Exogenous NGF Affects Cholinergic Transmitter Function and Y-Maze Behavior in Aged Fischer 344 Male Rats

Lawrence R. Williams, R. Jane Rylett, Hylan C. Moises and Andrew H. Tang

ABSTRACT: Chronic ICV administration of NGF stimulates the activity of the cholinergic neuronal markers, HACU and ChAT, as well as the evoked release of both endogenous and newly synthesized acetylcholine in the brain of aging Fischer 344 male rats. However, the pattern of cholinergic phenotype stimulation indicates an age-related differential regulation of ChAT, HACU, and ACh release between specific brain areas, with the largest effects found in the striatum. NGF treatment also increases the effectiveness of neurotransmission between basal forebrain cholinergic neurons and postsynaptic amygdaloid target neurons. The stimulation of central cholinergic transmitter function after NGF treatment affects behavior in a Y-maze brightness discrimination paradigm. NGF treatment does not affect the cognitive measure of brightness discrimination, but reduces the number of avoidance attempts, a measure of motor function.

RÉSUMÉ: Le NGF exogène influence la fonction de transmission cholinergique et le comportement de rats Fischer 344 mâles âgés dans un labyrinthe en Y. L'administration chronique ICV de NGF stimule l'activité des marqueurs cholinergiques neuronaux, HACU et ChAT, ainsi que la libération évoquée d'acétylcholine endogène et nouvellement synthétisée dans le cerveau de rats Fischer 344 mâles agés. Cependant, le profil de la stimulation phénotypique de nature cholinergique indique qu'il existe une régulation différentielle, reliée à l'âge, de la libération de ChAT, de HACU et de ACh entre des régions spécifiques du cerveau, les effets les plus importants se retrouvant dans le striatum. Un traitement par le NGF augmente également l'efficacité de la transmission nerveuse entre les neurones cholinergiques de la base du prosencéphale et les neurones cibles amygdaloïdes postsynaptiques. La stimulation de la fonction de transmission cholinergique centrale, aprés traitement au NGF, influence le comportement dans le paradigme de discrimination lumineuse d'un labyrinthe en Y. Le traitement par le NGF n'influence pas la mesure cognitive de la discrimination lumineuse, mais diminue le nombre de tentatives d'évitement qui sont une mesure de la fonction motrice.

Can. J. Neurol. Sci. 1991; 18: 403-407

The neurotrophic protein, nerve growth factor (NGF), is well known for its survival promoting and transmitter metabolism stimulating properties for both peripheral and central neurons.[1] Of clinical interest is the role that NGF plays in the maintenance of the central cholinergic neurons in the basal forebrain and striatum. These neurons comprise three cholinergic systems: i) the projection neurons of the medial septum and diagonal band of Broca (MS/DB) to the hippocampus, ii) the projection neurons of the nucleus basalis magnocellularis (NBM) to the cerebral cortex and amygdala, and iii) the interneurons of the striatum (c.f., 2, 3). The neurons of all three systems are particularly affected by Alzheimer's disease where there is a pronounced loss in the levels of cortical and hippocampal acetylcholine (ACh), choline acetyltransferase (ChAT), and high affinity choline uptake (HACU).[4,5] In many Alzheimer's brains there is also evidence of substantial atrophy or death of cholinergic neurons in the MS/DB, NBM, and striatum.[4,6] Abnormalities in NGF metabolism have been hypothesized to underlie the cholinergic dystrophy associated with AD,[7] and the treatment of AD patients with NGF is currently being evaluated by the National Institute on Aging (NIA).[8] However, the neurotrophic role of NGF endogenous to the CNS is not well understood, and considerable work needs to be done to elucidate the effects of exogenous NGF on the transmitter function and behavior.

Using animal models, we are examining the age-related deficits in the central cholinergic systems, and the effects of exogenous NGF on cholinergic transmitter function. There is a differential age-related regulation of the cholinergic phenotypes expressed by the three major cholinergic neuronal systems, and their sensitivity to exogenous NGF. In aged rats, significant losses in ChAT activity are found in the MS/DB and striatum, but there is no apparent loss of enzyme activity in the NBM.[2] In

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the frontal cortex and hippocampus of aged rats, there is a substantial loss of HACU, whereas in the striatum no HACU deficit is observed.[5] Intracerebroventricular (ICV) infusion of exogenous NGF into aged rats significantly increases ChAT and HACU in the hippocampus, frontal cortex, and striatum, with the largest stimulations found in the striatum.[5] We have extended these initial neurochemical findings to examine the effects of NGF on ACh release, cholinergic electrophysiology, and Y-maze behavior.

