FEATURED ARTICLE

NGF-Induced Enhanced Acquisition of a Spatial Learning Task in Young and Old F344 Rats is Time Dependent

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The aim of the present study was to determine whether the beneficial effects of NGF on measures of learning and memory were long-lasting and persisted significantly beyond the NGF treatment interval. Accordingly, artificial CSF or ß-nerve growth factor (NGF) was infused via osmotic pumps for 2 weeks into the lateral ventricles of young (3-6-month-old) and old (22-26-month-old) Fischer 344 rats. Subjects were then trained one trial per day for 14 days to learn the spatial location of a hidden platform in a Morris water maze. Two experiments were conducted to assess the immediate and long-term effects of NGF. Training in Experiment 1 commenced at the end of the treatment period (i.e., no delay), while training in Experiment 2 was initiated, on average, 3 weeks following the end of the CSF/NGF period. Treatment with NGF lead to enhanced performance in both age groups in Experiment 1 but failed to affect performance in Experiment 2. It is therefore concluded that NGF was effective at facilitating performance of a spatial task in both young and old animals, but that such effects were time-dependent.

Key Terms: *Nerve Growth Factor (NGF), Morris Water Maze, Spatial Learning and Memory*

It is now well accepted that many age-related learning and memory impairments relate to deficiencies of cholinergic neurotransmission (Bartus, Dean, Goas, & Lippa, 1980; Fischer, Bjorklund, Chen, & Gage, 1991; Fischer, Chen, Gage, & Bjorklund, 1992; Fischer et al., 1987; Luine & Hearns, 1990; Stone, Altman, Berman, Caldwell, & Kilbey, 1989). Given its specificity for cholinergic neurons (Hefti, 1983; Phelps et al., 1989), many have suggested that nerve growth factor (NGF) may prove neurotherapeutic in attenuating age-related cognitive deficits (Korsching, Auburger, Heumann, Scott, & Thoenen, 1985; Rennert & Heinrich, 1986; Richardson, Verge Issa, & Riopelle, 1986; Seiler & Schwab, 1984; Taniuchi, Schweitzer, & Johnson, 1986; Woolf, Gould, & Butcher, 1989). Further strengthening NGF's potential in treating age-related cognitive disorders are results revealing a significant inverse correlation between impaired spatial memory and low levels of hippocampal NGF (Henriksson, Soderstrom, Gower, Ebendal, Winblad, & Mohammed, 1992), and the

presence of decreased levels of NGF within the hippocampus and decreased levels of NGF mRNA in the forebrain region (Larkfors et al., 1987).

Much data supportive of NGF's neurotherapeutic potential have already been reported in the literature. Results from neuroanatomical (Fischer et al., 1991; Fischer et al., 1987; Hefti, 1986; Kromer, 1987; Williams et al., 1986), neurochemical (Dekker, Gage, & Thal, 1992; Dekker, Langdon, Gage & Thal, 1991; Dekker & Thal, 1992; Haroutunian, Kanof, & Davis, 1986; Haroutunian, Kanof, & Davis, 1989; Hefti, Dravid, & Hartikka, 1984; Santucci, Kanof, & Haroutunian, 1993; Williams, 1991; Williams, Rylett, Moises, & Tang, 1991) and behavioral (Dekker et al., 1992; Fischer et al., 1991; Fischer et al., 1987; Mandel, Gage & Thal, 1989) experiments have all coalesced to provide a convincing line of evidence suggestive of NGF's ability to enhance cholinergic activity, rescue cholinergic cells from degeneration, and reverse age-related and lesion-induced memory impairments.

Despite the growing body of literature strengthening the link between NGF treatment, enhancement of cholinergic systems, and improvements in learning and memory in aging, a full

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characterization of NGF's temporal durability in enhancing cognitive performance still remains to be determined. Recently, Niewiadomska, Komorowski, & Baksalerska-Pazera (2002) have reported morphometric and densitometric evidence indicating that cholinergic neurons are indeed responsive to NGF therapy in aged rats but that such therapeutics effects were dependent on the continuous supply of NGF. In contrast, Frick, Price, Koliastos, & Markowska (1997) have reported persistent changes in spatial recent memory in aged rats up to four weeks after termination of NGF treatment. The present study sought to determine whether NGF's behavioral efficacy in old animals trained on a spatial learning task is long lasting, as has been reported by Frick et al. (1997). In addition, the present investigation also studied whether NGF might afford some performance improvement in non-neurologically compromised, non-cognitively impaired young animals trained on the same spatial learning task. We report here the results from two experiments revealing that two weeks of exogenous NGF treatment was sufficient to enhance acquisition of a spatial learning task in both young and old rats, but that this enhancement was transitory.

