New Information About the Development of the Autonomic Nervous System

This simple nervous system provides a model for studying how neurons choose a neurotransmitter and form specific connections to each other and their targets

Building a brain would seem to be so complicated as to be impossible. Nevertheless, it happens all the time in the normal course of embryonic development. Somehow, a few primordial cells multiply to form billions of nerve cells, which come together in a precise three-dimensional arrangement and establish appropriate connections with one another.

This is the fourth in a series of occasional articles on recent developments in neurobiology. The first story dealt with the search for new psychoactive drugs (24 August, p. 774); the second concerned brain peptides (31 August, p. 886); and the third discussed sex hormones and brain development (7 September, p. 985).

Because the brain is so complicated, many researchers have not tackled the problem of its development directly but have instead focused on simpler nervous systems, such as the autonomic nervous system, which helps to regulate the more or less automatic activities of our internal organs, including the heart, blood vessels, and intestines. Says Dale Purves of the Washington University School of Medicine, "Many features of the central nervous system are still forbidding, but it, like the autonomic system, consists of a series of connections between neurons." However, the brain contains many different kinds of neurons, many of whose connections have not yet been traced, whereas the autonomic system consists of only a few kinds of nerve cells whose connections are understood for the most part.

Part of the reason for the interest in neural development is the relation of the research to the topic of nerve regeneration. Usually when a nerve tract is severed or severely injured, the damage is not repaired. The nerves may grow for a while in some haphazard and uncoordinated way but they rarely establish normal connections with their target organs. The result is permanent disability: A person whose spinal cord is severed, for example, will be paralyzed below the level of the injury.

Thus, neurobiologists hope that an understanding of the signals that guided the nerves to their targets in the first place and caused them to develop into one type of nerve cell or another may provide clues to the regeneration puzzle. As Ira Black of Cornell University Medical College puts it, "The important thing is that the research may shed light on a number of until now hopeless neurological problems."

Two of the prominent features of developing nervous systems are the migration of neurons from their point of origin to their target organs—often a long way—and the commitment of the neurons to produce one neurotransmitter or another. (Neurotransmitters are the chemicals by which nerve cells communicate.) Research on the autonomic nervous system is shedding some light on both of these phenomena. Investigators are finding that the environment encountered by the migrating neurons greatly influences the destination they attain and the neurotransmitter they produce when they get there.

Moreover, the neurons appear capable of changing their choice of neurotransmitter during development if exposed to the appropriate signals, a finding with a direct bearing on the regeneration problem. A neuron's neurotransmitter has generally been considered to be one of its immutable characteristics (although recent discoveries of more than one potential neurotransmitter in some neurons have presented a complication.) But if neurons are more "plastic" in this regard than was once thought, a better understanding of the signals influencing neurotransmitter choice may one day aid attempts to induce regeneration of damaged nerves.

The autonomic nervous system is divided into two parts: the sympathetic subdivision, which is mostly concerned with reaction to stress or danger, and the parasympathetic subdivision, which is more concerned with stimulating such everyday activities as digestion and excretion.

The nerve pathways of both subdivisions contain only two main neurons, one originating in the spinal cord or brain and extending out to the periphery, and the second forming the actual contact with the target organs. The first neurons in both branches are cholinergic—that is, they use the neurotransmitter acetylcholine. The second neurons of parasympathetic tracts are also cholinergic, but in the sympathetic tracts they are adrenergic, using norepinephrine (also called noradrenalin) to transmit signals to their targets.

All the neurons of the autonomic system develop from an area of the embryo called the neural crest, a group of cells extending along the upper surface of the neural tube. The neural tube stretches along the back of the embryo in roughly the location to be occupied by the spinal cord, one of the organs derived from the tube.

According to Nicole Le Douarin and her colleagues at the Institut d'Embryologie du CNRS et du Collège de France in Nogent-sur-Marne, the upper region—the top 15 percent or so—of the neural crest gives rise mainly to the cholinergic neurons of the parasympathetic system, the middle 40 to 50 percent of the crest yields adrenergic neurons of the sympathetic system, and the bottom segment produces both types. They based this conclusion on an elegant series of experiments in which they transplanted segments of the neural tubes from quail embryos into chick embryos and vice versa. Because the nuclei of chick cells look very different from those of quail cells, the two can be distinguished and the fate of the transplanted cells readily followed.

