BEHAVIORAL EFFECTS OF NEUROTROPHIC FACTOR SUPPLEMENTATION IN AGING

Gregory M. Rose

Neuroscience Drug Discovery
Bristol-Myers Squibb Co.
5 Research Parkway
Wallingford, CT 06492
Phone: 203-677-6144
Fax: 203-677-7569
E-Mail: roseg@bms.com

ABSTRACT

Neurotrophic factors are now recognized to play important roles in the normal function of the mature central nervous system. This knowledge has motivated experiments to evaluate the potential benefits of administering neurotrophic factors to the aged brain. This article provides a review of studies to date that have determined the behavioral effects of such treatments. Nerve growth factor (NGF) administration appears to reliably enhance learning and memory in aged rats, while glial-derived neurotrophic factor (GDNF) causes some improvement in motor function. Problems associated with neurotrophic factor administration to humans are discussed.

KEY WORDS

Neurotrophin, Nerve Growth Factor, Glia-Derived Neurotrophic Factor, Cognition, Motor, Behavior, Aging, Therapeutic

INTRODUCTION

A number of small proteins, collectively termed neurotrophins or growth factors, play important roles in promoting cell survival and differentiation in the developing nervous system (reviewed in 1-3). Strictly speaking, neurotrophins are members of a family of structurally related molecules that includes nerve growth factor (NGF; 4), while growth factors are agents, such as fibroblast growth factor (FGF), that were first recognized as having effects in non-neural tissues. For the remainder of this article, neurotrophins and growth factors will be considered together under the more general term, "neurotrophic factors".

Many neurotrophic factors have been identified, and it is likely that many others remain to be discovered. Until fairly recently, it was thought that neurotrophic factors had no significant role in the adult brain. However, we now recognize that these molecules are necessary for select populations of CNS neurons to function normally, and perhaps even to survive (4-6). Levels of some neurotrophic factors or their receptors decline in the aged brain (7-10), and brain regions that are targets of these factors (e.g., basal forebrain cholinergic neurons in the case of NGF) show age-related changes that

include neuronal atrophy and loss (9, 11, 12). Since infusion of NGF into the brains of young adult animals appeared to reduce cholinergic neuron death after experimentally-induced damage (13-15), Fischer et al. (11) tested whether NGF administration might enhance cholinergic neuron survival and function in aged rats. In this experiment, treatment with NGF improved cholinergic neuron morphology, although it did not reduce age-related neuron loss. However, perhaps the most impressive consequence of NGF treatment was that age-related memory impairment was lessened. The purpose of this article is to provide a review of the studies that have examined the effects of neurotrophic factor administration in aged subjects at a behavioral level, and to discuss the potential promise of, and problems with, this approach as a therapy for age-related behavioral dysfunction in humans.

Domains of Behavioral Impairment in Aging

It is well known that advanced aging is accompanied by many changes in the central nervous system that can result in behavioral impairments. Aged individuals can suffer from dysfunction of sensory registration (e.g., impairments in vision, hearing, sense of smell, touch or taste), as well as reduced muscular strength and motor coordination. In addition, problems with information processing are observed, perhaps the most salient of which are impairments in learning and memory. The central targets of known neurotrophic factors include brain structures that are involved in cognitive or psychomotor function. Because of this, studies performed to date examining the effects of neurotrophic factor administration to aged animals have focused on these behaviors. By far the greatest number of such studies reporting positive results (improvement) in aged subjects have evaluated the effect of NGF upon learning and memory.

Effects of Neurotrophic Factors Upon Cognitive Function

Spatial Learning. The most frequently used behavioral paradigm for evaluating the effects of neurotrophic factor treatment upon learning and memory has been the Morris water maze (16). In this task, animals (usually rats or mice) are placed in a pool of tepid water and must swim to find a hidden escape platform. They initially find

the platform by chance. However, after 10-20 training trials, young adult animals can swim more-or-less directly to the platform from any starting point at the edge of the pool. They are able to do this by identifying the platform's location in the pool with reference to visual, auditory and olfactory cues that are present in the environment surrounding the pool. Put another way, the animals learn the "place" where the escape platform can be found. Place, or spatial, learning is a well-developed ability in rodents, and critically depends upon the hippocampus (17) as well as connected cortical structures (18-22).

