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Sense and Synapse: [Dr. Paul Greengard]

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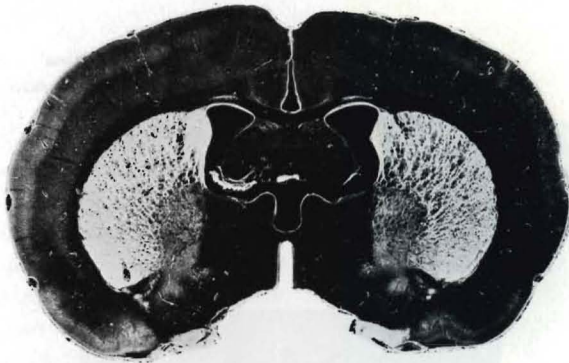
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THE ROCKEFELLER UNIVERSITY RESEARCH PROFILES

FALL 1987



Cross section of a rat's brain. The fluorescent staining marks the location of DARPP-32, a phosphoprotein involved in mediating the action of the neurotransmitter dopamine.

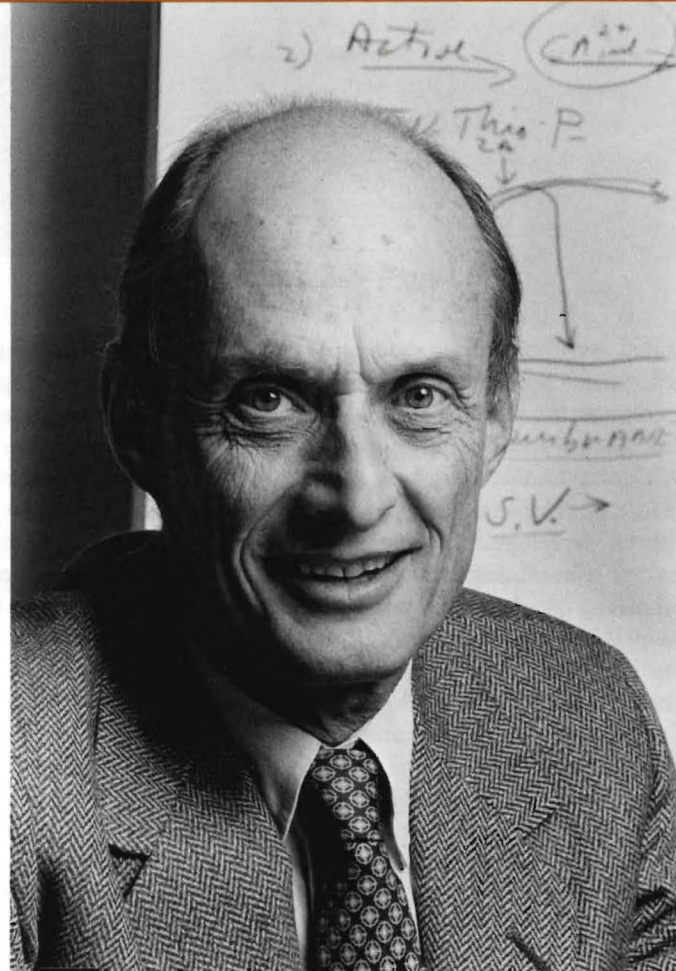
Sense and Synapse

In the living brain, electrical signals flash from nerve cell to nerve cell like lights in a pinball machine. In the human brain, with its hundred billion nerve cells, millions of cells are signaling one another at any given moment, each in touch with hundreds of other cells and each of those cells receiving hundreds of simultaneous messages. What prevents utter chaos? How does the brain achieve sense and order?

