delineation of a *mer* operator to a 26-bp region within the *mer* promoter, and indicate that the protein interacts with this operator in the presence and absence of the effector, mercuric ion. The purification protocol, which yields several milligrams of protein per gram of cells in two steps, may be applicable to other DNA binding proteins. The MerR protein–*mer* DNA system is now set for quantitative and high resolution probes of specific metal-protein and protein-DNA interactions.

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11. Single-letter abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; and X, any amino acid.
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Nerve Growth Factor Treatment After Brain Injury Prevents Neuronal Death

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Cholinergic neuronal degeneration after axotomy has been proposed to be due to the loss of a retrogradely transported neurotrophic factor, possibly nerve growth factor (NGF). To test this hypothesis, NGF was continuously infused into the lateral ventricles of adult rats that had received bilateral lesions of all cholinergic axons projecting from the medial septum to the dorsal hippocampus. After 2 weeks of NGF treatment, identification of cholinergic neurons by the presence of the biosynthetic enzyme choline acetyltransferase revealed a dramatic increase (350%) in the survival of the axotomized septal cholinergic neurons. Thus, NGF or an NGF-like molecule can act as a neurotrophic factor for these neurons.

NERVE GROWTH FACTOR (NGF) IS crucial for the normal development and maintenance of peripheral sympathetic and sensory ganglia (*1*). The administration of exogenous NGF seems to inhibit naturally occurring neuronal death (*2*) and to promote neuronal survival in these ganglia after axotomy (*3*). NGF may also have a physiological role in the mammalian central nervous system (CNS) as a trophic factor for basal forebrain cholinergic neurons. For example, these neurons have receptors for NGF (*4*) and exhibit selective uptake and retrograde transport of ^{125}I-labeled NGF injected into their CNS target regions, the hippocampus and neocortex (*5*), which contain significant concentrations of endogenous NGF and its messenger RNA that can be elevated in response to septal lesions (*6*). Long-term intraventricular injections of NGF elevate the biosynthetic enzyme for acetylcholine, choline acetyltransferase (CAT), in the septum and hippocampus of neonatal rodents but not of adult animals unless there is a partial lesion of the septo-hippocampal axonal pathways (*7*). NGF also seems to enhance the expression of cholinergic enzymes in explant cultures of embryonic telencephalic neurons, although it is still uncertain to what extent NGF can promote the survival of CNS cholinergic neurons in vitro (*8*). Since recent studies have begun to evaluate the role of NGF as a trophic molecule for forebrain cholinergic neurons after partial lesions of their axonal projections (*9*), the present experiments were conducted to determine whether the administration of exogenous NGF could prevent the degeneration of cholinergic neurons within the medial septum that normally occurs after complete lesions of the dorsal septo-hippocampal pathways (*10*). NGF was continuously infused into the lateral ventricle of adult rats for 2 weeks after complete bilateral transection of the supracallosal stria and fornix-fimbria, which contain all of the cholinergic septal afferents to the dorsal hippocampus. Immunocytochemical localization of CAT (*11*) was used to identify and quantitate the number of cholinergic neurons within the medial septum in normal, lesioned, and NGF-treated animals. The results demonstrate that administering exogenous NGF after CNS lesions can inhibit retrograde degeneration of axotomized septal cholinergic neurons in vivo.

Bilateral aspiration lesions of the supracallosal stria-cingulum bundle, dorsal fornix,

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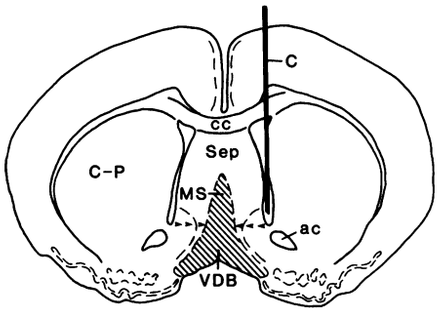