Spatial learning ability declines in aged rodents (23-25), as it does in aged humans (26-30). It has been hypothesized that this problem is at least partially due to reduced function of cholinergic inputs from the basal forebrain to hippocampus and cortex (31). This hypothesis is supported by two lines of evidence. First, lesions of cholinergic basal forebrain structures impair spatial learning (32-36). [Note however, that the results of studies using neurotoxins to selectively destroy neurons that supply cholinergic inputs to only the hippocampus or the cortex, but not both, have produced mixed effects in behavioral studies; compare (37) and (38).] Second, the morphology of basal forebrain cholinergic neurons changes during senescence, with most studies reporting shrinkage of cell bodies and loss of immunoreactivity in processes (e.g., 9, 39). These observations led to the idea that providing NGF to basal forebrain cholinergic neurons might enhance their function, and thereby reduce spatial learning impairments in aged rats.

An experiment testing this hypothesis was first published by Fischer et al. (11). These authors reported that intracerebroventricular administration of NGF improved spatial memory retention in the water maze in aged (approximately 24 month old) female Sprague-Dawley rats. Cholinergic (acetylcholinesterase-positive) cell body sizes were increased in the nucleus basalis and in the striatum of NGF-treated animals, although the number of cholinergic neurons lost in the aged rats was not lessened by NGF treatment. Later studies by Fischer et al. extended these initial findings. Intracerebroventricularly applied NGF improved spatial memory retention in 18 month- and 24 month-old female Sprague-Dawley rats, but had minimal effect in 30-month old animals (40). Cholinergic neuron sizes, but not numbers, were increased in NGF-treated rats, with the nucleus basalis and the striatum being most responsive. Similar behavioral findings in the water maze task were reported by Markowska et al. (41), using 23-month old male Fischer 344 rats, and by Chen et al. (42), using 26-month old female Fischer 344 rats. More recently, it has been shown that direct intrahippocampal administration of NGF improved spatial learning in 26 month old male Long-Evans rats (43).

More recent studies examining the effects of NGF administration to aged rats have explored alternative strategies to deliver the molecule to basal forebrain neurons. Chen et al. (44) and Martinez-Serrano et al.

(45, 46) found an improvement in spatial memory following the grafting of cells that had been genetically modified to secrete NGF into the basal forebrain region. Placement of NGF-secreting cells in either the medial septal region (which projects to the hippocampus) or the nucleus basalis (which projects to neocortex) were both effective, there appearing to be no clear advantage to one region over the other, or to having NGF-secreting cells grafted to both locations. Lindner et al. (47) found that NGF-secreting cells that were encapsulated in semipermeable membranes and then implanted in the lateral ventricles of aged rats also improved spatial learning. Finally, Bäckman et al. (48) conjugated NGF to a transferrin receptor antibody to facilitate transport of NGF into the brain following peripheral intravenous administration. Improved performance of impaired aged rats in the water maze task was seen after five weeks of twice-weekly injections. Interestingly, systemic administration of the NGF conjugate affected the morphology of cholinergic neurons in the medial septal region, but not in the nucleus basalis.

Taken together, the results of these studies suggest that improvements in the function (to the extent that they are indicated by morphological measures) of either medial septal or basal nucleus cholinergic neurons are sufficient to improve spatial learning or memory in aged rats. Relatively few studies have observed an enhancement of learning per se (43, 45, 46, 48). Instead, NGF administration resulted in improved memory (i.e., retention of spatial information). It has been argued that this dichotomy is a function of the dose of NGF administered, with enhanced learning seen only following higher doses (45).

