of influenza A or adenoviral origin. This may stem from the fact that these proteins have been extensively studied and are disproportionately represented in the computer library. Because of the lack of sequences available for vaccinia, measles, and swine flu proteins, the probability that the correct proteins have been sequenced is less than 10 percent. As more information becomes available on these viral proteins, we expect to find additional homologies with myelin proteins (*14*).

Our investigation suggests that spontaneous mutations in the continuously evolving viral proteins could result in fluctuations in the immunogenicity of the epitopes. This, in turn, would account for the variations in incidence of neural complications observed from year to year.

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14. Since the submission of this manuscript, R. S. Fujinami and M. B. A. Oldstone [*Fed. Proc.* **44**, 1921 (1985)] have reported the production of EAE in a rabbit sensitized with a synthetic octapeptide containing six residues (YGSLPQ) that are identical in DNA polymerase of hepatitis B and one of the three regions in BP known to induce encephalomyelitis in rabbits [R. Shapira, F. C-H. Chou, S. McKneally, E. Urban, R. F. Kibler, *Science* **173**, 736 (1971)].
15. The single letter code of abbreviations for amino acids is as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

16. We thank J. Brown and K. Walsh for the use of the computers and their programs, and R. S. Fujinami for helpful discussions. Supported in part also by a grant from the National Institutes of Health (AM07902).

28 March 1985; accepted 5 June 1985

Choline Acetyltransferase Activity in Striatum of Neonatal Rats Increased by Nerve Growth Factor

Abstract. *Some neurodegenerative disorders may be caused by abnormal synthesis or utilization of trophic molecules required to support neuronal survival. A test of this hypothesis requires that trophic agents specific for the affected neurons be identified. Cholinergic neurons in the corpus striatum of neonatal rats were found to respond to intracerebroventricular administration of nerve growth factor with prominent, dose-dependent, selective increases in choline acetyltransferase activity. Cholinergic neurons in the basal forebrain also respond to nerve growth factor in this way. These actions of nerve growth factor may indicate its involvement in the normal function of forebrain cholinergic neurons as well as in neurodegenerative disorders involving such cells.*

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Trophic factors are soluble molecules that influence the survival and differentiation of neurons in developing animals (*1, 2*). It has been suggested that abnormalities in the synthesis or utilization of trophic factors result in the dysfunction or death of specific neuronal populations which occurs in certain degenerative diseases of the central nervous system (CNS) (*1, 3*). A test of this hypothesis requires identification and characterization of trophic factors for the neurons of interest. Nerve growth factor (NGF) is a protein molecule whose neuronotrophic effects have been detailed in many investigations of the peripheral nervous system (PNS) (*4*). Recent studies of neonatal and adult rats showed that cholinergic neurons of the basal forebrain also respond to NGF (*5–8*). To determine whether a different population of forebrain cholinergic neurons, those of the corpus striatum, would also respond to a highly purified preparation of NGF, we measured the activity of choline acetyltransferase (ChAT), an enzyme that is

selectively localized in cholinergic neurons and that is involved in the synthesis of acetylcholine (*9*). Injections of NGF produced prominent, dose-dependent increases in ChAT activity in the corpus striatum of neonatal rats.

Sprague-Dawley rats were given NGF (30 μg, intracerebroventricularly) (*10–13*) on postnatal days 2, 4, 6, and 8 and were decapitated on day 12. Controls received the same dose of cytochrome c, a molecule physicochemically similar to NGF but lacking trophic activity, or the injection vehicle alone. The brains were quickly removed, dissected on ice (*14*), and analyzed for ChAT activity and protein content (*15, 16*). Also measured were the activities of tyrosine hydroxylase, a marker of peripheral sympathetic and central catecholaminergic neurons (*17*), and glutamate decarboxylase, a marker for neurons containing γ-aminobutyric acid (GABA), which are intrinsic to the corpus striatum and a number of other brain regions (*18*). NGF injections produced significant increases in ChAT activity in the corpus striatum, but did not alter the activity of tyrosine hydroxylase or glutamate decarboxylase (Fig. 1A). No change in ChAT activity was produced when NGF was injected peripherally (*19*) with the same dose and schedule used for the CNS injections.