Le Douarin says that the pattern of nerve development they observed is not the result of an early commitment of the crest cells to produce one type of neuron or the other. In fact, if the Nogent-sur-Marne workers transplant the uppermost region of the neural tube, the segment

0036-8075/79/1026-0434$00.75/0

that gives rise to the cholinergic parasympathetic nerve cells, into the lower region, which produces adrenergic sympathetic neurons, the transplanted tissue forms adrenergic neurons. Conversely, the adrenergic region, when transplanted into the cholinergic region, forms cholinergic neurons.

Le Douarin concludes that the potential to produce the two types of neurons exists throughout the crest. Apparently, cells from different crest segments migrate along different pathways to their final destination, and during this time their differentiation to either adrenergic or cholinergic neurons is directed by the local conditions they encounter.

Such a finding agrees with results obtained by other investigators, although at least one of them, Alan M. Cohen of the Johns Hopkins University School of Medicine, recently modified his earlier conclusion that the presence of some of the tissues through which the crest cells migrate is necessary for the cells to attain their adrenergic character.

In more recent experiments, Cohen finds that some neural crest cells, taken early in development before migration begins and grown in culture, become either adrenergic or cholinergic without any apparent influence by other tissues. "This does not mean," he says, "that the environment does not play an important role in neurotransmitter choice, but the environment guides it and does not determine it."

Le Douarin points out that the transplant studies cannot tell whether the same neural crest cell has the potential to be either adrenergic or cholinergic, depending on its environment. An alternative possibility is that the crest consists of a mixed population of cells, some of which may develop into cholinergic neurons and some into adrenergic neurons. In this case, the environment, by favoring the multiplication of one, would select in favor of that group of developing nerve cells at the expense of the other.

Experiments performed with cells grown in culture can give information about this issue. The experiments, which were independently performed by a group of investigators at Harvard Medical School (including Edwin Furschpan, Story Landis, Paul Patterson, and David Potter) and by Richard Bunge and Mary Johnson at Washington University School of Medicine, used neurons from the superior cervical ganglion of the rat. This ganglion consists of a collection of cell bodies of neurons that are almost exclusively adrenergic neurons of the sympathetic system. But when they are grown in cultures with some types of nonneuronal cells, such as heart or skeletal muscle cells, they change direction and produce acetylcholine instead of norepinephrine.

White leghorn chick embryo into which a section of quail neural tube has been transplanted. The embryo has a band of pigmented quail-like feathers because the neural tube gives rise to pigmented cells that migrate into the skin, in addition to autonomic neurons and a number of other cell types. The location of the pigmented band depends on the location of the neural tube graft. [Source: Nicole Le Douarin, Institut d'Embryologie du CNRS et du Collège de France]

Since the suggestion that a neuron might switch its choice of neurotransmitter went counter to the conventional wisdom, it had to be carefully buttressed. Says Potter, "We spent much effort in showing that single neurons are plastic." There was always the possibility that the adrenergic cells were dying in the cultures and being replaced by the multiplication of an originally small population of cholinergic neurons.

None of the investigators found any evidence for death or multiplication of the cultured cells, a result incompatible with the selective growth of cholinergic nerves. In addition, the Harvard workers have followed the fates of individual neurons. They can show a progression from adrenergic to adrenergic plus cholinergic capabilities in individual cells. Although they think that these dual-function neurons eventually become purely cholinergic, they have not yet directly demonstrated this change.

There is also some evidence for chemical plasticity of autonomic neurons in the living animal. Black and his colleagues at Cornell have demonstrated the appearance of adrenergic nerves in the gut, where they are not normally found in the mature animal, between the 11th and 12th days of gestation in the rat. By the 13th day of gestation they have apparently disappeared. Black has evidence that the neurons themselves are not lost, but that they persist, although without the capacity to produce norepinephrine.