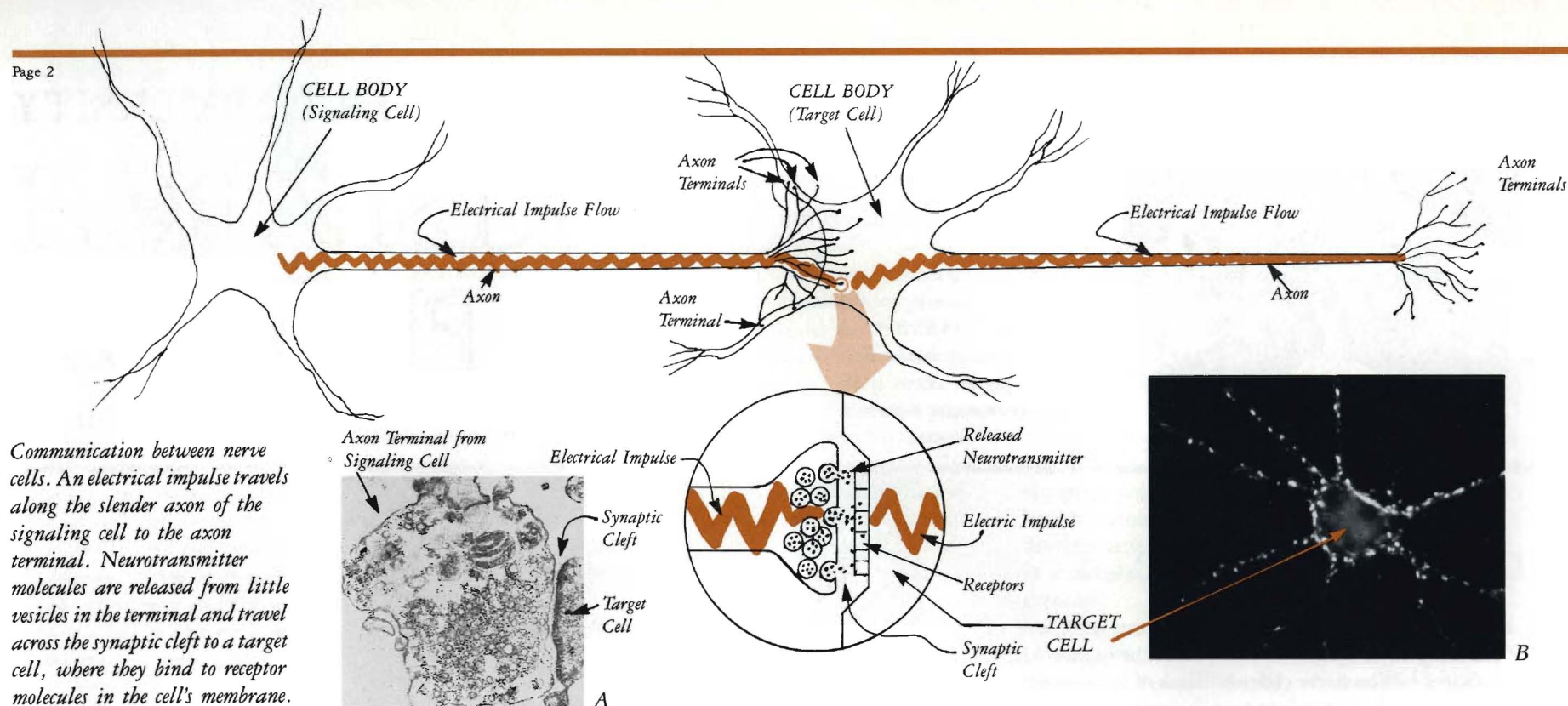
Unlike signals generated in a machine, nerve signals change form, from electrical to chemical, to negotiate the tiny fluid gulf—the synapse—that separates a nerve cell, or neuron, from its neighbor cells. Once across the synapse, the chemical signal switches back to electrical and the process continues. Through each successive flash-squirt-flash, occurring in a few thousandths of a second across a distance of less than a millionth of an inch, nerve cells relay their messages like racers passing a baton.