Thus, studies from a number of laboratories have demonstrated that administration of NGF to the brain of aged rats results in improvements in spatial learning or memory. This effect appears to show some dose-dependence (i.e., higher doses of NGF produce greater improvement; 41). Beneficial effects of NGF administration have been observed after only 1-1 ½ weeks in cases where NGF-secreting cells have been implanted directly into cholinergic nuclei (44, 46). However, when NGF was delivered intracerebroventricularly, at least two weeks of infusion have been necessary for significant behavioral improvement to be observed (11, 40, 49, 50). The magnitude of this behavioral improvement, while statistically significant compared to vehicle-treated aged animals, has not been sufficient to restore performance to the level of young adults. Finally, NGF administration has not improved age-related sensorimotor deficits (41, 47), suggesting some specificity for the "trophism" of this molecule.

Few studies of the effects of other neurotrophic factors upon learning and memory in aged rats have been performed, or at least described. Fischer et al. (49) found that, like NGF, NT-3 improved spatial learning when infused intracerebroventricularly, but that administration of an equivalent amount NT-4/5 produced only a marginal behavioral improvement, while BDNF was

not effective at all. This pattern of results was mirrored in the change in cholinergic neuron morphology produced by the respective agents: NGF and NT-3 increased cell body size, while BDNF and NT-4/5 had no effect. Pellemounter et al. (43) also found BDNF infusion to be ineffective in improving spatial learning in aged rats.

Working Memory. Working memory tasks require temporary mental manipulation of information. This information may be spatial (e.g., where you parked your car today) or factual (e.g., a telephone number just looked up). The important aspect of working memory is that the information has value for only a limited time, usually a period of minutes in studies designed for rodents. Aged rats have deficits in working memory (25, 51, 52), and chronic NGF administration has been shown to improve performance in several different working memory tasks. Working memory for spatial location is improved in aged male Fischer 344 rats in alternation tasks involving 2-choice "T"-mazes, either on land or in the water (41, 50). Lindner et al. (47) showed that performance in a repeated acquisition version of the water maze was also improved in aged male Fischer 344 rats that had received chronic NGF treatment at a dose that was either too low, or too briefly applied, to improve long-term spatial memory. While NGF administration did not affect performance of aged male Fisher 344 in a brightness discrimination maze (53), it did improve the ability of aged male Wistar rats to recognize the difference between newly presented objects and objects presented one hour previously (54).

Effects of Neurotrophic Factors On Motor Function

Improving sensory or motor function in aged subjects would be beneficial in its own right, as well as potentially providing the foundation for improvement in more complex behaviors such as cognition. However, there has not been nearly as much published experimental work to date that deals with this topic. Several studies have noted that motor-related parameters (such as swimming speed in the water maze) were not affected by treatment with NGF. In a more detailed study, Markowska et al. observed that intraventricular administration of NGF, at either of two doses that improved memory, had no effect upon the performance of aged male Fischer 344 rats in a battery of sensorimotor tests (41).

Thus, NGF does not appear to have a beneficial effect on aged sensory or motor systems. In contrast, another neurotrophin, glial cell line-derived neurotrophic factor (GDNF), which has been demonstrated to promote the survival of midbrain dopamine-containing neurons (55, 56), has also been shown to reduce motor deficits in aged rats. In these studies, behavioral effects have been detected after injections of GDNF into the substantia nigra (57), the neostriatum (58, 59) or the lateral ventricle (60) of aged animals. The results of GDNF administration were to enhance general locomotor activity (57, 60) as well as to improve performance in more

specific tests such as traversing an inclined balance beam (58) and bar pressing for food reward (59). However, GDNF administration did not improve muscle strength or performance in the rotarod or in a food pellet retrieval task (the staircase test; 59). Further, the ability of GDNF to improve motor performance appears to be age-related. Improvement in the balance beam test was observed in 16-17 month old Fischer 344 rats (58), but not in somewhat older (approximately 21 month old) animals (59).