Fig. 1. Schematic diagram of a transverse section through the region of the septal nucleus in an adult rat illustrating: (i) the location of cholinergic neurons within the septal-diagonal band region (crossed-hatched area) that project to the hippocampal formation and (ii) the placement of the infusion cannula (C) into the right lateral ventricle. For these experiments either 2.5S NGF or cytochrome c, dissolved in sterile saline (250 ng/μl), was infused into the lateral ventricle at a constant rate (0.5 μl/hour) for a period of 14 days (total of 40 μg in 2 weeks). Cytochrome c was used as a control protein for these studies because it has a similar molecular weight and isoelectric point as NGF but has no neurotrophic activity. Since it is difficult to identify the boundary between the medial septum (MS) and the vertical limb of the diagonal band (VDB), an imaginary horizontal line (arrowheads) was drawn between the ventral extent of the lateral ventricles to separate the medial septal neurons from the diagonal band at the same location in all specimens used for cell counts. Only those CAT immunoreactive neurons located within the medial septum were included in the statistical analyses. Abbreviations: ac, anterior commissure; cc, corpus callosum; C-P, caudate-putamen; and Sep, septal region.

and fimbria were made in adult female Sprague-Dawley rats (n = 14) to completely transect all cholinergic axons in the dorsal septo-hippocampal pathways (*12*). Immediately after this surgery two groups of animals with lesions (n = 5 each) had a stereotaxically implanted cannula (connected to an Alzet minipump) positioned in the right lateral ventricle at the level of the anterior septum (Fig. 1). The remaining animals constituted a lesioned, but nontreated group. Half of the rats with cannulas received a constant infusion of 2.5S NGF for 14 days, and the remaining animals received cytochrome c under the same conditions. Three groups of unlesioned control animals were also prepared: one group received no treatment (n = 4), and the remaining two groups (n = 3 each) received either NGF or cytochrome c as above. All animals were allowed to survive for 2 weeks before being prepared for Nissl staining and for CAT immunocytochemistry to identify cholinergic neurons in the medial septum and vertical limb of the diagonal band (Fig. 1). At the termination of the NGF treatments the residual NGF remaining in the minipumps was pooled and assayed for biological activity as described (*13*).

Evaluation of the tissue sections stained for Nissl substance or CAT by immunocytochemistry (Fig. 2) indicated an obvious difference in the number and density of cholinergic (CAT-positive) and small-to-medium-sized (10 to 20 μm) noncholinergic neurons within the medial septum and vertical limb of the diagonal band in several of the experimental groups. In general, the number of CAT neurons within the medial septum and vertical limb of the diagonal band is similar in all intact animals (that is, NGF- and cytochrome c–treated animals and untreated controls) and the distribution of these cells seems comparable to that observed by others (*14*). This result suggests that the intraventricular infusion of NGF into normal unlesioned animals does not induce the expression of CAT in septal neurons that normally do not contain this protein. These observations are in agreement with studies indicating that intraventricular injections of NGF into normal adult rats produce only slight increases in CAT within the septum (*7*).

Although cholinergic neurons in both the medial septum and diagonal band undergo retrograde degeneration after fornix-fimbria transections, greatest cell death occurs within the medial septal region (*10, 12*). Thus, this region was selected to quantitate differences in the number of CAT-positive neurons (*15*) in the different experimental groups (Fig. 1 and Table 1). Cell counts from the three groups were evaluated with a group-by-side analysis of variance; both the group [$F(2,9) = 75.73$] and side [$F(1,9) = 36.26$] main effects were signifi-