This process, at work in the brain and throughout the nervous system, is called neurotransmission. The chemicals that traverse the synapse, called neurotransmitters, produce or suppress the electrical impulses of nerve cells. What controls the production, the release, and the actions of neurotransmitters? Twenty years ago, Paul Greengard had an idea what the answer might be.

Dr. Greengard directs the Rockefeller University's laboratory of molecular and cellular neuroscience. A recent transplant from Yale, he is one of a number of leaders in neurobiology and other fields of research who have joined the Rockefeller



Paul Greengard



Communication between nerve cells. An electrical impulse travels along the slender axon of the signaling cell to the axon terminal. Neurotransmitter molecules are released from little vesicles in the terminal and travel across the synaptic cleft to a target cell, where they bind to receptor molecules in the cell's membrane. The neurotransmitter then either stimulates or suppresses an electrical impulse in the target cell. Dr. Greengard's research has demonstrated that the activity of neurotransmitters is mediated by a biochemical process called protein phosphorylation.

A

Electron micrograph showing molecules of the phosphoprotein Synapsin I (black specks) on neurotransmitter vesicles (clear circles) in axon terminal of a signaling neuron.

B

Light micrograph showing target neuron outlined by nerve terminals (white specks) containing Synapsin I.

faculty over the past few years bringing with them new insights into nature's puzzles and new approaches toward understanding and correcting nature's anomalies. Certainly one of the most difficult and compelling of nature's puzzles is the brain.

Rodolfo Llinás, chairman of physiology and biophysics at New York University Medical Center, who has collaborated with Dr. Greengard in some of his research, wrote several years ago: "It is becoming clear that most of the diseases of the brain and many psychiatric disorders result from a disruption of synaptic communication or are associated with such a disruption. The synapse is the weakest link in brain activity; it is the first part of a neuronal chain to be fatigued by a high level of message transmission, and it is the site of action for most of the drugs that affect the brain."

The work in Dr. Greengard's laboratory is helping to explain the synaptic events that occur in healthy and unhealthy brains. He and his colleagues are applying what they are learning to improve diagnostic techniques and drugs for such conditions as schizophrenia, Parkinson's disease, and Alzheimer's disease. All of this research, as well as similar studies going on in many

other laboratories, began when Dr. Greengard looked at some liver cells and saw a way into the brain.

THE CHEMICAL CONUNDRUM

That nerve cells generate electrical impulses was recognized more than a century ago. Beginning around the 1920s and progressing with the development of electronics and high-speed computers, physiologists have achieved the ability to record and analyze the electrical signals of individual neurons and track neural networks throughout the body and within the brain.

Of the chemistry of the brain, as early as 1890 the philosopher-psychologist William James wrote, "chemical action must of course accompany mental activity, but little is known of its exact nature." Not much more was known of it nearly sixty years later when Paul Greengard decided to move from mathematics and physics, which he had studied as an undergraduate, to physiology, and it first became "obvious" to him that "if you want to understand how nerve cells function you have to understand the biochemistry." The difficulties would become equally obvious.

He had been accepted into the biophysics laboratory at Johns Hopkins then under the direction of Detlev Bronk. One of the pioneers of nerve research, Bronk was also an innovative educator and firm believer in scientific self-determination, as he later demonstrated during his fifteen-year presidency of Rockefeller.

"Although the laboratory's orientation was strictly physiological," says Dr. Greengard, "Bronk agreed to let me do my Ph.D. research on the chemical changes associated with degeneration and loss of function in nerve axons." (Axons are the slender threads along which the electrical signals of neurons travel to reach the synapse.) His thesis advisor was Frank Brink, also later to come to Rockefeller and now professor emeritus. "It was a kind of first effort," Dr. Greengard explains, "to understand the relationship of nerve function and biochemistry."

After Hopkins he spent five years in England, at the University of London working with one of the few people doing biochemical research on the nervous system, at Cambridge studying protein chemistry, and at the National Institute of Medical Research, one of the few places in the world that had a department equipped for both biophysical and biochemical investigations. He made some fruitful findings about the effects of biochemical manipulation on physiological functioning, but the central question concerning the mechanisms of neurotransmission remained as elusive for him as it had for others.