The Cornell workers are currently investigating whether the adrenergic neurons eventually become cholinergic. Such an occurrence would be consistent with what the Harvard and Washington University researchers are seeing in cultured cells. Black's work supports the contention that the persistence of a particular neurotransmitter may well be influenced by the neuron's environment.

Cholinergic neurons may also have the ability to reverse direction and become adrenergic, according to Le Douarin. Experiments in which cholinergic cells from a parasympathetic ganglion of the quail were transplanted into chick embryos in the region of neural crest that gives rise to adrenergic cells suggested this possibility. The transplanted cells migrated and produced adrenergic neurons even though, before transplantation, they were beyond the stage when migration occurs. The chick embryos were in an earlier stage of development than the quail cell donors. But, because individual cells could not be followed, Le Douarin notes that the ganglion might have contained a small population of undifferentiated cells that performed these feats.

Just how long developing neurons retain their chemical plasticity is unclear, although it is probably not indefinitely. Cells taken from newborn rats during the first few days after birth still have the capacity to become cholinergic in culture. But this capacity is greatly reduced in nerve cells taken from 3-week-old rats, according to Bunge and Johnson. The researchers speculate that some signal causes the neurons to become fixed in the adrenergic state.

A large part of investigators' efforts is now directed toward identifying the signals in the embryonic environment that influence neurotransmitter choice and the other aspects of nerve development. Materials produced by the target organs and the tissues through which the immature nerve cells are migrating may be important. The work with cultured rat gan-

glion cells has shown that adrenergic cells do not have to be incubated with nonneuronal tissue in order to become cholinergic, provided the incubation medium has previously been in contact with the tissue. This result suggests that the tissue secretes a "factor" into the medium that induces the change. According to the Harvard group, the factor is a protein that they are now trying to isolate and characterize. They know, however, that it is not nerve growth factor (NGF), a protein that has long been known to be necessary for the normal development and maintenance of the peripheral sympathetic nervous system.

Moreover, there appears to be a need for some way to prevent adrenergic neurons from becoming cholinergic in the living animal. Most of the tissues that secrete the factor that elicits acetylcholine production in cultured nerve cells are innervated by cholinergic nerves. Thus, the factor might help the tissues to establish appropriate neural connections. Glial cells also produce it, however, and they are closely associated with many types of nerve cells, including adrenergic ones. If adrenergic neurons are exposed to cholinergic signals in vivo, then something must prevent them from converting to acetylcholine production.

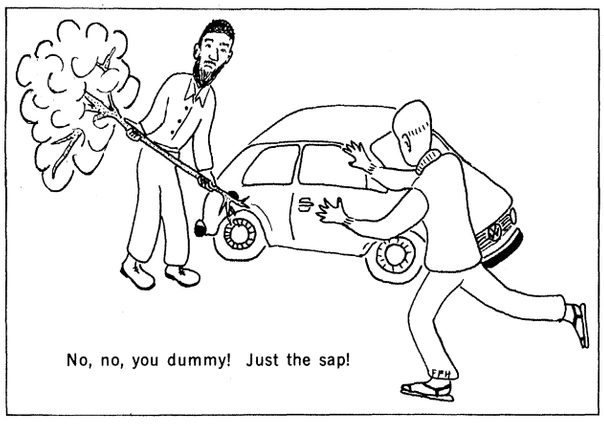
Cultured adrenergic nerve cells do not receive the normal innervation that they would have in the living animal. This lack of stimulation may underlie their ability to develop cholinergic characteristics. The Harvard workers have shown that stimulating cultured neurons electrically causes them to become resistant to the cholinergic signal and retain their initial adrenergic character.

In earlier work, Black and his colleagues demonstrated that stimulation by the first (cholinergic) nerves in a sympa-

Speaking of Science

Unlike Money, Diesel Fuel Grows on Trees

From the man and the country that brought us the petroleum plant comes now the diesel tree. Three years ago, Nobel laureate Melvin Calvin of the University of California at Berkeley returned from Brazil and reported that he had located several members of the genus *Euphorbia* which produce significant quantities of a milk-like emulsion of hydrocarbons in water (*Science*, 1 October 1976, p. 46). Calvin has been to Brazil again, and told the American Chemical Society last month that he observed a tree in the jungle that produces virtually pure diesel fuel.