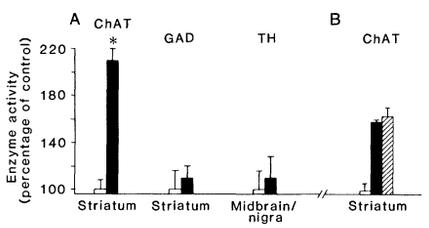

Fig. 1. (A) Effect of NGF treatment on the activity of neurotransmitter enzymes in the corpus striatum and ventral midbrain and substantia nigra of neonatal rats. Animals received 30 μg of NGF (closed bars) or the same dose of cytochrome c or injection vehicle alone (open bars) on postnatal days 2, 4, 6, and 8 and were decapitated on day 12. There was no significant difference in ChAT activity between controls that received cytochrome c and those that received vehicle alone. Preincubation of the ChAT assay mixture from control animals with 1 to 30 μg of NGF had no effect on enzyme activity. Values are means + standard errors. The asterisk indicates a significant difference ($P < 0.001$, Student's *t*-test). Control values for enzyme activity were as follows: ChAT, 105 ± 8 nmol/hour per milligram of protein; glutamate decarboxylase (GAD), 9.5 ± 1.4 nmol/hour; and tyrosine hydroxylase (TH), 1997 ± 290 pmol/hour. (B) ChAT activity in the corpus striatum of neonatal rats injected with different NGF preparations. Animals were injected with 10 μg of routinely prepared NGF (closed bar) or with NGF further purified by electrofocusing and gel filtration (shaded bar) in the schedule used in (A). (*11*). Enzyme activity was determined after decapitation on day 12. Results for both NGF preparations were significantly different from the control value ($P < 0.05$, Student's *t*-test).

Most procedures for isolating mouse NGF yield preparations contaminated with renin (*20*), γ-globulin (*21*), and possibly other molecules that may produce significant physiological and behavioral effects. For example, after intracranial injections of one of these NGF preparations, the renin contaminant elicited polydipsia, increased appetite for sodium, and increased brain ornithine decarboxylase activity through production of angiotensin II (*22*). Although very little renin activity was present in our NGF samples (*11*), we submitted NGF to two further chromatographic steps: preparative electrofocusing followed by gel filtration (*11*). NGF prepared in this manner was devoid of renin activity and, as shown by polyacrylamide gel analysis, was several percent purer than the starting material, with no change in bioactivity in vitro (*11*). This isolation scheme ensures that the activity must reside in molecules that are very similar if not identical to NGF. The preparation was compared to the starting material for its effects on ChAT activity in the corpus striatum. The same schedule and route were used to deliver repeated 10-μg doses. Virtually identical increases in ChAT activity were detected (Fig. 1B).

The dose-response relation between NGF and ChAT activity is shown in Fig. 2. Even small NGF doses (1 to 3 μg) produced increases in ChAT activity; more robust effects were seen at higher doses. The dose-dependent response of cholinergic neurons to NGF suggests that the effect was receptor-mediated. Further studies will be required to identify and characterize NGF receptors on striatal cholinergic neurons and to determine the mechanism responsible for the effect on ChAT activity.

Several observations suggest that endogenous NGF plays a physiological role in basal forebrain and striatal cholinergic neurons. Significant levels of messenger RNA encoding NGF (*23*) and of material that reacts with antibodies to NGF (*24*) have been found in the brain. Trophic material reactive with NGF antibodies is also present in cultures of striatal astrocytes (*25*). In addition, NGF receptors have been detected in the CNS (*26*), and their level appears to be developmentally regulated in the chick forebrain (*27*). Our ability to demonstrate prominent changes in a specific marker for cholinergic neurons, but not in markers for catecholaminergic or GABA-containing cells, is in keeping with the results of prior neurochemical studies of the effect of NGF on forebrain neurons (*5, 28*). These findings complement morphological studies showing that NGF is selectively transported retrogradely to neurons of the basal forebrain cholinergic complex (*29*), and they suggest that, in the CNS, endogenous NGF and its receptor may selectively mediate events in cholinergic neurons. The strongest evidence for the importance of endogenous NGF to peripheral neurons has come from experiments in which antibodies to NGF were administered to developing animals. Antibody-mediated sequestration of NGF produced destruction of sensory and sympathetic neurons (*4, 30*). Before concluding that endogenous NGF does play a role in the function of CNS cholinergic neurons, it will be necessary to demonstrate that the viability or function of these neurons is affected by intracerebral injection of NGF antibodies.