"We were faced with a situation," he explains, "where every nerve cell looked the same as every other nerve cell, and so far as we could tell was using the same neurotransmitters. There didn't seem to be any way even to begin to ask intelligent questions about how the cells connect to one another or to distinguish one cell from another. It was as if, in electronics, you were confronted with resistors without any color-coding, or every resistor were painted the same color."

Stymied, he returned to the United States and took a post as director of biochemistry at Geigy Research Laboratories, in Ardsley, New York, where he thought that "at least I could use my skills in an effort to develop new medicines to help people." Over the next several years, he worked on the development of imipramine, one of the major anti-depression drugs, and served as a professor of pharmacology at the Albert Einstein College of Medicine, where he was involved in studies that led

to major advances in understanding of the mode of action of local anesthetics. He also followed with close interest reports of some new developments in hormone research coming from Vanderbilt University, in Tennessee.

"IT OCCURRED TO ME . . ."

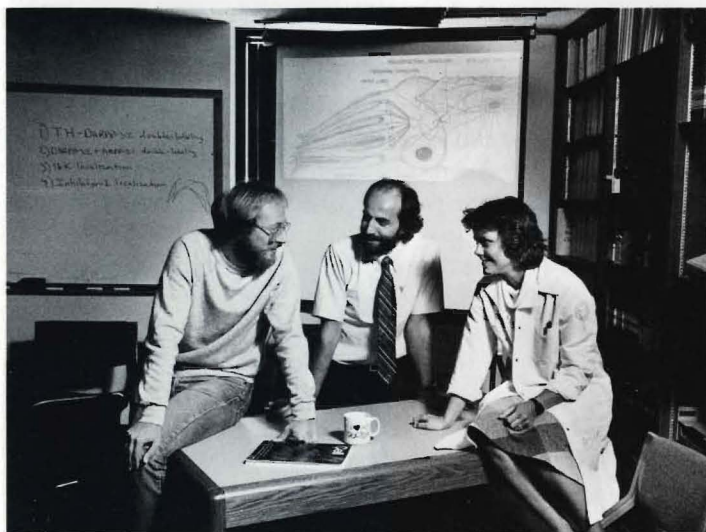
In the same way that neurotransmitters are the short-distance messengers across the synapse in the nervous system, hormones are the long-distance emissaries of the endocrine system, traveling through the bloodstream from the body's glands to their target cells, where they are caught and bound by antenna-like receptors on the cell's surface.

Earl Sutherland, an endocrinologist at Vanderbilt, had found a molecule, called cyclic AMP, in liver cells, which somehow mediated the hormone's message to the cells concerning whether they should store carbohydrate or release it into the bloodstream. (He was awarded a Nobel Prize for this discovery in 1971.) Edwin Krebs, now at the University of Washington, in Seattle, took Sutherland's findings an important step further and showed that cyclic AMP works by activating an enzyme—a catalytic protein—called a kinase. "It occurred to me," Dr. Greengard says, "that similar things might be going on in nerve cells."

Just about the time he was thinking of asking Sutherland if he might come and see these events at first hand, he was invited to accept a visiting professorship in Sutherland's laboratory as part of a National Institutes of Health research program. While at Vanderbilt, he received an appointment at Yale, where he set up his own laboratory and began looking in the brain for Krebs's kinase. He found it in high concentrations. "And much higher concentrations at the synapse than anywhere else, which made me feel pretty confident," he says, "that whatever it was doing in the brain it wasn't just breaking down carbohydrates."

What Dr. Greengard was seeing was the cellular machinery of a biochemical phenomenon called protein phosphorylation. Protein phosphorylation means, simply, the placing of a phosphate molecule on a protein. Its consequences were to prove far from simple.