Neurotrophic Factor Administration to Humans

The success of experimental studies in animals implies that neurotrophins might be effective for treating age-related nervous system dysfunction in human beings. Germane to this review, NGF has been suggested as a potential treatment for Alzheimer's disease (e.g., 61, 62) and GDNF has been proposed as a therapy for Parkinson's disease (e.g., 63). Given the fanfare accorded these new therapeutic approaches, published reports of their use in humans are remarkably scarce. Several papers have been published describing positive results of intracerebroventricular administration of NGF to a single patient with Alzheimer's disease (64-66), while, to my knowledge, no literature exists describing clinical experiences with GDNF. However, the paucity of written material is compensated for by a plethora of rumors that NGF and GDNF are ineffective in humans, or that their administration produces unacceptable side effects. Profound side effects have been observed in clinical trials with other neurotrophic factors (most notably, associated with the administration of ciliary neurotrophic factor, (CNTF) for the treatment of amyotrophic lateral sclerosis (67). In addition, muscle pain and hyperalgesia were reported by healthy human volunteers that received a single dose of either subcutaneously or intravenously administered NGF. In rodent studies, weight loss following intracerebroventricular administration of NGF (68), as well as both mechanical and thermal hyperalgesia following systemic administration of NGF (69, 70) have been reported.

Thus, a critical aspect of the design of human studies must be recognition that neurotrophic factors have effects other than upon the target of interest. For example, evidence is emerging that NGF plays an important role in immune and endocrine system regulation, as well as modulating the function of sensory neurons in the peripheral nervous system (71, 72). To avoid potential "nonspecific" effects of neurotrophic factor administration to the brain, delivery systems must be developed that provide very targeted dosing. Studies in rats indicate that intracerebroventricular administration does not meet this criterion (73). In addition, other experimental work indicates that while delivery of very low doses of neurotrophic factors (directly into the brain parenchyma) for long time periods might be an effective treatment strategy, administration of high doses for short periods probably is not. Injections of cells that have been genetically modified to manufacture and secrete neuro-

trophic factors (44, 46, 74-76) might provide an avenue to implement the latter approach.

Finally, it must be recognized that side effects are a possibility with virtually every pharmacologically-based therapy that is currently available. In some treatments, such as cancer chemotherapy, side effects are virtually inevitable and are quite severe. However, these side effects are considered acceptable because: 1) there is no alternative treatment, and 2) the benefit of treatment outlasts the period of drug delivery when side effects are present. It can currently be argued that, although it is possible to provide a certain degree of symptomatic relief, there are no effective treatments for age-related neuropathological diseases such as Alzheimer's or Parkinson's disease. It has also been reported that, at least for NGF, some beneficial effects were detectable as long as one year after treatment (66). If further work supports these initial observations, it would seem reasonable to consider neurotrophic factor treatment a viable clinical alternative, particularly if the side effects are treatable.

CONCLUSION

In summary, a great deal of experimental evidence indicates that the administration of neurotrophic factors to aged rodents has beneficial effects on certain types of behavior, particularly cognition. Preliminary studies in human beings suggest that the beneficial effects of neurotrophic factors are not limited to experimental animals. However, it will undoubtedly be many years before the therapeutic potential of these molecules can be thoroughly evaluated. Continued basic research into the effects of neurotrophic factors in aging is necessary to expand knowledge of potential targets, to refine dosing parameters, and to provide a complete understanding of how these agents achieve their effects.

