

NERVE GROWTH FACTOR INDUCES A DOSE-DEPENDENT
AND LONG-LASTING INCREASE OF CHOLINE
ACETYLTRANSFERASE ACTIVITY IN THE SEPTAL AREA
AND HIPPOCAMPUS OF UNINJURED RATS

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Abstract. The effect of nerve growth factor (NGF) on the intact septohippocampal cholinergic system of adult rats was studied. Nerve growth factor was continuously infused at different doses (5-100 µg) for two weeks into the lateral ventricle of adult rats. Controls received intracerebroventricular infusion of equal amounts of cytochrome c. Nerve growth factor treatment was capable of inducing a dose-dependent increase of choline acetyltransferase activity (ChAT) in septal area and ventral hippocampus. In both areas, the NGF-induced rise of ChAT activity was sustained for at least one week after infusion, then it progressively declined towards control values. By three and five weeks, using NGF at 25 and 100 µg respectively, ChAT increase was still significant in both septum and ventral hippocampus. The present findings corroborate a role for NGF in adult septohippocampal cholinergic system and indicate that the "pharmacological" modulation of these neurons by NGF may last several weeks following withdrawal of this trophic factor.

Increasing body of evidence supports the view that nerve growth factor (NGF) plays a physiological role in the central nervous system (CNS). In this regard, several neuronal populations are now known to

be responsive to NGF including retinal ganglion cells (5, 24), striatal and basal forebrain cholinergic neurons (1, 8, 37). Investigations focused on the action of NGF in the cholinergic systems of the basal forebrain have indicated that (i) a correlation exists between cholinergic innervation in the forebrain and the distribution of endogenous NGF, its receptor and the mRNA encoding NGF and NGF receptor (4, 9, 19, 20, 23, 32, 40, 45, 46); (ii) basal forebrain cholinergic neurons of both neonatal and adult rats respond to exogenous NGF with a selective and prominent increase in choline acetyltransferase (ChAT) activity (7, 14, 18, 29), the enzyme involved in the synthesis of acetylcholine; (iii) injury to the septohippocampal cholinergic pathway, such as transection of the fimbria-fornix, results in changes in the levels of NGF, its mRNA and its receptor (13, 21, 30, 35, 41); (iv) exogenously administered NGF antagonizes the disappearance (and/or death) of axotomized septal cholinergic neurons (15, 22, 30) and (v) intracerebroventricular administration of anti-NGF antibodies in neonatal and adult animals produces specific alterations of forebrain cholinergic neurons (12, 39).

In addition, we recently showed that continuous infusion of NGF for two weeks (via miniosmotic Alzet pumps) in adult CNS, elevates ChAT activity not only in septal cholinergic neurons with a prior lesion but also in unlesioned animals, suggesting that NGF is required for the maintenance and function of mature forebrain cholinergic neurons (11). Furthermore, the effect elicited by NGF is dose-dependent. For example, NGF at 5 $\mu\text{g/pump}$ did not significantly increase ChAT activity in septal area. However in the same region NGF at 25 and 50 $\mu\text{g/pump}$ increased ChAT activity by 46% and 79%, respectively, when compared to control animals (Fig. 1). The increase of ChAT activity induced by 25 $\mu\text{g/pump}$ NGF was sustained for one week; thereafter it declined towards control values. By 3 weeks ChAT increase was less evident but still significant in the septum (Fig. 2). Similar results were obtained in the hippocampus where NGF at 25 and 50 $\mu\text{g/pump}$ was able to elicit a significant increase of ChAT activity (Fig. 1). Also in this area the effect observed using 25 μg NGF was sustained for one week after infusion and then it declined towards control values (Fig. 2).

To further investigate the role of NGF on uninjured septohippocampal cholinergic neurons in terms of dose-response effect and maximal duration of the effects on ChAT activity, we have extended our studies by utilizing an NGF dose higher than previously reported. In particular, young adult rats were treated for two weeks with 100 μg NGF and then sacrificed at 7 and 35 days following the termination of infusion. At both times, a significant effect of NGF on ChAT activity was observable in the septum and ventral hippocampus (Fig. 3). In both areas the effect