Natives of the forest have known about the species, *Cobaifera langsdorfii*, for a long time, Calvin says. They drill a 5-centimeter hole into the 1-meter thick trunk and put a bung into it. Every 6 months or so, they remove the bung and collect 15 to 20 liters of the hydrocarbon. Since there are few Rabbit diesels in the jungle, the natives use the hydrocarbon as an emollient and for other nonenergy-related purposes. But tests have shown, he says, that the liquid can be placed directly in the fuel tank of a diesel-powered car.

A cross section of the trunk shows that the hydrocarbons collect in thin capillaries that may extend the full 30-meter height of the tree. A hole drilled into the tree probably collects hydrocarbons from capillaries ruptured by the drilling, Calvin speculates, so that it may be possible to increase the yield by drilling additional holes. An acre of 100 mature trees might thus be able to produce 25 barrels of fuel per year. Unfortunately, in the United States the tree would probably grow only in southern Florida, but the Brazilian government has already established experimental plantations.

Calvin concedes that *Cobaifera* will probably never represent a significant source of diesel fuel for the U.S. It is of interest chiefly as an example of the great diversity of materials produced by plants. Calvin's primary interest remains *Euphorbia*. On his northern California ranch he has a stand of *Euphorbia lathyrus* that is currently producing the equivalent of 10 barrels of petroleum per acre. He argues forcefully, however, that the yield could be improved dramatically by breeding and genetic selection and cites in support of this contention the tenfold increase in the yield of rubber plants achieved during the 1950's and 1960's. Already, he says, a Japanese group is obtaining 15 barrels per acre from a plantation on Okinawa.

Even at 10 barrels per acre, Calvin projects a price of about $40 per barrel for the finished product, roughly twice the cost of crude oil. But that comparison is misleading, he insists. The hydrocarbons from *Euphorbia* are primarily a blend of C_{15} compounds (terpene trimers) that, when subjected to catalytic cracking, yield various products virtually identical to those obtained by cracking naphtha, a high-quality petroleum fraction that is one of the principal raw materials of the chemical industry. Naphtha, he says, now costs $50 per barrel, a price that makes oil from *Euphorbia* competitive, even with the current yields.

The California chemist has spent 3 years promoting the potential of *Euphorbia*, and he thinks he has had some success. Several companies have approached him for advice and some, particularly mining companies, have begun conducting their own studies. *Euphorbia* requires relatively little water for cultivation and thus might prove a near-ideal species with which to reseed the surface after a strip mine has been closed down. What could be more appropriate, once the hydrocarbon resources have been removed from beneath the soil, than to begin growing new ones on the surface?—THOMAS H. MAUGH II

0036-8075/79/1026-0436$00.50/0

thetic pathway helps to regulate the development of the second (adrenergic) nerve cells in vivo. If the first nerves are cut, then the second nerves fail to mature normally. Conversely, if the second nerves are severed or destroyed, the preceding cholinergic nerves do not undergo normal maturation. Thus, the maturation of all the nerves in a sympathetic tract depends on their mutual interactions. Black says, "During development, information travels in both directions across the synapse."

Moreover, the Cornell workers find that the influences extend all the way from the brain and spinal cord to the target organs. If the central neurons connecting with the first sympathetic nerves are cut, the subsequent cells in the tract fail to develop normally. And if the target organ to be innervated by the pathway is removed, both the cholinergic and adrenergic nerve cells fail to develop properly. Black points out that these results have an important clinical implication: a neurological abnormality may be the result of a defect in development at some distant site in the nervous system.

The key to normal maturation in the forward direction of the sympathetic nerve pathway may be stimulation of the second neuron by the first. In the reverse direction, the means by which the target influences the development of the incoming nerve cells is unknown, although NGF would be a prime suspect on just about anyone's list. Black says his results suggest that NGF is probably not acting alone but that some other signal may also be involved.