REFERENCES

1. Maness, L. M., Kastin, A. J., Weber, J. T., Banks, W. A., Beckman, B. S., and Zadina, J. E.: The neurotrophins and their receptors: structure, function, and neuropathology. *Neurosci. Biobehav. Rev.* 18: 143-59, 1994.
2. Davies, A. M.: The role of neurotrophins during successive stages of sensory neuron development. *Prog. Growth Factor Res.* 5: 263-89, 1994.
3. Ip, N. Y., and Yancopoulos, G. D.: The neurotrophins and CNTF: two families of collaborative neurotrophic factors. *Annu. Rev. Neurosci.* 19: 491-515, 1996.
4. Cowen, T., and Gavazzi, I.: Plasticity in adult and ageing sympathetic neurons. *Prog. Neurobiol.* 54: 249-88, 1998.
5. Svendsen, C. N., and Sofroniew, M. V.: Do central nervous system neurons require target-derived neurotrophic support for survival throughout adult life and aging? *Perspect. Dev. Neurobiol.* 3: 133-42, 1996.
6. Rylett, R. J., and Williams, L. R.: Role of neurotrophins in cholinergic-neurone function in the adult and aged CNS. *Trends Neurosci.* 17: 486-490, 1994.
7. Tagliatela, G., Caprioli, A., Giuliani, A., and Ghirardi, O.: Spatial memory and NGF levels in aged rats: natural variability and effects of acetyl-L-carnitine treatment. *Exp. Gerontol.* 31: 577-87, 1996.
8. Henriksson, B. G., Soderstrom, S., Gower, A. J., Ebendal, T., Winblad, B., and Mohammed, A. H.: Hippocampal nerve growth factor levels are related to spatial learning ability in aged rats. *Behav. Brain Res.* 48: 15-20, 1992.
9. Bäckman, C., Rose, G. M., Bartus, R. T., Hoffer, B. J., Mufson, E. J., and Granholm, A. C.: Carrier mediated delivery of NGF: alterations in basal forebrain neurons in aged rats revealed using antibodies against low and high affinity NGF receptors. *J. Comp. Neurol.* 387: 1-11, 1997.
10. Hasenohr, R. U., Soderstrom, S., Mohammed, A. H., Ebendal, T., and Huston, J. P.: Reciprocal changes in expression of mRNA for nerve growth factor and its receptors TrkA and LNGFR in brain of aged rats in relation to maze learning deficits. *Exp. Brain Res.* 114: 205-13, 1997.
11. Fischer, W., Victorin, K., Björklund, A., Williams, L. R., Varon, S., and Gage, F. H.: Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. *Nature.* 329: 65-68, 1987.
12. Fischer, W., Chen, K. S., Gage, F. H., and Björklund, A.: Progressive decline in spatial learning and integrity of forebrain cholinergic neurons in rats during aging. *Neurobiol. Aging.* 13: 9-23, 1992.
13. Hefti, F., and Weiner, W. J.: Nerve growth factor and Alzheimer's disease. *Ann. Neurol.* 20: 275-281, 1986.
14. Williams, L. R., Varon, S., Peterson, G. M., Victorin, K., Fischer, W., Björklund, A., and Gage, F. H.: Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria fornix transection. *Proc. Natl. Acad. Sci.* 83: 9231-9235, 1986.
15. Kromer, L. F.: Nerve growth factor treatment after brain injury prevents neuronal death. *Science.* 235: 214-216, 1987.
16. Morris, R.: Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Meth.* 11: 47-60, 1984.
17. Moser, M.-B., Moser, E. I., Forrest, E., Andersen, P., and Morris, R. G. M.: Spatial learning with a minislab in the dorsal hippocampus. *Proc. Natl. Acad. Sci.* 92: 9697-9701, 1995.