Method

Subjects

Seventeen young (3-4-month-old) and 15 old (22-23-month-old) male Fischer 344 (F344) rats (ages relative to the time of surgery), obtained from the NIA aging colony at Harlan Sprague Dawley (Indianapolis, IN), were used as subjects in Experiment 1. Experiment 2 employed 16 young (6-7-month-old) and 16 old (25-26-month-old) F344 rats from the same source. Subjects were housed in a self-contained temperature regulated stay-clean housing system in groups of two per polycarbonate cage (55.9 cm x 30.5 cm x 20.3 cm high) under a 12:12 hr light/dark cycle. Subjects had free access to Purina Rat Chow and water throughout the investigation. A minimum two-week acclimation period was imposed prior to using the animals. The IACUC of the Bronx Veteran Affairs Medical Center approved the use of the animals.

Surgery

All subjects were prepared with a permanently implanted 25 g cannula directed at the left lateral ventricle (AP from bregma: -0.8 mm, ML: +1.7 mm, DV from skull: -4.5 mm) under 30 mg/ml/kg (im) ketamine (KETALAR, Parke-Davis, Morris Plains, NJ) and 8 mg/ml/kg (ip) xylazine (ROMPUN, Mobay Corp., Shawnee, Kansas) anesthesia. During the same surgical session an Alzet mini-osmotic pump (model #2002 pump with a nominal 2-week pump duration) was positioned subcutaneously between the scapulas and connected to the implanted cannula with PE 60 tubing. In order to eliminate the additional surgical stress and risk associated with another dose of anesthetic, pumps were not removed after the 2-week flow period to determine whether their contents were emptied.

In Experiment 1, 8 young and 7 old animals received pumps filled with 6.1 μg of angiotensin-free ß-NGF (0.36 μg/day at a nominal flow-rate of 0.47 μl/h; Dr. R. W. Stach, U. of Michigan-Flint; Stach, Garian, & Olender, 1979), while 9 young and 8 old rats received pumps filled with artificial cerebrospinal fluid (CSF, the vehicle). During training, two rats in the old/CSF group and one rat in the old/NGF group died during training (on days 9 and 13, and on day 4, respectively), thus reducing the number of subjects that completed all 14 days of training in each of these groups to 6.

In the second experiment, 8 subjects in each age group received pumps filled with ß-NGF while 8 subjects in each age group received pumps filled with CSF. In this experiment, 3 rats from the old/CSF and 2 rats from the old/NGF conditions died on day 11 (n = 3 in the old/CSF group and n = 1 in the old/NGF group) and day 13 (n = 1 in the old/NGF group), thus reducing the number of subjects that completed all 14 days of training in these two groups to 5 and 6, respectively.

Aseptic surgery was performed using a Kopf stereotaxic instrument with the upper incisor bar set level with the intraaural line. ß-NGF was prepared fresh from a frozen stock solution just prior to surgery and contained 0.1 mg rat serum albumin (Sigma, St. Louis, MO) per ml of artificial CSF. Artificial CSF was mixed fresh from refrigerated stock solutions just prior to surgery.

Apparatus

A 91.4-cm-diameter and 63.5-cm-high circular pool, located in an isolated experimental room with black-white wall hangings and ceiling florescent lights, served as the Morris water maze. The circular wall of the pool was constructed of non-translucent white Plexiglas while the floor was a blue-colored plastic tarp lining. An adequate amount of Carnation Coffee-mate non-dairy creamer was added to the water to make it opaque. A round 17.8-cm-diameter platform positioned upon a 25.4-cm-high stand was immersed in the water 1.3 cm below the surface. Water was maintained at room temperature and was changed every 1 to 2 days.