We find it interesting that developing cholinergic neurons of both the basal forebrain and the striatum responded to NGF injections, for, although they use the same neurotransmitter, these neuronal populations differ in several ways. Cholinergic neurons in the striatum are large and have extensively arborized local axon collaterals. They appear to be local circuit neurons (*31*). By contrast, neurons of the basal forebrain project their axons over long distances. Those of the medial septal nucleus and vertical limb nucleus of the diagonal band send fibers to the hippocampus, while those of the substantia innominata and nucleus basalis project to the neocortex and amygdala (*32*). Striatal neurons that can be tentatively identified as cholinergic (*33*) are tonically active (three to six

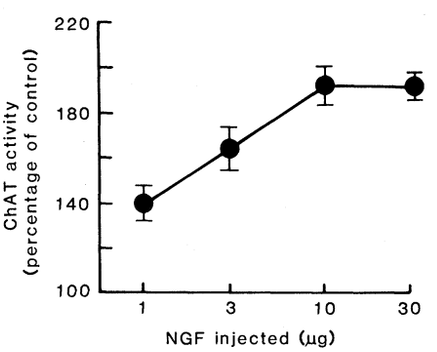

Fig. 2. Dose-response curve for NGF and ChAT activity in the corpus striatum of neonatal rats. The injection schedule and time of sacrifice are given in the legend to Fig. 1. Control injections were as described in the legend to Fig. 1A. Values are mean ± standard errors.

impulses per second) and seldom show any response to limb movements during performance of a task (*34*). On the other hand, neurons of the nucleus basalis have a higher tonic firing rate and show responses to a variety of task-related events (*35*). It is unclear whether specific features of CNS neurons can be used to predict whether they will respond to a particular neuronotrophic factor. The fact that developing cholinergic neurons of both the basal forebrain and striatum responded to NGF suggests that differences in morphology and physiology may not preclude a response to the same trophic agent. The same pattern was observed for the response of PNS neurons to NGF and to another trophic agent (*1, 2, 4*).

Our study did not address the response of mature striatal cholinergic neurons to NGF; however, cholinergic neurons of the basal forebrain do respond beyond the neonatal period (*5*). Interestingly, NGF produced much greater increases in ChAT activity in the septum of mature animals in which partial transection of the fimbria had produced axotomy of some septal fibers (*7*). It appears that the sensitivity of lesioned cholinergic neurons to NGF was, in part, restored to the level found in developing animals. It will be important to compare the responses of intact and lesioned mature striatal neurons to NGF.

Dysfunction or death of cholinergic neurons appears to be a key feature of several human neurodegenerative disorders, including Alzheimer's disease (*36*) and Huntington's chorea. In the latter disorder, involuntary choreiform movements are combined with changes in personality and mentation and later with progressive dementia (*37*). A hallmark of the disorder is neuronal loss in the caudate and putamen. Small neurons are primarily affected; however, large neurons are often shrunken or show degenerative changes, and in advanced lesions they may be lost (*37, 38*). Neurochemical studies have consistently demonstrated deficits in glutamic acid decarboxylase and ChAT activity (*39*). That the cholinergic deficit may be functionally significant was suggested by a clinical study in which chorea was improved by administration of physostigmine, a centrally acting anticholinesterase (*40*). There are, as yet, no data to indicate a role for NGF in Alzheimer's disease or Huntington's chorea. Nevertheless, the demonstration that the cholinergic neuronal groups affected in these disorders respond to NGF should stimulate a search for regional abnormalities in the synthesis or utilization of NGF or a similar factor. Moreover, if animal studies continue to show that lesioned central cholinergic neurons respond to NGF, this will suggest that administration of NGF may help to maintain the function of these populations in the neurodegenerative disorders that involve them.

