Nerve Growth Factor Acts in Brain

Nerve growth factor may be necessary for the development and survival of brain neurons, including the tracts that degenerate in Alzheimer's and Huntington's diseases

CONVENTIONAL wisdom has generally held that nerve growth factor (NGF) does not act in the brain, but only on peripheral nerve cells. Conventional wisdom is sometimes wrong, however, and this seems to be one of those times. Over the past year or two, several converging lines of evidence have led neurobiologists to a new consensus that NGF is active in the brain. "It now appears that nerve growth factor is probably critical for normal brain development and probably for the maintenance of function, too," says Ira Black of Cornell University Medical College in New York City.

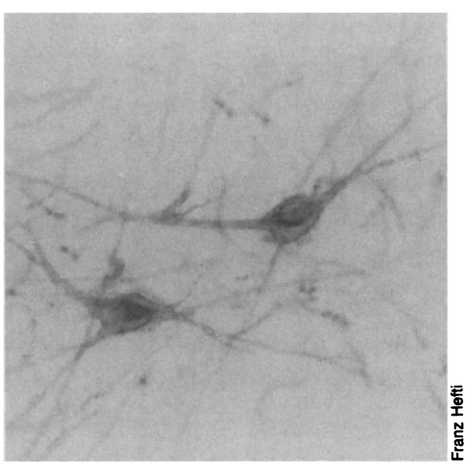
Moreover, the research could lead to a better understanding of—and perhaps eventually therapies for—Alzheimer's and Huntington's diseases, two serious and currently incurable conditions that are caused by the progressive degeneration of brain neurons. The neuronal tracts affected by NGF include one that deteriorates in Alzheimer's disease and another that degenerates in Huntington's disease.

The findings raise the possibility that the nerve cells are lost either because the brain fails to make enough of the growth factor to maintain them or because they no longer respond to it normally. Even if aberrant NGF responses do not contribute directly to the etiology of the diseases, the growth factor may still have the potential for preventing or slowing the neuronal losses.

NGF, which is a protein with a molecular weight of 13,000, has a long history. It was originally discovered nearly 40 years ago by Rita Levi-Montalcini of Washington University School of Medicine. In the peripheral nervous system, it is needed for the development and maintenance of sympathetic nerve cells that use catecholamine neurotransmitters such as norepinephrine and dopamine. It also acts on some sensory neurons that make neuroactive peptides.

Researchers had thought, logically enough, that if the protein had effects in the brain, it would work on catecholamine-producing neurons there, too. Years of trying failed to produce any evidence that this was the case, however. As Black puts it, "For at least a quarter of a century, people jumped through hoops trying to find an effect in brain."

It now turns out that they were concentrating their efforts on the wrong types of nerve cells. More recent work has shown that in the brain NGF acts on cholinergic neurons, which use the neurotransmitter acetylcholine. For example, Franz Hefti, working first with Hans Thoenen at the Max Planck Institute for Psychiatry in Munich, Germany, and now at the University of Miami School of Medicine, has shown that injecting NGF into the brains of newborn rats results in increased concentrations of choline acetyltransferase, a key enzyme needed for synthesizing acetylcholine, in certain brain regions. Hefti and others have found that the protein has a similar effect on the neurons when they are grown in tissue culture.



Franz Hefti

NGF receptors in the human brain: *These neurons from the nucleus basalis have been stained with a monoclonal antibody to the NGF receptor.*

Choline acetyltransferase is a marker for cholinergic neurons. Whether NGF increases the concentration of the enzyme by stimulating the growth of those nerve cells in rat brain or by improving the functional capability of the cells already present is currently unclear. There are some indications that the protein can cause the cholinergic neurons to grow new projections in culture.