Procedure

Animals were required to find the spatial location of a submerged hidden platform in a Morris water maze (Morris, 1981). Subjects were lowered into the water at the wall of the pool facing the center and were required to find the platform and remain on it for 15 s. All animals were placed in the pool at the same start position on every trial. In addition, the platform was located in the far right quadrant of the pool relative to the animal's start position on every trial. Animals were tested 1 trial per day for 14 days (5 consecutive days per week) and the escape latency (s) served as the dependent measure. Subjects were allotted 10 m on each trial to find the platform. If they did not find the platform they were placed upon it for 15 s and an escape latency of 600 s was recorded. Over the course of the two experiments rats failed to find the platform within the allotted 600 s on less than 1% of the trials (6 out of 859 trials). Testing in Experiment 1 commenced exactly 14 days after pump implantation surgery at the end of the CSF/NGF nominal flow period, while training in Experiment 2 was initiated approximately 3 weeks (mean = 19.7 days) following the end of the CSF/NGF nominal flow period. All data were analyzed with *Statistica v. 6.0* computer software (StatSoft, Tulsa, OK).

Results

Experiment 1

A 2 (AGE) x 2 (NGF) x 14 (DAY) mixed design ANOVA with repeated measures on the last variable was used to analyze escape latencies (see Figure 1). This analysis revealed a significant DAY effect [$F(13, 364) = 2.72, p < .01$] with latencies of all the animals generally decreasing over trials (days 1-14 means: 126.8, 104.8, 85.3, 96.1, 98.6, 75.7, 63.2, 82.2, 75.9, 54.5, 70.8, 63.2, 38.9, and 29.1 s). More relevant to the present experimental hypothesis was a significant main effect of NGF [$F(1, 28) = 3.86, p = .05$] with animals (young + old) treated with NGF (mean = 58.6 s) finding the platform faster than those treated with CSF (mean = 93.6 s). No other statistical effects were significant (all Fs < 1.15, all ps > .05).

To determine whether NGF-induced improvement was evident during early versus late trials, further examination of the data was performed with the latencies from the first and last 7 days of training analyzed with separate 2 (AGE) x 2 (NGF) x 7 (DAY) mixed design ANOVAs with repeated measures on the last variable. Results from these analyses revealed a significant main effect of NGF [$F(1, 28) = 4.49, p < .05$] over the first 7 days, with NGF-treated animals (young + old) exhibiting lower

escape latencies (mean CSF = 118.1 s; mean NGF = 67.8 s). A similar enhancement effect was not observed when the latencies from the last 7 days of training were analyzed ($p > .05$), although this was probably due to the fact that many of the animals were already performing with a high degree of proficiency. Finally, the ANOVA performed on data from the last 7 days of training revealed a significant main effect for the DAY variable [$F(6, 168) = 3.07, p < .01$] with latencies for all animals generally decreasing over trials (days 8-14 means: 82.2, 75.9, 54.5, 70.8, 63.2, 38.9, and 29.1 s).
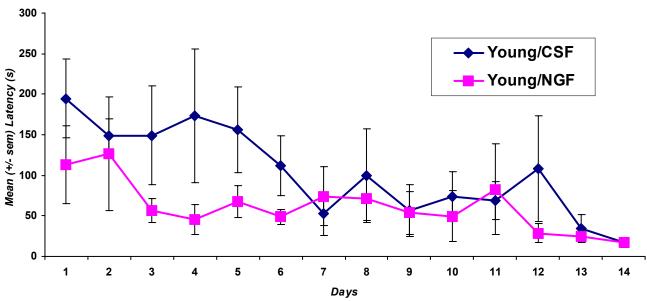
Experiment 2

Mean (±SEM) escape latencies to find the submerged platform for the four groups of subjects are presented in Figure 2. These data were analyzed with a 2 (AGE) x 2 (DRUG) x 14 (DAY) mixed design ANOVA with repeated measures on the last variable. As in Experiment 1, the findings from the present analysis revealed a significant main effect of DAYS with latencies of all the animals generally decreasing across trials [$F(13, 364) = , p < .01$] (days 1-14 means: 152.8, 116.6, 114.9, 105.5, 87.4, 60.6, 81.2, 65.3, 75.6, 92.3, 78.2, 74.9, 77.5, and 62.7 s). No other statistical effects were detected, including a failure of NGF to facilitate acquisition (all ps > .05), a result that contrasts with the findings from Experiment 1. Analysis of the data from the first and last 7-day block failed to reveal any statistically significant effects (all ps > .05) except for a main effect of DAYS over the first 7 days of training [$F(6, 168) = 3.17, p < .01$] (mean days 1-7: 152.8, 116.6, 114.9, 105.5, 87.4, 60.6, and 81.2 s).