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11. NGF was isolated from the submandibular glands of adult male Swiss-Webster mice by a modification of a described method (*10*). NGF was eluted from the second carboxymethyl cellulose column (CM-52, Whatman; column dimensions, 2.5 by 17 cm; flow rate, 60 ml/hour) with a gradient of sodium chloride prepared by mixing equal (150 ml) volumes of 50 m*M* tris-HCl (*p*H 9.0) with the same buffer containing 0.4*M* sodium chloride in a linear gradient mixer (Pharmacia GM-1). Purity was 91 to 94 percent by polyacrylamide isoelectric focusing (IEF) and 92 percent by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (*12*). It was fully active in the in vitro biological assay and contained 2 parts in 10^5 of the renin activity in gland homogenates (*12*). Some samples were submitted to preparative electrofocusing in a gradient of *p*H 7 to 10 and then to gel filtration by the method of T. L. Darling and E. M. Shooter [in *Cell Culture Methods for Molecular and Cellular Biology*, D. W. Barnes, D. A. Sirbasku, G. H. Sato, Eds. (Liss, New York, 1984), vol. 4, pp. 79–93]. This NGF preparation was at least 97.5 percent pure by IEF and at least 94 percent pure by SDS-PAGE, was fully active in the in vitro assay, and was devoid of renin activity.
12. NGF samples were submitted to IEF analysis by the procedure of L. A. Greene *et al.* [*Neurobiology* **1**, 37 (1971)], except that *N,N,N′,N′*-tetramethylethylenediamine (0.18 percent; Bio-Rad) and ammonium persulfate (0.03 percent; Bio-Rad) catalyzed polymerization. Ampholytes (*p*H 3.5 to 10; LKB Ampholines) were included at a concentration of 2 percent. The anode solution was 10 m*M* phosphoric acid and the cathode solution 20 m*M* sodium hydroxide. Electrophoretic runs were terminated when cytochrome c (Sigma) migrated to the bottom of the gel. Gels were fixed and rinsed over several days in 20 percent trichloroacetic acid (TCA), stained with 0.1 percent Naphthol Blue Black (Sigma) in 10 percent acetic acid, and destained in the same solvent. The procedure of U. K. Laemmli [*Nature (London)* **227**, 680 (1970)] was used for SDS-PAGE with 15 percent polyacrylamide slab gels. Fixation in 20 percent TCA preceded staining (*10*). Gels were scanned at 635 nm (Shimadzu CS 930 scanning densitometer). Peaks were quantified by cutting out and weighing the peaks plotted by the densitometer. Values were corrected by referring to standard curves of staining intensity for known amounts of added NGF. Purity values were adjusted to account for staining sensitivity. In vitro biological activity was measured in the dissociated chick dorsal root ganglion assay of R. J. Riopelle and J. C. Kennedy [*Can. J. Physiol. Pharmacol.* **60**, 707 (1982)]. Assays were read 24 to 72 hours after plating. For determination of renin activity, lyophilized samples of the gland homogenate or NGF were redissolved in 25 μl of 0.2 percent acetic acid. The volume was brought to 150 μl with additions such that final conditions for the assay were 0.1*M* sodium phosphate (*p*H 7.4), 1.0 m*M* EDTA, 1.6 m*M* dimercaprol, 2.2 m*M* 8-quinolinol sulfate, and 1.6 n*M* hog plasma renin substrate (Miles-Pentex). Angiotensin I (Sigma) was labeled with ^{125}I by a modification of the method of W. M. Hunter and F. C. Greenwood [*Nature (London)* **194**, 495 (1962)]. Angiotensin I generation was assayed at selected times by the method of E. Haber *et al.* [*J. Clin. Endocrinol. Metab.* **29**, 1349 (1969)]. The activity of homogenate under these conditions was 1014 nmol/hour per milligram of protein. Recovery of added angiotensin I approximated 100 percent.
13. The syringe needle (27 gauge; Hamilton) was placed approximately 2.0 mm anterior to the interaural line and 1.5 mm lateral to the sagittal suture and an intraventricular injection was made after direct transcutaneous puncture in restrained, unanesthetized pups. On each day trial animals from the same litter received 1 percent Blue Dextran (Sigma) in phosphate-buffered saline to confirm that injected material perfused the entire ventricular system. Lyophilized NGF was dissolved in 0.2 percent acetic acid and an equal volume of phosphate-buffered saline was added (final *p*H 7.0). Ten microliters was injected over approximately 20 seconds. Injections were well tolerated.
14. Brains were sagittally sectioned and dissected on a chilled glass plate. In addition to the corpus striatum (caudoputamen) the ventral one-third of the midbrain was dissected. The latter is herein referred to as ventral midbrain and substantia nigra.
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16. Pups receiving 30 μg of NGF, but not controls, demonstrated a reduction in the rate of increase in body weight and length. At 12 days of age, body weight was 75 to 85 percent of normal. No significant change was seen in brain weight or in brain protein per unit of tissue weight. Diminished growth rate was also apparent in animals injected with highly purified NGF. NGF-treated pups were normally active and demonstrated no grossly observable change in brain morphology.
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41. Supported in part by PHS grants NS00603, NS17642, and ES07094 and by a grant from the March of Dimes Birth Defects Foundation to M.V. J. We thank J. Holaday, J. Long, C. Kitt, D. Price, S. Mitchell, M. DeLong, and E. Shooter for many helpful discussions. O. Rogers and J. Hermann provided expert technical assistance. In conducting this research we adhered to the recommendations of the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

