, or glial cells.
Below: An isolated progenitor cell before it has begun to differentiate. After it had grown in culture for two weeks, it differentiated into the hundreds of neurons and glial cells seen on page 14.**

Does any of this have a prayer of working?

them with nutrient molecules called growth factors, which bind to receptors on the surface of the cells and stimulate them to divide. The alternative way is to manipulate the cell from within—provide the cells with a gene, called an oncogene, whose product increases cell division. Recent experiments with both of these techniques have made it possible to generate large quantities of progenitor cells in the laboratory, starting with very small numbers of cells.

As I said earlier, neural progenitor cells are found in developing embryos. Experiments with animal models have indicated that such fetal cells behave much better than do adult cells when grafted into an adult brain, probably because they're more primitive and, therefore, more plastic. But in order to treat human diseases, we need to start with human cells (animal cells will be rejected by the immune system). In Sweden, where such experiments have been generating a lot of excitement recently, reports have indicated that human fetal cells transplanted into the brains of aged human patients (in this case to treat Parkinson's disease) are actually able to survive, grow, and differentiate over a period of years. In this country, there are moral, political, and practical barriers to doing research with human fetal tissue, but these techniques that allow us to grow large quantities of progenitor cells in the laboratory could allow us to generate an essentially infinite supply from a small sample of human fetal cells obtained on a one-time-only basis. That is, a single sample of fetal tissue could provide a potentially inexhaustible supply of progenitor cells.

New research also shows that the adult human brain contains a small reservoir of these progenitor cells or stem cells. This discovery overturned dogma that had been accepted in neurobiology for a century. The existence of stem cells in the adult human brain opens up the possibility that we might be able to transplant a patient's own stem cells from a healthy part of the brain into the part that's diseased. Current research is focusing on ways to accomplish this.

Does any of this have a prayer of working? Although it's not yet clear whether this kind of cell-replacement therapy will work for Alzheimer's disease, dramatic progress has been made using it in Parkinson's disease. This is encouraging, but we're not going to know definitely whether this kind of therapy can work until we have gained more understanding of the fundamental properties of neural progenitor cells. This is the kind of research that we do here at Caltech. Even though it doesn't have a medical school, it's a place where biological research goes on that is relevant to human medicine and human disease. The concept of using replacement cells in therapy for diseases such as Alzheimer's and Parkinson's has emerged directly from the kind of basic research into the fundamental biology of these fascinating cells that we, and our colleagues in other institutions, carry out. □

David J. Anderson earned his AB from Harvard in 1978 and his PhD from Rockefeller University in 1983. Following a postdoctoral appointment at Columbia University, he came to Caltech as assistant professor of biology in 1986 and was named associate professor in 1992. He is also an associate investigator of the Howard Hughes Medical Institute and an adjunct assistant professor of anatomy and cell biology at the University of Southern California School of Medicine.