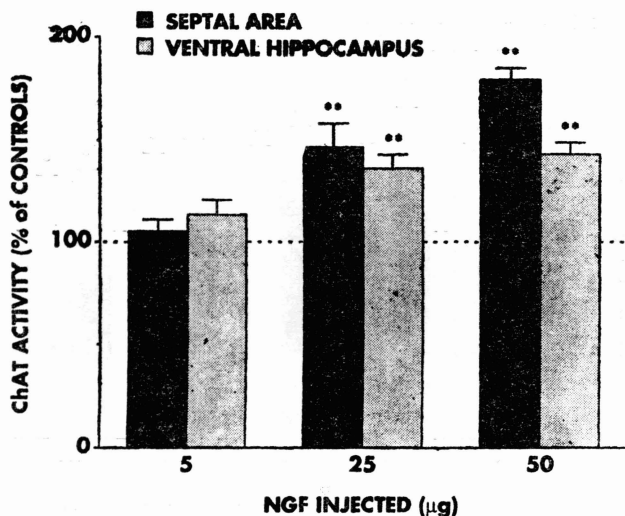


Fig. 1. Dose-related effect of NGF on ChAT activity in the septal area and ventral hippocampus of unlesioned animals. Mouse NGF (2.5 S) was purified from male mouse submaxillary gland according to the procedure of Bocchini and Angeletti (2). For experimental purposes, NGF was dissolved in phosphate-buffered artificial cerebrospinal fluid, with 0.01% bovine serum albumin as a carrier protein, as described by Williams et al. (44). Adult, male, Sprague-Dawley rats weighing about 220 g were used. Under sodium pentobarbital anesthesia (50 mg/kg, i.m.), a cannula was stereotaxically inserted in the lateral ventricle of the brain, permanently fixed, and connected to a loaded Alzet miniosmotic pump (model 2002, flow rate about 0.5 μ l/h, mean fill volume 200 μ l). Animals were continuously infused for 14 days with NGF at the indicated doses or equal amount of cytochrome c dissolved in the same infusion vehicle. At the end of the treatment, NGF remaining in the pumps (mean residual volume about 35 μ l) was still biologically active when assessed *in vitro* (34). Following 14 days of NGF infusion rats were sacrificed by cervical dislocation and brains rapidly removed. Hippocampi were dissected on ice and subdivided into three sections of equal size along their septotemporal axis; only the ventral portion of hippocampus (shown to be the most sensitive to NGF treatment, see Fusco et al. 11) was analyzed. In this study, the septal area refers to the medioventral part of a brain slice obtained by coronal section of the forebrain at the level of the most rostral portion of genu corpus callosum (landmark of the rostral section) and the optic chiasm (landmark of the caudal section). Brain regions were kept at -70°C until assay. Choline acetyltransferase activity was determined by previously described procedures with minor modifications (10, 36). The protein content was determined according to Markwell et al. (25). All measurements were made in triplicate and the data were evaluated by Student's *t*-test. Each value represents mean \pm SEM ($n = 4-8$). ** $p < 0.01$ vs. controls. (Data modified from Fusco et al. (11)).

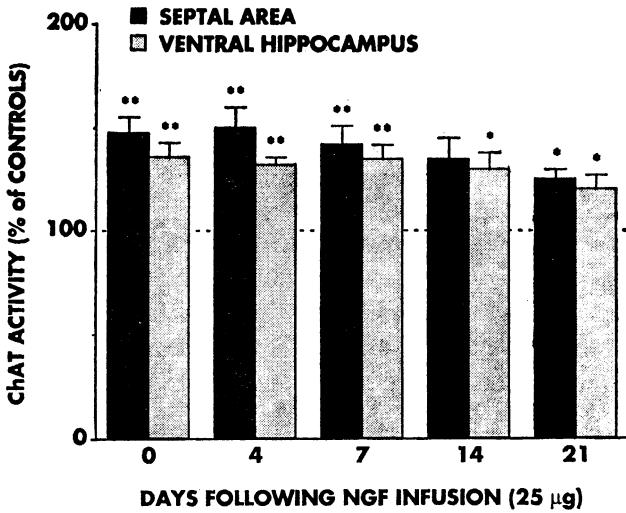


Fig. 2. Time-course of NGF effect on ChAT activity in septal area and ventral hippocampus of unlesioned rats. In this study NGF was administered at 25 µg/pump for two weeks. Minipumps were removed after termination of infusion and animals sacrificed at the indicated times. For other technical details see Fig. 1. Each value represents mean \pm SEM ($n = 4-5$). ** $p < 0.01$ vs. controls. (Data modified from Fusco et al. 11).