Materials and Methods

ICV Cannula Implantation and NGF Solutions

Male Fischer 344 rats 4 to 24 months of age were obtained from the NIA colony at Harlan/Sprague-Dawley (Indianapolis, IN). Renin-free 2.5s NGF was purchased from Bioproducts for Science (Indianapolis, IN). Animals were anesthetized and implanted with a preformed cannula device, and infused with control and NGF solutions.[9] Animals were infused for two weeks using Model 2002 (0.5 µl/hr) Alzet osmotic pumps (Alza Corp., Palo Alto, CA) or four weeks using Model 2ML4 pumps (Y-maze) or paraffin-dipped[9] Model 2002 pumps (electrophysiology). The concentration of NGF was adjusted according to the particular pump flow rate so that the dose of NGF to the rat brain in all experiments was 1.2 µg/day, a dose found to maximally stimulate basal forebrain ChAT activity in axotomized young adult Sprague-Dawley female rats.[3] Control animals were infused with the vehicle, the albumin (1 mg/ml) serving as a non-specific protein.

Analysis of ACh Release

At the end of 2 weeks of ICV vehicle or NGF infusion rats were decapitated, and the brains were quickly removed and rinsed in ice-cold buffered 0.32 M sucrose. Frontal cortex, hippocampus and striatum were dissected from the right hemisphere and then either prepared as slices for the chemiluminescent measurement of release of endogenous ACh,[10] or as synaptosomes for the measurement of release of newly-synthesized ^{3}H-ACh following preloading with ^{3}H-choline.[11]

Electrophysiology

Conventional intracellular recordings were obtained from pyramidal cells in the basolateral amygdaloid (BLA) nucleus in vitro using 450 µm slice preparations of ventral forebrain containing the amygdala prepared from 4 month old rats.[12] Glass microelectrodes filled with 2 M potassium acetate and having resistances of 90-160 MΩ were used to impale single neurons identified as pyramidal cells by their characteristic discharge of a short burst of action potentials to a brief intracellular pulse of depolarizing current and by the presence of a prominent after hyperpolarization following a current evoked burst of spikes. To activate cholinergic afferents from basal forebrain to the BLA, 500 ms trains of stimuli were delivered via a stimulating electrode placed into the NBM, or by brief tetanic stimulation of the external capsule (EC). The ability of NBM or EC stimulation to elicit a slow muscarinic depolarization in BLA pyramidal neurons was assessed in slices from control (untreated and vehicle-treated) and NGF-treated animals.

Y-Maze Brightness Discrimination Behavior

The effect of vehicle or NGF treatment on the behavior of 24-month-old rats was tested using the three-arm symmetrical Y-maze.[13] A series of nine 25-trial avoidance sessions began on the fifteenth day following NGF or vehicle treatment. Each session consisted of 25 trials separated by 40-sec inter-trial interval periods. Two response measures were used to describe the behavior: i) *Percent Avoidance Attempts*, the percentage of trials in a session in which at least one alley entry was made before shock (regardless of whether the movement was to the correct or incorrect alley); and ii) *Percent Correct Discrimination*, the percent of trials where the first entry, whether an avoidance or escape attempt, was to the correct alley.

Results

NGF treatment stimulates release of both endogenous and newly synthesized ACh.

In 24-month-old rats, a substantial decrease in ChAT (31%) is measured in striatum, and HACU is decreased in frontal cortex (30%) and hippocampus (23%) compared to 4 month controls.[5] The age-related loss of striatal ChAT activity correlates with significant decrease (30%) in the evoked release of endogenous ACh in slices of striatum compared to 4 month controls (Figure 1). However, there was no decrease in the evoked release of newly synthesized ^{3}H-ACh from striatal synaptosomes. There also is no decrease in the release of ACh in aged frontal cortex or hippocampus by either method of measurement (Figure 1).