The regions of the rat brain in which Hefti observed the NGF effects include the septal area of the basal forebrain, the hippocampus, and the cortex. The brains of individuals who die of Alzheimer's disease show a loss of cholinergic neurons from comparable brain areas. The neurons in question have their cell bodies in the nuclei of the basal forebrain, which include the medial septum and nucleus basalis, and extend axonal projections to the hippocampus and the cortex, two areas that contribute to memory formation. The degeneration of the cholinergic tract may be a major contributor to the memory and other mental deficits of the patients.

Moreover, the cholinergic tract of the basal forebrain is not the only one affected by NGF. According to William Mobley of the University of California at San Francisco (UCSF), Michael Johnston of the University of Michigan School of Medicine in Ann Arbor, and their colleagues, the protein also increases choline acetyltransferase in the cholinergic neurons of the corpus striatum of the rat brain. The comparable region of the human brain deteriorates in Huntington's disease. Black has made a similar observation with neurons from the corpus striatum that are maintained in tissue culture.

Simply showing that injected NGF has effects on nerve cells in the brain does not necessarily prove that it has a normal role there. However, a great deal of additional evidence supports that hypothesis and shows that the behavior of the protein in the brain generally parallels its behavior in the peripheral nervous system, except for the differences in the responding neurons.

In the periphery, NGF is made by the target tissues of the nerve cells on which it acts. After release by the target cells, the protein binds to specific receptors on the neurons and is then transported backward up the axons to the nerve cell bodies, where it exerts its effects. Investigators, including Sigrun Korsching of the Thoenen group and Louis Reichardt of UCSF, have detected the messenger RNA for NGF primarily in the hippocampus and cortex, the target areas innervated by the basal cholinergic neurons. The finding indicates that the NGF

gene is active in the expected tissues. NGF itself can be detected in the septum and nucleus basalis as well as in the hippocampus and cortex.

Meanwhile, Martin Schwab of the Munich group has shown that NGF can be transported from its proposed site of synthesis in the hippocampus to the cholinergic cell bodies in the basal forebrain. And Eugene Johnson of Washington University School of Medicine has demonstrated that rat brains contain NGF receptors that are indistinguishable from those on peripheral nerve cells. Although the receptors are somewhat more concentrated in the medial septum than elsewhere, they are distributed throughout the brain, which may mean that additional neurons will turn out to be responsive to NGF. Hefti has also found NGF receptors in the human brain.

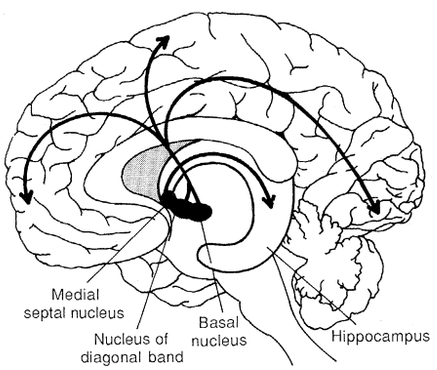
Taken together, these findings show that all the elements needed for an active NGF system in the brain are in place. "Nerve growth factor is made in the brain; the message is in the brain; we have shown there are receptors in the brain. It's hard to imagine that all this is going on for nothing," Johnson says.

NGF may be needed in the embryonic and newborn animal for the development of the cholinergic brain neurons and later in life for maintenance of the nerve cells. This, too, would be consistent with its role in the peripheral nervous system. One indication of a developmental role for the protein comes from Reichardt, who finds that an increase in the protein in appropriate brain regions precedes the increase in the cholinergic activity of the developing neurons.

In addition, preliminary results from Mobley and Uwe Otten of the University of Basel (Switzerland) indicate that antibodies to NGF inhibit the development of the cholinergic neurons of the basal forebrain. In these experiments the antibodies were injected into rat fetuses at day 15 of gestation, a time when the blood-brain barrier is relatively open, and the brains of the animals were examined 2 months later.

Other investigators have been unable to show that antibodies to NGF, when injected into the brains of older animals, counteract the effects of the protein. This may be because the antibodies are simply unable to reach the sites of NGF action under those conditions. Antibodies to NGF do inhibit its effects on cultured brain neurons.