Discussion

Results from Experiment 1 illustrate the ability of a 2-week NGF administration regimen to facilitate acquisition of a spatial place-learning task by young and aged rats. This finding extends the work of several investigations indicating NGF's effectiveness at enhancing spatial memory and increasing basal forebrain neural integrity in 24-month-old and 18-month-old female Sprague-Dawley rats (Fischer et al., 1991; Fischer et al., 1987), and facilitating acquisition of place learning in adult male F344 rats with cholinergic lesions of the nucleus basalis of Meynert (Dekker et al., 1992; Mandel et al., 1989). The NGF administration regimen in these four previously published studies, however, was at least 4-weeks in duration with behavioral assessment initiated during the middle of the treatment period. Therefore, the
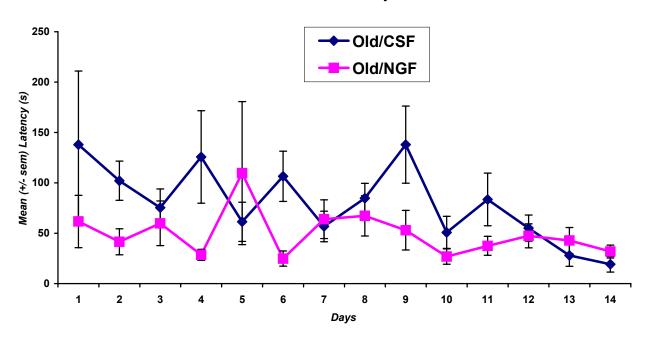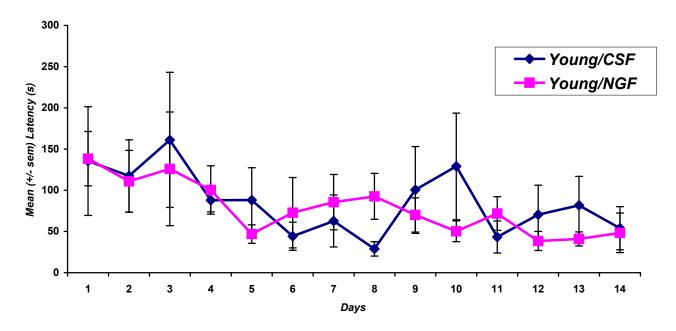
No Delay

No Delay

Figure 1. Effects of CSF and NGF on acquisition of a spatial learning task by young (Top Panel) and old (Bottom Panel) F344 rats in Experiment 1. Both young and old animals learned this task with equal proficiency in that their latencies generally decreased as a function of trials ($p < .01$) and did not differ from one another ($p > .05$). More importantly, overall, NGF treatment produced shorter escape latencies. This effect was observed over the full course of 14 days of training and over the course of the first 7 days of training ($ps \leq .05$).
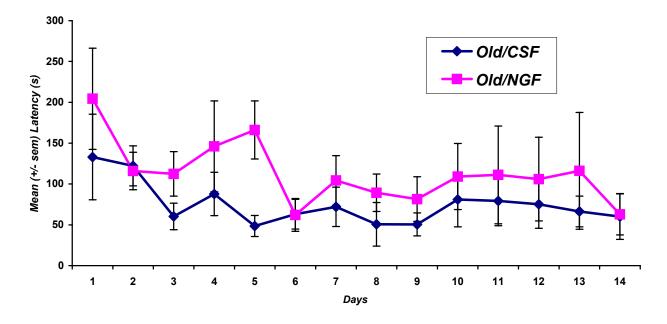
3-Week Delay

3-Week Delay

Figure 2. Effects of CSF and NGF on acquisition of a spatial learning task by young (Top Panel) and old (Bottom Panel) F344 rats in Experiment 2. No differences existed with respect to age or CSF/NGF condition. All animals generally learned the task equally well and exhibited significant decreases in escape latencies over the first 7 days of training ($p < .01$), and over the entire 14 days of training ($p = .01$).

importance of the present study is the demonstration that NGF administered for 2-weeks was sufficient to enhance task acquisition, but that this regimen was inadequate when a delay was imposed between the end of the treatment period and the start of behavioral assessment.