Treatment of aged rats with NGF for 2 weeks results in stimulations of ChAT activity in frontal cortex, hippocampus, and striatum, 30% compared to young controls. HACU activity in NGF-treated aged rats is increased in frontal cortex and striatum similar to ChAT, but there is no effect of NGF on HACU in the aged hippocampus.[5] The evoked release of endogenous ACh is increased only in the striatum of NGF-treated aged rats, 30% compared to control aged rats. However, the release of newly synthesized ACh is stimulated in all three brain regions after NGF infusion with the rank order of increase being striatum > frontal cortex > hippocampus (Figure 1).

NGF Increases Responsiveness of Amygdala Neurons to NBM Electrical Stimulation

The ability of NBM or EC stimulation to elicit a slow cholinergic depolarization was examined in 43 BLA pyramidal neurons in slices prepared from untreated (n=4) or vehicle-treated (n=4) 4 month old rats (Table 1). In the absence of eserine, depolarizing responses were recorded in 25% of the neurons after NBM stimulation and in 52% after activation of cholinergic fibres within the EC. In contrast, among the 33 neurons studied in slices from rats treated at least 3 weeks with NGF (n=8), 67% showed a cholinergic depolarization in response to NBM stimulation, and 80% responded to stimulation of the EC.

NGF Treatment Inhibits Y-Maze Avoidance Acquisition

After 15 days of treatment with vehicle or NGF at 1.2 µg/day, 24 month old rats were tested in the Y-maze paradigm. The control group of animals quickly learned to avoid the shock

https://doi.org/10.1017/S0317167100032546 Published online by Cambridge University Press

by initiating more avoidance attempts (Figure 2) and by selecting the correct alley to enter (Figure 3). In the NGF-treated group, the avoidance movement was retarded in response to the light change (Figure 2). The number of avoidance responses did not increase until near the end of experiment when the control animals already reached an asymptotic level of performance. In spite of a poorer movement initiation, the NGF treatment had no effect on the acquisition of brightness discrimination (Figure 3).

Discussion

Infusion of NGF into the brain of rodents at a dose of 1.2 µg/day for two to four weeks has a profound stimulatory effect on the molecules regulating ACh synthesis, and the release of ACh from cholinergic nerve terminals. This stimulation of cholinergic transmitter metabolism correlates with the increased synaptic efficacy found in the NBM-amygdalopetal projection system after NGF treatment, and with inhibitory effects on animal behavior. The locomotor initiation necessary for shock avoidance response in the Y-maze was retarded while the acquisition of a brightness discrimination was unaffected.

The results indicate a differential age-related regulation of ChAT, HACU, and ACh release by the cholinergic neurons in the basal forebrain and striatum. In the frontal cortex and hippocampus of 24-month-old rats, there is a substantial loss of HACU but not ChAT, as compared to 4-month-old controls, whereas in the striatum there is a substantial loss of ChAT, but not HACU.[2,5] The decreases in the activities of these potentially rate limiting processes do not necessarily result in decreased presynaptic stores of ACh, as indicated by the measure of ACh evoked release. In fact, an age-related decrease in ACh release is only observed for endogenous ACh in the striatum (Figure 1). This decrease correlates with the significant loss of striatal ChAT activity.

NGF treatment stimulates ChAT and HACU in both young and aged Fischer male rat brain, particularly in the striatum and frontal cortex.[5] However, the stimulation of these regulatory molecules does not correlate necessarily with a stimulation of ACh release using the administration protocol used in the present experiments. A stimulation of endogenous ACh release is only