Following page, near photo, from left: Martin Babler, Pietro De Camilli, and Teresa McGuinness, who study the phosphoprotein Synapsin I, which controls the release of neurotransmitters in all cells of the nervous system. Projected on the screen behind them is a drawing of a squid. Invertebrates like the squid are frequently used in such research because their nerve cells are larger and more accessible than those of mammals.



COLOR-CODING THE BRAIN

Proteins are the molecular workhorses of cells. Some serve as structural material, others—the critical ones from a biochemical standpoint—are responsible for carrying out cellular activities. “Today we know,” says Dr. Greengard, “that in order for many proteins to become active they must be switched on by another molecule, a sort of ‘master molecule’ called a protein kinase, which, by adding phosphate to them, alters their shape.” A protein thus affected is called a substrate.

“In protein phosphorylation, the scheme goes like this. The hormone or neurotransmitter, which we call the ‘first messenger,’ locks onto the receptor, which causes a change in the level of concentration of a second messenger, like cyclic AMP. That activates a kinase, which then adds a phosphate molecule to a substrate protein in the cell. From there on, an enormous variety of things can happen depending on the nature of the substrate and the type of cell.”

After having seen the process at work in two biological systems, it seemed logical to him to take a look at other kinds of cells. “We discovered the same thing going on in every tissue in organisms throughout the animal kingdom.” Later, he began to wonder whether there were other second messengers (four

have been found to date) that activated other kinases (about a dozen have been identified).

But demonstrating that protein phosphorylation was occurring in nerve cells did not prove that it was controlling the conversion of nerve signals. “To find that out,” Dr. Greengard says, “we would have to put kinases directly into cells that were not receiving neurotransmitters and see if the kinases had the same effect on physiological activity as neurotransmitters.

“We had a few problems. The first was getting pure kinases, so that we could be sure that they alone were causing the effect. The second was the difficulty of injecting them into single cells and recording the electrical activity. In most of our earlier studies we used invertebrate nerve cells, which are much larger than mammalian cells so it’s easier to stick things into them. In the last few years, a much more refined technique called patch clamping has been developed in Germany, making it easier to work with smaller cells. The biggest problem was convincing physiologists whose collaboration we needed that we weren’t crazy.”

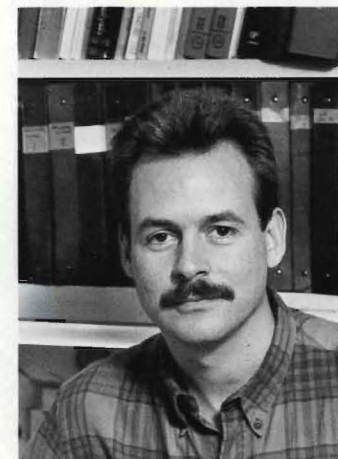
Angus Nairn, a member of Dr. Greengard’s group since 1979, arrived at Yale “just about the time Paul had managed to persuade some people to inject protein kinases into neurons. Today,” he says, “you go to a neuroscience meeting and find out that everyone’s injecting kinases and discovering that kinases are regulating the particular system they’re studying.”

TOWARD A MOLECULAR MEDICINE

Protein phosphorylation is now generally accepted as not only the central regulatory mechanism of nerve cells, but as a basic biochemical activity in *all* communication systems in cells. “There was no sudden ‘breakthrough,’” Dr. Greengard states, “just years of work in which the evidence accumulated.”

How can so many different things happen in so many different kinds of cells by means of one kind of biochemical reaction? The answer is that substrate proteins in different tissues differ from one another. “There are hundreds of different proteins in the nervous system and other systems,” says Dr. Greengard, “and many different proteins in many different kinds of nerve cells in the brain.”

As he and his colleagues learn what second messengers are



Richard Haganir, who studies the events that occur in nerve cells receiving acetylcholine, a neurotransmitter that may be involved in the scrambled messages of Alzheimer’s disease.