18. Hardman, R., Evans, D. J., Fellows, L., Hayes, B., Rupniak, H. T., Barnes, J. C., and Higgins, G. A.: Evidence for recovery of spatial learning following entorhinal cortex lesions in mice. *Brain Res.* 758: 187-200, 1997.
19. Rogers, D. C., and Hunter, A. J.: Photothrombotic lesions of the rat cortex impair acquisition of the water maze. *Pharmacol. Biochem. Behav.* 56: 747-54, 1997.
20. Sutherland, R. J., Whishaw, I. Q., and Kolb, B.: Contributions of cingulate cortex to two forms of spatial learning and memory. *J. Neurosci.* 8: 1863-1872, 1988.
21. Kolb, B., Sutherland, R. J., and Whishaw, I. Q.: A comparison of the contributions of the frontal and parietal association cortex to spatial localization in rats. *Behav. Neurosci.* 97(1): 13-27, 1983.
22. Whishaw, I. Q., and Kolb, B.: Decortication abolishes place but not cue learning in rats. *Behav. Brain Res.* 11: 123-134, 1984.
23. Rasmussen, T., Schliemann, T., Sorensen, J. C., Zimmer, J., and West, M. J.: Memory impaired aged rats: no loss of principal hippocampal and subicular neurons. *Neurobiol. Aging.* 17: 143-7, 1996.
24. Rapp, P. R., and Gallagher, M.: Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. *Proc. Natl. Acad. Sci.* 93: 9926-30, 1996.
25. Frick, K. M., Baxter, M. G., Markowska, A. L., Olton, D. S., and Price, D. L.: Age-related spatial reference and working memory deficits assessed in the water maze. *Neurobiol. Aging.* 16: 149-160, 1995.
26. Denney, N. W., Dew, J. R., and Kihlstrom, J. F.: An adult developmental study of the encoding of spatial location. *Exp. Aging Res.* 18: 25-32, 1992.
27. Kirasic, K. C., Allen, G. L., and Haggerty, D.: Age-related differences in adults' macrospatial cognitive processes. *Exp. Aging Res.* 18: 33-39, 1992.
28. Robbins, T. W., James, M., Owen, A. M., Sahakian, B. J., McInnes, L., and Rabbitt, P.: Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. *Dementia.* 5: 266-81, 1994.
29. Schaie, K. W., and Willis, S. L.: Age difference patterns of psychometric intelligence in adulthood: generalizability within and across ability domains. *Psychol. Aging.* 8: 44-55, 1993.
30. Sharps, M. J., and Golin, E. S.: Memory for object location in young and elderly adults. *J. Gerontol.* 42: 336-341, 1987.
31. Bartus, R. T., Dean III, R. L., Beer, B., and Lippa, A. S.: The cholinergic hypothesis of geriatric memory dysfunction. *Science.* 217: 408-417, 1982.
32. Robinson, J. K., Wiley, R. G., Wenk, G. L., Lappi, D. A., and Crawley, J. N.: 192IgG-saporin immunotoxin and ibotenic acid lesions of nucleus basalis and medial septum produce comparable deficits in delayed nonmatching to position in rats. *Psychobiology.* 24: 179-186, 1996.
33. Shen, J., Barnes, C. A., Wenk, G. L., and McNaughton, B. L.: Differential effects of selective immunotoxic lesions of medial septal cholinergic cells on spatial working and reference memory. *Behav. Neurosci.* 110: 1181-6, 1996.
34. Waite, J. J., Chen, A. D., Wardlow, M. L., and Thal, L. J.: Behavioral and biochemical consequences of combined lesions of the medial septum/diagonal band and nucleus basalis in the rat when ibotenic acid, quisqualic acid, and AMPA are used. *Exp. Neurol.* 130: 214-29, 1994.
35. Janis, L. S., Bishop, T. W., and Dunbar, G. L.: Medial septal lesions in rats produce permanent deficits for strategy selection in a spatial memory task. *Behav. Neurosci.* 108: 892-8, 1994.
36. Kelsey, J. E., and Vargas, H.: Medial septal lesions disrupt spatial, but not nonspatial, working memory in rats. *Behav. Neurosci.* 107: 565-574, 1993.
37. Baxter, M. G., Bucci, D. J., Gorman, L. K., Wiley, R. G., and Gallagher, M.: Selective immunotoxic lesions of basal forebrain cholinergic cells: effects on learning and memory in rats. *Behav. Neurosci.* 109: 714-22, 1995.
38. Leanza, G., Nilsson, O. G., Wiley, R. G., and Bjorklund, A.: Selective lesioning of the basal forebrain cholinergic system by intraventricular 192 IgG-saporin: behavioural, biochemical and stereological studies in the rat. *Eur. J. Neurosci.* 7: 329-43, 1995.
39. Armstrong, D. M., Sheffield, R., Buzsaki, G., Chen, K. S., Hersh, L. B., and Nearing, B. G., FH.: Morphologic alterations of choline acetyltransferase-positive neurons in the basal forebrain of aged behaviorally characterized Fisher 344 rats. *Neurobiol. Aging.* 14: 457-470, 1993.
40. Fischer, W., Bjorklund, A., Chen, K., and Gage, F. H.: NGF improves spatial memory in aged rodents as a function of age. *J. Neurosci.* 11: 1889-1906, 1991.
41. Markowska, A. L., Koliatsos, V. E., Breckler, S. J., Price, D. L., and Olton, D. S.: Human nerve growth factor improves spatial memory in aged but not in young rats. *J. Neurosci.* 14: 4815-4824, 1994.