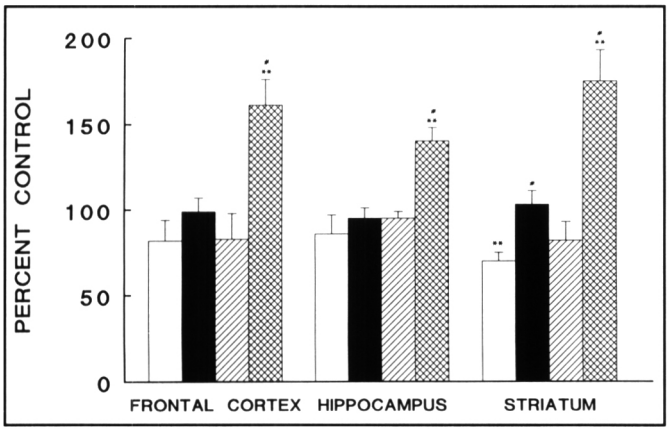

*Figure 1 — The Effect of NGF Treatment on ACh Release in Aged Rats. NGF was administered continuously into the right lateral ventricle of 24-month-old animals for two weeks, then both evoked release of endogenous ACh (E-ACh) from brain slices and evoked release of newly synthesized ACh (N-ACh) from synaptosomes were measured as described in results. Data are presented as the mean ± S.E.M. of percentage changes from the values measured in 4 month untreated animals for frontal cortex (E-ACh = 134 ± 12 pmol ACh/mg prot, N-ACh = 5235 ± 394 dpm/mg prot), hippocampus (E-ACh = 120 ± 19 pmol ACh/mg prot, N-ACh = 9060 ± 466 dpm/mg prot), and striatum (E-ACh = 245 ± 15 pmol ACh/mg prot, N-ACh = 7335 ± 468 dpm/mg prot). The number of rats in each group is indicated in parenthesis. Open bar - Endogenous release from 24 M Untreated rats n=4; Solid bar - Endogenous release from NGF-treated rats n=8; Diagonal bar - Newly synthesized release from untreated rats n=9; Hatched bar - Newly synthesized release from NGF-treated rats n=7. **p<0.01 compared to 4-month-old untreated animals; #p<0.01 compared to 24-month-old untreated animals.*

Table 1. NGF Treatment Increases the Percentage of BLA Pyramidal Neurons Showing Responses to Cholinergic Pathway Stimulation

	NBM Stimulation	EC Stimulation
Untreated and Vehicle-Treated	5/20 neurons 25%	12/23 neurons 52%
NGF-Treated	12/18 neurons 67%	12/15 neurons 80%

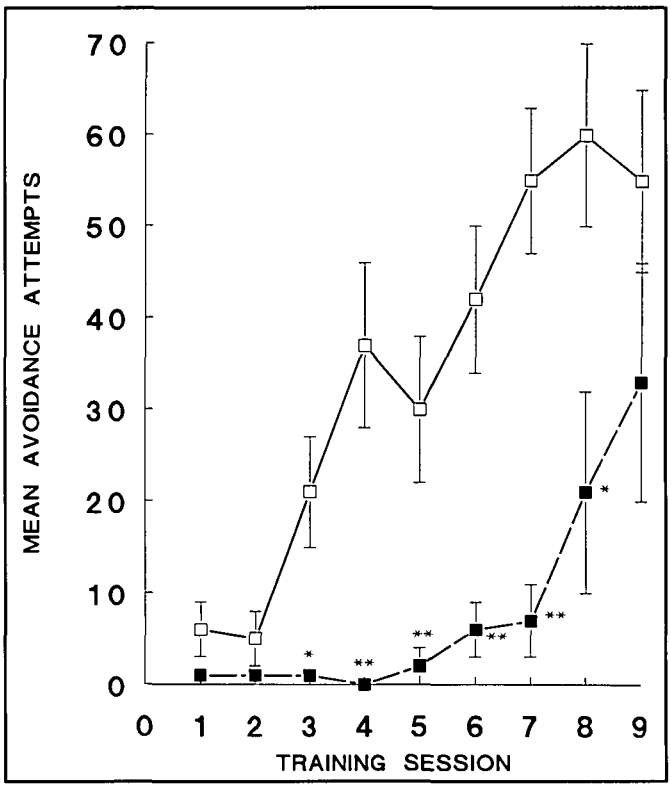

*Figure 2 — The Effect of NGF on Y-Maze Avoidance Acquisition in Aged Rats. Aged rats were treated ICV for 15 days with vehicle or NGF and then were tested in the Y-maze discrimination task on the next 9 consecutive working days. Data are presented as the mean ± S.E.M. number of avoidance attempts for each session. The values for vehicle-treated animals are shown in open squares and solid line, n=5. The values for NGF-treated animals are shown in closed squares and broken line, n=5. *p<0.05 compared to 24- month-old vehicle-treated rats; **p<0.01 compared to 4-month -old vehicle-treated animals.*

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found in the aged striatum. NGF treatment stimulates the release of newly synthesized ACh in all three brain areas. The level of stimulation correlates more with the stimulation of ChAT activity than of HACU.