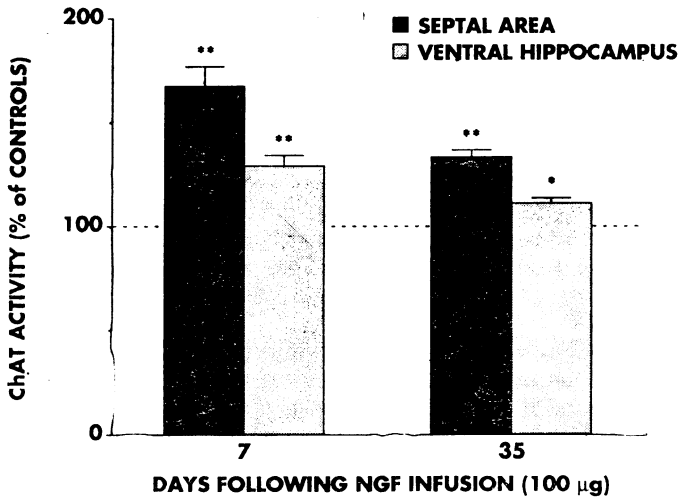


Fig. 3. Effect of NGF administration (continuous infusion, 100 µg over 2 weeks) on ChAT activity in the septal area and ventral hippocampus. Animals were sacrificed at 7 and 35 days after termination of infusion. For other technical details see Fig. 1. Each value represents mean \pm SEM ($n = 6$). * $p < 0.05$, ** $p < 0.01$ vs. controls.

was more prominent at 7 days after termination of treatment (+67% in the septum, +29% in the hippocampus). By 35 days, ChAT increase was less evident but still significant in both regions (+33% in the septum, +11% in the ventral hippocampus).

Data from different laboratories have clearly shown that NGF treatment increases ChAT activity in the forebrain of neonatal animals (6, 14, 18, 28, 29, 39). Similar results were observed in young adult rats following injury to the cholinergic pathways (16, 42), in the hippocampus of rats receiving transplants of foetal septal tissue (38) and in aged rats (43). These data have led to the hypothesis that neuronal injury and age may be relevant in activating NGF-related mechanisms regulating cholinergic metabolism. Our studies demonstrate that continuous infusion of NGF elicits a prominent, dose-dependent and long-lasting increase of ChAT activity in both the septum and the ventral hippocampus of adult uninjured rats. In agreement with previous observations (14, 18), these data confirm and extend the concept that a prior lesion is not necessary to trigger NGF-induced functional modifications of basal forebrain cholinergic neurons. In relation to the dose-response effect of NGF, it was observed that a dose of 100 μg did not further increase the effects on septal and hippocampal ChAT activity observed using NGF at 50 μg . This suggests that, using the protocol described above, the largest effect on ChAT activity can be observed in adult animals with NGF doses of about 50 μg . The dose-dependent and saturable effect observed with the NGF on ChAT activity strongly suggests that specific and thus saturable NGF receptors are likely to be involved. Concordantly, recent data indicate that high affinity NGF receptors are present in the basal forebrain and hippocampus (19, 32, 33). Noteworthy is that, in the basal forebrain, NGF itself can modulate at both transcriptional and translational levels expression of its own receptors (6). However, the possible causal and temporal interrelationship between the NGF-induced up-regulation of NGF receptors and the increase of ChAT activity has yet to be determined in detail.

No indication of the general mechanism of action of NGF can be inferred from these experiments. It should be stressed, however, that the elevation of ChAT activity in both septum and hippocampus is transient (see Fig. 2 and 3) and, more importantly, is not accompanied by a concomitant increase of acetylcholinesterase (AChE) activity (11). Since both ChAT and AChE are located in the cholinergic terminals and synapses (27), an increase in hippocampal ChAT without a concomitant elevation of AChE activity argues against a promotion of sprouting induced by NGF. As concerns specifically the NGF-induced increase of ChAT activity, one can hypothesize that events at both transcriptional

and translational levels may concur in producing such an effect. We have recently found that, in both neonatal and adult animals, the NGF-induced increase of ChAT enzymatic activity is associated with an enhancement of ChAT gene transcription (6). Consistent with this finding, an NGF-related increment of ChAT mRNA in adult septum has been recently reported using *in situ* hybridization procedures (17). Additionally, since post-translational mechanisms, such as phosphorylation, have recently been suggested to play a role in the physiological action of ChAT (3), the possible involvement of NGF in these processes remains to be ascertained.

In summary, our findings indicate that a marked and long-lasting increase of ChAT can be achieved in both septum and hippocampus of adult rats by continuous intracerebroventricular administration of NGF and, as such, corroborate a functional role for NGF in the control of adult basal forebrain cholinergic neurons. Since deficits of these neurons represent one of the most consistent neuropathological features in Alzheimer's disease, the potential therapeutical use of NGF in this disease has become a topic of discussion (26, 31). Furthermore, in relation to the multifaceted concerns that the clinical use of NGF will raise, our results can be viewed as an additional insight regarding aspects related with NGF pharmacokinetics after intracerebroventricular administration in mammals.

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