10 December 1984; accepted 22 April 1985

Memory Processing of Serial Lists by Pigeons, Monkeys, and People

Abstract. *List memory of pigeons, monkeys, and humans was tested with lists of four visual items (travel slides for animals and kaleidoscope patterns for humans). Retention interval increases for list-item memory revealed a consistent modification of the serial-position function shape: a monotonically increasing function at the shortest interval, a U-shaped function at intermediate intervals, and a monotonically decreasing function at the longest interval. The time course of these changes was fastest for pigeons, intermediate for monkeys, and slowest for humans.*

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The U-shaped serial-position function is a prominent benchmark of our understanding of memory processing. Typically recognition or recall memory is better for the first list items (primacy effect) and the last list items (recency effect) than it is for the middle items (*1*). The primacy effect has been traditionally thought to index long-term memory and the recency effect to index short-term memory. The form of the serial-position function along with the analyses of its primacy and recency effects has contributed to the support or demise of many theories of memory, from the early association network theories (*2*) to the more recent dual-process theories (*3*).

The importance of the serial-position function in testing theories of human memory processing makes it a natural choice for testing animal memory. Only recently have procedures been developed that allow researchers to test animal serial-position functions (*4*). Variables with proven effects on human serial-position functions can now be tested on animal serial-position functions to compare memory functions and cognitive processes and to examine the evolution of cognition.

We now report similar changes in the form of the serial-position function for pigeons ($n = 4$), monkeys ($n = 2$), and humans ($n = 6$) when retention interval was controlled. An immediate test revealed no primacy effect, but the effect emerged at intermediate tests to produce a U-shaped function, and the recency effect dissipated at the longest intervals. This qualitative similarity implies similar memory mechanisms.

The task for all three species was a serial-probe-recognition task. Trials were begun by pressing down a three-position T lever (monkeys and humans) or pecking on a 9 by 9.3 cm clear window (pigeons). Lists of color slides were rear-projected one at a time on the upper of two 12 by 9 cm screens separated 17 cm (center to center). Each of four memory items was displayed for 1 second (humans and monkeys) or 2 seconds (pigeons) with a 1-second interval between items. A probe item was projected on the lower screen after a delay (retention interval) from the last list item. If the probe item was a repeat of one of the list items ("same" trial), a correct response by humans or monkeys was a lever movement to the right and by pigeons a peck to a right disk (lighted red). Otherwise (on "different" trials) a left lever movement or a left disk (lighted green) peck was correct. Humans sat in a chair and held the lever box on their laps, monkeys were restrained in a primate chair, and pigeons worked in a Skinner box. Monkeys' correct responses were rewarded with a tone (500 Hz) plus a banana pellet or orange juice, pigeons with tone plus 2.8 seconds of mixed grain, and humans with tone only. Incorrect responses produced a lighted time-out period (5 seconds for humans and monkeys and 10 seconds for pigeons).

Test items for the pigeons (*Columba livia*) and monkeys (*Macaca mulatta*) were travel slides unique to that trial (limited to one trial per session) from a collection of 3000. Test items for the humans (two male and four female, 21 to 41 years old) were trial-unique kaleidoscope slides from a collection of 550. Kaleidoscope patterns prevented what would have been a performance ceiling effect with travel slides (*5*). Sessions were randomized sequences of ten "same" and ten "different" trials with the probe delay constant. Pigeons and monkeys were tested in four randomized blocks of six delays. Humans were tested in two randomized blocks of eight delays; the delays and sequence used at each delay were counterbalanced within and across human subjects. One sequence of particular items was used to test pigeons, two to test monkeys, and four to sixteen to test the humans.

The average serial-position functions are shown in Fig. 1. For each species, the 0-second delay functions show that memory for the first serial position was poor but progressively improved toward the end of the list. These serial-position functions markedly changed with probe delay; the primacy effect appeared, and by the middle two probe delays the serial-position functions had become U-shaped, showing primacy and recency effects for all three species. Further probe delay increases produced a progressive decline in memory toward the end of the list. These serial-position function changes were significant ($P < 0.03$) as tested by polynomial trend analyses (*6*).

These serial-position function changes are similar for all three species, but take place in about 10 seconds in pigeons, 30 seconds in monkeys, and 100 seconds in humans. This difference is important in the understanding of animal cognitive processes; the time scale for animals seems to be compressed relative to ours.

Human serial-position functions are typically obtained from recall (not recognition) tests. The retention interval is not precisely controlled (there is a free recall period), which probably accounts for subjects' showing only one of the effects described here: dissipation of the recency effect (*7*, *8*). First-item recall is "de-