The stimulation of cholinergic metabolism in the NBM projection system, as evidenced by the stimulation of newly synthesized ACh release in the frontal cortex, correlates with the increased synaptic efficacy found in the electrophysiologic experiments. After NGF treatment, there is a substantial increase (42%) in the number of amygdala pyramidal neurons responding to NBM electrical stimulation.

The stimulation of central cholinergic neurotransmission by NGF treatment might affect presumptive cholinergically-mediated animal behaviors such as memory and extrapyramidal motor behaviors. NGF treatment at 0.12 µg/day (7s NGF) was reported to ameliorate age-related impairments in the Morris spatial memory maze.[14] Similarly, treatment with 2.5s NGF at 0.3 µg/day improved spatial memory performance in rats with NBM lesions.[15] In the Y-maze experiments, the aged rats were treated with 2.5s NGF at 1.2 µg/day, the dose found to maximally stimulate ChAT activity.[3] At this dose, NGF had no effect on the presumed cognitive measure of brightness discrimination.

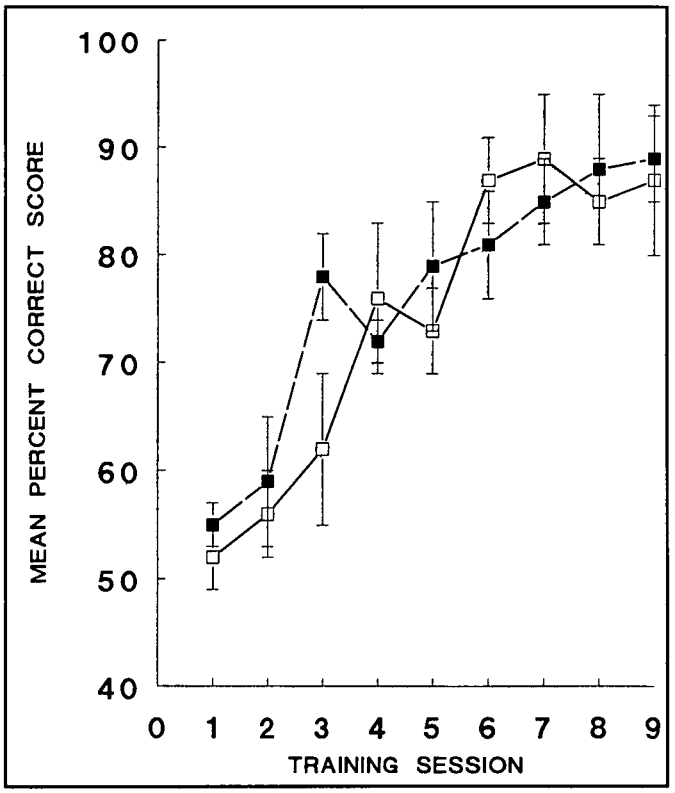

*Figure 3 — The Effect of NGF on Y-Maze Brightness Discrimination Acquisition in Aged Rats. Aged rats were treated ICV for 15 days with vehicle or NGF and then were tested in the Y-maze discrimination task on the next 9 consecutive working days. Data are presented as the mean ±S.E.M. percentage of correct choices for each session. The values for vehicle-treated animals are shown in open squares and solid line, n= 5. The values for NGF-treated animals are shown in closed squares and broken line, n=5. *p<0.05 compared to 24 month old vehicle-treated rats; **p<0.01 compared to 4 month old vehicle-treated animals.*

However, the NGF treatment significantly reduced the rate of acquisition of the locomotor avoidance response.