Angus Nairn, whose research focuses on the phosphoproteins that regulate dopamine transmission. This system becomes disrupted in Parkinson’s disease and schizophrenia.

Reflecting the diversity of approaches in the Greengard laboratory, Charles Ouimet, left, is a neuroanatomist who is working to localize different phosphoproteins in the brain; Jean-Antoine Girault, right, is a neurologist who has also completed a Ph.D. studying the regulation of neurotransmitter release.



affected by what neurotransmitters and what kinases depend on what second messengers and what sorts of proteins they phosphorylate in what kinds of cells it is becoming possible to begin to color-code the brain and to determine the chemical types of nerve cells that contact each other.

There are some phosphoproteins that are present in every nerve cell in every mammalian nervous system and that regulate activities common to all nerve cells. Such a protein is Synapsin I, which Dr. Greengard and his group discovered and have been studying since 1972, and which they have shown to regulate neurotransmitter release.

Synapsin I is localized at nerve terminals next to the synapse on the tiny sacs, or vesicles, in which nerve cells store neurotransmitters. In a series of elegant experiments, Dr. Greengard and his student Teresa McGuinness, in collaboration with Rodolfo Llinás, demonstrated that when a dephosphorylated form of Synapsin I is injected into a cell it prevents the cell from releasing neurotransmitter into the synapse. Conversely, injection of the kinase that phosphorylates Synapsin I causes a five- to tenfold increase of neurotransmitter release.

There are some phosphoproteins that are found exclusively

in the brain and, in fact, only in specific parts of the brain. Angus Nairn and Ivar Walaas, another member of Dr. Greengard's group, have identified seventy of them. "We didn't know exactly what we'd find when we began," Dr. Nairn says. "We just thought it would be easier, since the brain is so complicated, to break it down into smaller parts to see what might be going on with one particular neurotransmitter or one particular area.

"We spent a couple of years categorizing and classifying here and there. Then, about the time we moved to Rockefeller, we decided to concentrate on the basal ganglia, one of the areas in the brain where the neurotransmitter dopamine is active. We chose it because Ivar knew a lot about its anatomy and because of Paul's interest in Parkinson's disease and schizophrenia."

In Parkinson's disease, neurons receive too little dopamine. In schizophrenia, there is increasing evidence of excessive dopamine transmission. The first drugs that were effective in treating these conditions were developed in the black-box era of brain biochemistry by essentially hit or miss tinkering. In 1973, research by Dr. Greengard confirmed dopamine involvement by showing that the drugs modified kinase activity in the dopamine system. Since then, he and his group have been able to explain some of the clinical effects, both good and bad, of a variety of psychoactive drugs, including hallucinogens and antidepressant drugs, in terms of their effects on phosphorylation.

In parkinsonism and in the progressive and fatal neurological destruction of Huntington's chorea, the disease that killed folksinger Woody Guthrie, specific cells of the basal ganglia are missing. In schizophrenia, the problem may involve faulty receptors. By pinpointing neurotransmission molecularly, the laboratory hopes to devise drugs that, for example, exactly replace missing molecules, and that can correct abnormalities in the cell-signaling process.

Among the phosphoproteins Dr. Nairn and Dr. Walaas found in the basal ganglia, one called DARPP-32 appears to be very specifically associated with dopamine regulation. (DARPP stands for dopamine and cyclic AMP-regulated phosphoprotein. The 32 refers to its molecular size.)

"At first," Dr. Nairn says, "we were looking in rat brains. Once we found the protein it wasn't practical to try to purify



Andrew Czernik, left, and Michael Browning, who are investigating the relationship between protein phosphorylation and alcoholism. A phosphoprotein, called protein III, appears abnormal in brain tissue taken from alcoholics who died sober but normal in alcoholics who died while intoxicated. Among the questions Drs. Browning and Czernik would like to answer: Is the abnormality a reflection of the biochemical changes that occur when alcohol-dependent brain cells are deprived of alcohol, or is it a physiological defect that alcohol corrects? Is the abnormality inherited or acquired? If it is genetic in origin, is it significant in the development of alcoholism?