Alternatively, the antibodies may have failed to work in the brain because the effects attributed to NGF are really caused by some contaminant in the preparations used for the experiments. This seems unlikely in view of the large body of evidence now favoring a brain role for the protein. Moreover, Mobley was skeptical about the early reports of NGF activity in the brain and thought that people might be seeing the effects of a contaminant that would disappear if the NGF were sufficiently purified. But that did not happen. "We kept trying to get NGF cleaner and cleaner," he says, "but the effect did not go away."



Cholinergic nerve tracts of the basal forebrain: *The neurons have their cell bodies in the nuclei of the basal forebrain (the medial septal nucleus, the nucleus of the diagonal band, and the basal nucleus) and send fibers to the cortex and hippocampus. Deterioration of the neurons may contribute to the dementia of Alzheimer's disease.* [*Redrawn with permission from* Scientific American, *January 1985, p. 73*]

Once the cholinergic neurons of the basal forebrain develop, they apparently require a continuing source of NGF to survive. As already mentioned, the protein is made in the hippocampus. If the nerve fibers running from the basal forebrain to the hippocampus are severed in rat brains, thereby interrupting the transport of NGF and other materials up the fibers, the nerve cells degenerate within about 2 weeks.

Several investigators, including Hefti, Fred Gage of the University of California at San Diego, and Lawrence Kromer of Georgetown University School of Medicine, have shown that infusion of NGF into the brain can largely prevent the cell destruction, especially of the cholinergic neurons. "You can save 90 to 100% of the cells from dying," says Gage, who is collaborating on the work with Anders Björklund of the University of Lund, Sweden, and Silvio Varon, who is also at San Diego.

The investigators are now trying to find out whether first stimulating the brain neurons by infusing NGF and then replacing the target tissue by grafting embryonic hippocampus into the cavity made by the lesion will lead to the long-term survival of the nerve cells without giving additional NGF. Preliminary results are promising, but Gage cautions that more work is needed to confirm them.

Rats in which the nerve tract from the basal forebrain to the hippocampus is severed provide a model for human Alzheimer's disease. The animals also suffer from deficits in learning and memory. NGF infusion into the brains of such rats may counteract their learning deficiencies, presumably by preventing the neuronal losses. For example, Hefti with Bruno Will of the University of Strasbourg (France), finds that infusing NGF into the brains of rats with the lesion improves their ability to learn a particular maze test. In addition, Gage and his colleagues have identified a subset of aged rats that are naturally impaired in their learning abilities. Grafts of basal forebrain that contain the cholinergic neurons improve the learning performance of the animals—as does infusion of NGF.

Although the potential implications of these animal studies for Alzheimer's disease are obvious, there is a major caveat. There is now no direct evidence for involvement of NGF in the genesis of the human disease. Attempting clinical trials with the protein in human patients would be premature at this time.

Investigators will no doubt look at NGF concentrations in the brains of Alzheimer's victims, however. Finding less NGF than normal might mean that too little is being produced, thus resulting in loss of the sensitive neurons; finding more than normal might mean that the brain is making large quantities in an attempt to compensate for an inability of the nerve cells to respond to the protein.

Of course, the deterioration of the cholinergic neurons in the brains of Alzheimer's patients may have nothing to do with aberrant NGF responses. The loss of the nerve cells might be secondary to the deterioration of neurons elsewhere in the brain or be caused by exposure to some toxic agent. Even if NGF is not involved, it may be possible to preserve the neurons by treating with NGF or perhaps with some as yet undiscovered drug that mimics the growth factor's activity in the brain. With an estimated 1.5 to 2 million Americans now suffering from Alzheimer's disease, it is a safe bet that the possible connection between the condition and NGF will be actively pursued. ■ **JEAN L. MARX**

ADDITIONAL READING

F. Hefti and W. J. Weiner, "Nerve growth factor and Alzheimer's disease," *Ann. Neurol.*, in press.

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