It is well recognized that cholinergic drugs affect the balance of extrapyramidal motor systems. In rats, scopolamine, the muscarinic antagonist causes an increase in avoidance responses or hyperactivity, while physostigmine, an acetylcholinesterase inhibitor which potentiates cholinergic transmission, results in a reduced number of avoidance responses or hypoactivity.[16] Thus, the effect of NGF treatment in reducing the number of avoidance responses in the Y-maze paradigm is very much in line with known cholinergic pharmacology. That is, NGF treatment probably stimulates striatal cholinergic interneuron synaptic efficacy resulting in an extrapyramidally-mediated hypoactivity in the Y-maze. The inability to demonstrate an enhancement in cognitive behavior after NGF treatment in the brightness discrimination task may be a limitation of the behavioral paradigm (e.g., the aged animals may not have been sufficiently impaired), or be related to the dose of NGF used in the experiment. A lower dose of NGF such as used in earlier experiments and a more cognitively demanding task (e.g. spatial memory) may shed further light on the functional significance of the cholinergic enhancement from NGF treatment. Cholinergic agonists can aggravate ataxia associated with AD.[17] Care must be taken if NGF is used to treat AD patients,[8] as consequential stimulation of cholinergic neurotransmission may exacerbate extrapyramidal symptoms.

References

1. Whittemore SR, Seiger Å. The expression, localization and functional significance of ß-nerve growth factor in the central nervous system. Brain Res Rev 1987; 12: 439-464.
2. Williams LR. Exogenous nerve growth factor stimulates choline acetyltransferase activity in aging Fischer 344 male rats. Neurobiol Aging 1990; in press.
3. Williams LR, Jodelis KS, Donald MR. Axotomy-dependent stimulation of choline acetyltransferase by exogenous mouse nerve growth factor in rat basal forebrain. Brain Res 1989; 498: 243-256.
4. Katzman R. Alzheimer's disease. N Eng J Med 1986; 314: 964-973.
5. Williams LR, Rylett RJ. Exogenous nerve growth factor increases the activity of high affinity choline uptake and choline acetyltransferase in the brain of Fischer 344 male rats. J Neurochem 1990; 55: 1042-1049.
6. Williams LR, Varon S, Peterson GM, et al. Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria-fornix transection. Proc Nat Acad Sci (USA) 1986; 83: 9231-9235.
7. Hefti F, Weiner WJ. Nerve growth factor and Alzheimer's disease. Ann Neurol 1986; 20: 275-281.
8. Phelps, CH, Gage FH, Growdon JH, et al. Potential use of nerve growth factor to treat Alzheimer's disease. Neurobiol Aging 1989; 10: 205-207.
9. Williams LR, Vahlsing HL, Lindamood T, et al. A small gauge cannula device for continuous infusion of exogenous agents into the brain. Exp Neurol 1987; 95: 743-754.
10. Israel M, Lesbats B. Application to mammalian tissues of the chemiluminescent method for detecting acetylcholine. J Neurochem 1982; 39: 248-250.
11. Meyer EM, Cooper JR. Correlations between Na^+-K^+ ATPase activity and acetylcholine release in rat cortical synaptosomes. J Neurochem 1981; 36: 467-475.

12. Washburn MS, Moises HC. Muscarinic responses of basolateral and lateral amygdaloid neurons recorded in vitro. Soc Neurosci Abstr 1989; 15: 525.
13. Tang AH, Franklin SR. Disruption of brightness discrimination in a shock avoidance task by phencyclidine and its antagonism in rats. J Pharmacol Exp Therap 1983; 225: 503-508.
14. Fischer W, Wictorin K, Björklund A, et al. Intracerebral infusion of Nerve Growth Factor ameliorates cholinergic neuron atrophy and spatial memory impairments in aged rats. Nature 1987; 329: 65-68.
15. Mandel RJ, Gage FH, Thal LJ. Spatial learning in rats: Correlation with cortical choline acetyltransferase and improvement with NGF following NBM damage. Exp Neurol 1989; 104: 208-217.
16. Anisman, H. Cholinergic mechanisms and alterations in behavioral suppression as factors producing time-dependent changes in avoidance performance. J Comp Physiol Psychol 1973; 83: 465-477.
17. Harbaugh RE, Reeder TM, Senter HJ, et al. Intracerebroventricular bethanechol chloride infusion in Alzheimer's disease. J Neurosurg 1989; 71: 481-486.

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