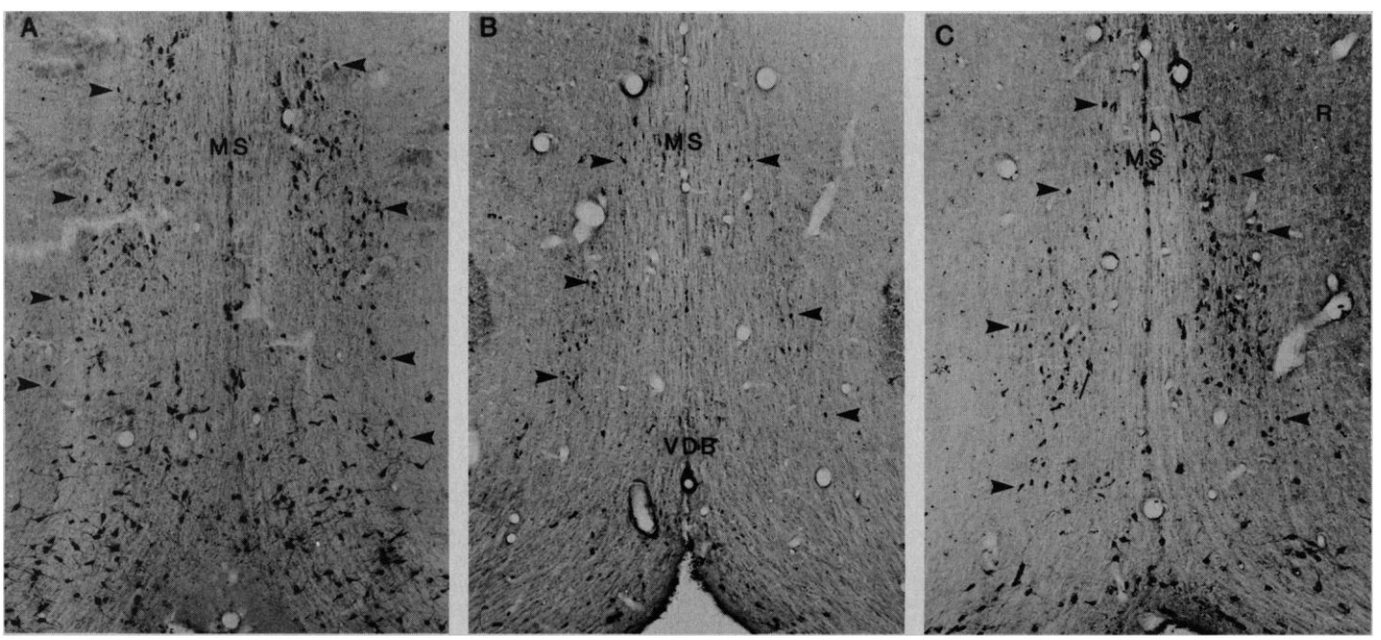

Fig. 2. Transverse sections through the region of the medial septum (MS) and vertical limb of the diagonal band (VDB) (about the same level as in Fig. 1) stained to visualize CAT immunoreactive neurons. (**A**) Normal distribution of CAT neurons (arrowheads) in a specimen with an intact fornix-fimbria. (**B**) Specimen with a bilateral fornix-fimbria transection and no NGF treatment. Very few CAT cells (arrowheads) are present within the medial septum. (**C**) Specimen with a bilateral fornix-fimbria lesion that received a continuous intraventricular infusion of NGF for 2 weeks after the lesion. There is extensive survival of CAT neurons (arrowheads) within the medial septum on both the left and the right (R) sides. CAT cells within the vertical limb of the diagonal band were not included in the analysis of cell survival (Table 1).

Table 1. Cholinergic neuronal survival in the medial septum after axotomy (means ± SEM). The control group indicates the number of CAT-positive medial septal neurons present in the entire nucleus and in its right and left counterparts. There is no significant difference in the number of CAT neurons present in control animals ($n = 4$) and unlesioned specimens treated with NGF. The lesioned group ($n = 4$) includes animals that received only bilateral fornix-fimbria lesions. No significant difference was observed between this group and animals with lesions plus cytochrome c treatment ($n = 4$). In both of these groups approximately 81% of the CAT-positive neurons within the medial septum underwent retrograde degeneration by 2 weeks after complete bilateral lesions of the dorsal septo-hippocampal pathways. This type of cell loss was also observed in adjacent sections stained for Nissl substance. In the group with lesions plus NGF there was a highly significant survival of cholinergic neurons with only about a 15% loss in total cell number.