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it from rats. They're convenient to work with but their basal ganglia are very small. So we went to a slaughterhouse and got four or five hundred cow brains. After we got the protein purified, we worked out its molecular structure and we think we know how it works. We've gone back to the rat brain now to see if we're right. If we are, regulation of DARPP-32 could become important therapeutically."

Synapsin I is a phosphoprotein that works at the presynaptic end of nerve communication. Richard Huganir's research centers on postsynaptic events at the receptor sites of receiving cells. "There are two ways you can study how cell communication works," he explains. "You can modulate the amount of neurotransmitter that's released from the first cell or you can modulate the sensitivity of the second cell to a signal, which is what I'm doing."

In the case of the neurotransmitter acetylcholine, the receptor molecule is itself a phosphoprotein. "We've been able to determine," he says, "that phosphorylation of the receptor makes cells less sensitive to the effects of acetylcholine; it desensitizes them through a kind of negative feedback system when there is too much activity along the neuronal pathway." Dr. Huganir's research may help to clarify whether or not the phosphoprotein is involved in Alzheimer's disease, which has been found to be associated with abnormal acetylcholine transmission.

CROSS-CONNECTIONS

Like the science he directs, the members of Dr. Greengard's laboratory are mostly young. They come from a variety of disciplines. Added to the biochemists, biophysicists, pharmacologists, and neuroanatomists, there are molecular biologists studying the genes that code for phosphoproteins, and on the clinical side, there are neurologists and psychiatrists looking at cerebral spinal fluid from patients with various neurological and mental illnesses. Goran Sedvall, chairman of the Department of Psychiatry at the Karolinska Institute, spent a year, from 1984 to 1985, in Dr. Greengard's laboratory as the first participant in an exchange program between the University and the Karolinska, supported by the Nicholson Exchange Fund for Biomedical Scientists. Teresa McGuinness, who says she "has never been able to separate my clinical and basic science

interests," and who now holds both an M.D. and a Ph.D. degree, continues to work on Synapsin I and is planning a residency in psychiatry.

Such multi-pronged science requires not only a lot of people but also a lot of space and equipment. The chance to expand his facilities and range of investigations was one of the inducements that brought Dr. Greengard to Rockefeller. Another was the kind of lab-crossing collaborations typical of Rockefeller research. Members of Dr. Greengard's group are currently working with a number of other groups at the University. They are applying protein phosphorylation studies to research on the brain's steroid hormones, on vision, and on the embryonic development of the nervous system, and using the techniques of molecular biology to study genetic regulation of phosphoproteins.

There have been a number of rather special dates on Dr. Greengard's 1987 calendar. In January, he received the Pfizer Biomedical Research Award, which carries a half-million-dollar research stipend, in recognition of his contributions to the understanding of phosphoproteins and their role in disorders of the central nervous system; in April, the prestigious 3M Life Sciences Award, presented at the annual meeting of the Federation of American Societies for Experimental Biology; in June, an honorary Doctor of Medicine degree from the Karolinska Institute; in September, the National Mental Health Association's Research Achievement Award; and in October, election as a Foreign Member of the Royal Swedish Academy of Sciences.

In the last several years, a handful of key discoveries like Dr. Greengard's has opened the way to new research possibilities in neurobiology and drawn talented young scientists to its ranks in unprecedented numbers, each determined to find answers to questions once considered unanswerable. In high school, Rick Huganir did a project on protein synthesis and memory, "which of course didn't work," he says, "because it was based on an incorrect theory around at that time that when you learn something a protein gets made. Then, when I was a sophomore in college, I read a Greengard paper on protein phosphorylation and I said to myself, 'okay, this is it. This is a handle.'"