An alternative interpretation of the water maze data from Experiment 1 is that NGF decreased latencies simply by facilitating swimming motor ability. Such an interpretation was discounted, however, for several reasons. First, there exists no evidence in the literature suggesting hyperactivity following NGF administration, and, in fact, Williams et al. (1991) have reported hypoactivity in aged rats treated with NGF. Moreover, in the Frick et al. (1997) study in which NGF's effects on swim time were explicitly examined, no facilitation was reported. Finally, informal observations of CSF and NGF treated animals revealed no perceptible difference in swim rates, although admittedly such changes may be imperceptible to human observation.

The time-dependent nature of our findings is in contrast to the behavioral results reported by Frick et al. (1997). These authors found 40 μg of recombinant human NGF infused into the ventricles of 22-month-old rats for 4 weeks to be effective in producing improvements in spatial recent memory when subjects were assessed on a delayed-nonmatch-to-position task administered in a Morris water maze. This memory improvement effect was manifested not only during the last week of NGF treatment, but persisted for up to 4 weeks following discontinuation of treatment. Although the inconsistency between our results and that of Frick et al. (1997) may be due to important procedural differences such as the use of different forms of NGF and different assessment tasks, a more parsimonious reason would be the length and dose of NGF administration. It is fully possible that 4-weeks of NGF treatment at a dose more than six-times that used in the present study is necessary to yield long-lasting cognitive effects that extend beyond the point at which treatment is discontinued.

Although reasonable at face value, an interpretation that suggests that NGF's effects are simply dose-dependent may not fully account for all of the data reported in the literature. For example, Niewiadomska et al. (2002) found that a relatively high dose (100 μg) of 2.5S mouse NGF administered over 4 weeks produced improvements in cholinergic cell marker parameters, but such effects were dependent on the continuous supply of the protein. Additionally, we have reported that hippocampal, frontal cortical, and striatal cholinergic neurochemical findings derived from studying the brains of the animals used in the present investigation and described elsewhere in an earlier publication (Santucci, Kanof, & Haroutunian,

1995) were also time-dependent in that enhancement of cholinergic parameters were dependent on the time since NGF treatment. If, indeed, future research confirms the time-dependent nature of NGF's effects on the nervous system and on learning and memory, then NGF's clinical utility in producing long-term cognitive-enhancing effects would be severely limited.

The fact that no obvious age-related impairment was detected in the old animals studied here should be addressed. Although somewhat unusual not to find an age-related cognitive impairment (but see Fischer et al., 1987), it is very likely that our subjects simply were not sufficiently challenged with a difficult enough task (see Finger, 1978). For example, animals in the present investigation always started from the same location and were required to swim to an escape platform that was consistently placed in the same quadrant of a maze whose diameter was approximately 25% smaller than those Morris swimming pools used in other studies. These procedural details contrasts drastically with the requirements of other spatial tasks such as the radial arm maze wherein animals need to remember 8 to 12 spatially-distinct locations, or other "place" learning versions of the Morris water maze which employ four different start locations and a larger diameter pool (e.g., Dekker et al., 1992; Fischer et al., 1991; Fischer et al., 1987; Mandel et al., 1989). It would be of interest to determine whether "task difficulty" might modulate NGF's cognitive-enhancing effects when using a 2-week administration protocol in young and old animals trained on tasks of varying levels of difficulty.

In conclusion, the present findings contribute additional evidence to the literature supporting NGF's putative cognitive-enhancing effects. The important contribution of the present data, however, is to underscore the time-dependent nature of NGF that may severely limit this protein's clinical utility. Other studies are needed in order to determine the extent and degree to which these results generalize to other doses, administration durations, ages, and behavioral paradigms.

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