Side	Control	Lesioned		Lesioned + NGF	
	Cells (n)	Cells (n)	Survival (%)	Cells (n)	Survival (%)
Right	1300 ± 101	245 ± 47*	18.8	1104 ± 23†	84.9
Left	1036 ± 77	190 ± 18*	18.3	868 ± 73†	83.8
Total	2336 ± 169	435 ± 64*	18.6	1971 ± 89†	84.4

*Significantly different from controls, $P < 0.01$. †Significantly different from animals with lesions alone, $P < 0.01$.

cant ($P < 0.001$). Comparisons between individual groups were made with the Newman-Keuls test (*15*). By 2 weeks after lesioning, the number of cholinergic neurons within the medial septum was significantly reduced (81%, $P < 0.01$) (Fig. 2) in animals that received complete bilateral lesions of the dorsal cholinergic septo-hippocampal pathways as compared with control animals, regardless of whether these animals also received an intraventricular infusion of cytochrome c (Table 1). In contrast, in animals that received the NGF treatment, the number of surviving CAT neurons within the medial septum (Fig. 2) increased significantly over those of the lesioned animals ($P < 0.01$). Since the animals had bilateral fornix-fimbria lesions, but only unilateral cannulas for the infusion of NGF, the number of surviving CAT neurons was evaluated both ipsilateral and contralateral to the site of NGF infusion. These data indicate extensive diffusion of NGF throughout the septal region since approximately the same percentage of CAT neurons were rescued from cell death ipsilateral and contralateral to the infusion site.

Although numerous investigators have attempted to determine whether NGF is a neurotrophic factor for neurons in the adult mammalian CNS, only recently has evidence accumulated that exogenous NGF may influence the survival of acetylcholinesterase-containing septal neurons after a partial lesion of the fornix-fimbria (*9*). Since it is difficult to quantify the survival of neurons in a partial lesion procedure, I made complete bilateral lesions of the dorsal septo-hippocampal pathways to transect all cholinergic axons projecting to the dorsal hippocampus before administering NGF. Furthermore, immunocytochemical staining for CAT, rather than staining for acetylcholinesterase, was used to identify true cholinergic neurons since the latter enzyme is also present in noncholinergic neurons within the CNS. This study was also designed to mimic clinical situations in which treatment with neurotrophic factors would most likely begin after an actual CNS injury when the sequence of events normally triggering retrograde neuronal death has already been initiated. Thus, results extend earlier studies of partial lesions (*9*) and demonstrate that a continuous intraventricular infusion of exogenous NGF begun immediately after axotomy can promote the survival of septal cholinergic neurons at least during the period of NGF treatment. This study also suggests that noncholinergic septal neurons survive in specimens receiving the NGF treatment. Since it is uncertain whether NGF receptors are present only on cholinergic septal neurons (*4*), the survival of the noncholinergic neurons may be due either to a secondary transneuronal effect related to the NGF-induced survival of the cholinergic cells or to a direct effect of NGF on these neurons.

The present study establishes that the continuous intraventricular administration of exogenous NGF can significantly reduce the retrograde neuronal death of septal cholinergic neurons that would normally result from axotomy. Moreover, it may be feasible to develop treatment procedure utilizing exogenous trophic factors to promote neuronal survival after a variety of CNS injuries.

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15. The intensity of the PAP staining often made it difficult to identify a nucleus within the CAT-positive cells. Thus, the number of cholinergic neurons within the medial septum was determined by counting all CAT-positive cells that contained an identifiable nucleus or a well-defined soma and proximal dendritic processes. These neurons were then localized to either the left or the right side of the septum. For consistency, all cells located directly on the midline were assigned to the right septum in every specimen. The cell counts were performed on every third section and were corrected to reflect the total number of CAT neurons present within the entire medial septum. The data were evaluated by a group-by-side analysis of variance with repeated measures on the second factor. Comparison of individual means was carried out with the Newman-Keuls test [B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1962), p. 310].
16. Preliminary data from this study were presented at the symposium "Cell and Tissue Transplantation into the Adult Brain," sponsored by the New York Academy of Sciences, New York, 2 to 4 April 1986.

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