Neurotransmitter choice is only one facet of neuronal development. Of equal interest are the ways in which neurons establish both the right kinds and the right numbers of connections.

Neuronal specificity, which is partly a question of what guides the developing nerve cells to contact the appropriate targets, has long interested—and baffled—neurobiologists. Currently, there are two schools of thought, which are not necessarily mutually exclusive, about what those signals might be. One is that long projections (axons) sent out by the neurons obtain directional clues, either mechanical or biochemical in nature, from the surfaces they contact as they grow toward their targets. The other is that the targets secrete chemicals, called tropic factors, that attract the axons. Evidence acquired by Rita Levi-Montalcini of the Laboratory of Cell Biology in Rome, Italy, suggests that NGF is a tropic factor guiding some sympathetic neurons to the targets they innervate. Other investigators have evidence for the existence of tropic factors for parasympathetic neurons. The role of these factors is still controversial, however, and more work will be needed before the basis for the guidance of nerve axons to their targets is clear.

Another aspect of specificity is the question of how a developing neuron, once it has come into the general vicinity of its destination, links up with one neuronal partner and not another. Again, because of its simplicity, the autonomic nervous system provides a useful model for addressing this problem. The Purves group, for example, is studying the pattern of innervation of individual adrenergic neurons in the superior cervical ganglion of the guinea pig.

In general, they find that each nerve cell is innervated predominantly by neurons originating in a single spinal cord segment; the input from neurons from adjacent spinal segments decreases with their distance from the dominant segment. The researchers are still trying to identify the signals that allow neuronal partners to recognize one another, but think that the positions occupied by two synapsing cells are among the influences determining specificity. Another possible contributor to specificity is that the potential partners bear chemical labels that allow them to recognize one another.

Neurons not only have to innervate the right targets, they also have to make the appropriate number of connections to those targets. Cell death—of as many as 50 percent of the original neurons—is a common feature of the development of nervous systems, and the autonomic nervous system is no exception, according to Lynn Landmesser of Yale University and Guillermo Pilar of the University of Connecticut. Their results also show that contact with the target is required for the survival of the nerve cells that do live.

This result implies that the targets may secrete some material needed to maintain the neurons. The identity of the hypothetical material for the parasympathetic nerve cells studied by Landmesser and Pilar is unknown, but there is a firm precedent for the existence of such a substance.

The adrenergic neurons of the sympathetic system depend on NGF for their maintenance, as shown by the pioneering studies of Levi-Montalcini and others, including Purves, Ian Hendry of the Australian National University in Canberra, and Hans Thoenen of the Max-Planck-Institut in Munich. The fact that NGF maintains the neurons does not necessarily mean that it guided them to their targets in the first place, however.

The cholinergic nerve cells of the parasympathetic system are not dependent on NGF in this way. One candidate for a cholinergic maintenance factor is a material currently being investigated by Darwin Berg of the University of California at San Diego. He and his colleagues have shown that skeletal muscle produces a substance that prevents the death of cholinergic neurons in culture. A similar substance is under investigation by Silvio Varon, also at San Diego. The two materials may or may not be the same.

Many nerve cells, including those of the autonomic system, send out several projections to a number of different target cells during development. Loss of some of the connections and concurrent formation of new synapses is another important way of establishing the final number of autonomic neuronal connections, according to Purves and Jeff Lichtman at the Washington University School of Medicine. These changes usually occur in such a way that the nerve cell terminals become concentrated on a smaller number of target cells. Lichtman and Purves hypothesize that this kind of synapse elimination is a developmental strategy to ensure that each target receives an adequate number of synaptic contacts and that each innervating nerve connects with no more target cells than it can handle. They speculate that competition for a maintenance factor produced by the targets, possibly a material similar to NGF, although not NGF itself, may aid in the rearrangement of a neuron's connections.

The development of even a simple nervous system such as the autonomic system is clearly a complicated business. Nevertheless, neurobiologists have made a good deal of progress in identifying the influences regulating autonomic development. They do not have all the answers, but they think they are on the right track.—JEAN L. MARX

"The important thing is that the research may shed light on a number of until now hopeless neurological problems."