42. Chen, K. S., Masliah, E., Mallory, M., and Gage, F. H.: Synaptic loss in cognitively impaired aged rats is ameliorated by chronic human nerve growth factor infusion. *Neurosci.* 68: 19-27, 1995.
43. Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Gollub, M., and Wellman, C.: The effects of intrahippocampal BDNF and NGF on spatial learning in aged Long Evans rats. *Mol. Chem. Neuropathol.* 29: 211-26, 1996.
44. Chen, K. S., and Gage, F. H.: Somatic gene transfer of NGF to the aged brain: behavioral and morphological amelioration. *J. Neurosci.* 15: 2819-2825, 1995.
45. Martinez-Serrano, A., Fischer, W., and Björklund, A.: Reversal of age-dependent cognitive impairments and cholinergic neuron atrophy by NGF-secreting neural progenitors grafted to the basal forebrain. *Neuron*. 15: 473-484, 1995.
46. Martinez-Serrano, A., Fischer, W., Soderstrom, S., Ebendal, T., and Björklund, A.: Long-term functional recovery from age-induced spatial memory impairments by nerve growth factor gene transfer to the rat basal forebrain. *Proc. Natl. Acad. Sci.* 93: 6355-60, 1996.
47. Lindner, M. D., Kearns, C. E., Winn, S. R., Frydel, B., and Emerich, D. F.: Effects of intraventricular encapsulated hNGF-secreting fibroblasts in aged rats. *Cell Transplantation*. 5: 205-223, 1996.
48. Bäckman, C., Rose, G. M., Hoffer, B. J., Henry, M. A., Bartus, R. T., Friden, P., and Granholm, A. C.: Systemic administration of a nerve growth factor conjugate reverses age-related cognitive dysfunction and prevents cholinergic neuron atrophy. *J. Neurosci.* 16: 5437-42, 1996.
49. Fischer, W., Sirevaag, A., Wiegand, S. J., Lindsay, R. M., and Björklund, A.: Reversal of spatial memory impairments in aged rats by nerve growth factor and neurotrophins 3 and 4/5 but not by brain-derived neurotrophic factor. *Proc. Natl. Acad. Sci.* 91: 8607-11, 1994.
50. Markowska, A. L., Price, D., and Koliatsos, V. E.: Selective effects of nerve growth factor on spatial recent memory as assessed by a delayed nonmatching-to-position task in the water maze. *J. Neurosci.* 16: 3541-8, 1996.
51. Means, L. W., and Kennard, K. J.: Working memory and the aged rat: deficient two-choice win-stay water-escape acquisition and retention. *Physiol. Behav.* 49: 301-307, 1991.
52. Caprioli, A., Ghirardi, O., Giuliani, A., Ramacci, M. T., and Angelucci, L.: Spatial learning and memory in the radial maze: A longitudinal study in rats from 4 to 25 months of age. *Neurobiol. Aging*. 12: 605-607, 1991.
53. Williams, L. R., Rylett, R. J., Moises, H. C., and Tang, A. H.: Exogenous NGF affects cholinergic transmitter function and Y-maze behavior in aged Fischer 344 male rats. *Canad. J. Neurol. Sci.* 18: 403-7, 1991.
54. Scali, C., Casamenti, F., Pazzagli, M., Bartolini, L., and Pepeu, G.: Nerve growth factor increases extracellular acetylcholine levels in the parietal cortex and hippocampus of aged rats and restores object recognition. *Neurosci. Lett.* 170: 117-120, 1994.
55. Lin, L.-F. H., Doherty, D. H., Lile, J. D., Bektesh, S., and Collins, F.: GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science*. 260: 1130-1132, 1993.
56. Bowenkamp, K. E., Lapchak, P. A., Hoffer, B. J., Miller, P. J., and Bickford, P. C.: Intracerebroventricular glial cell line-derived neurotrophic factor improves motor function and supports nigrostriatal dopamine neurons in bilaterally 6-hydroxydopamine lesioned rats. *Exp. Neurol.* 145: 104-17, 1997.
57. Hebert, M. A., and Gerhardt, G. A.: Behavioral and neurochemical effects of intranigral administration of glial cell line-derived neurotrophic factor on aged Fischer 344 rats. *J. Pharmacol. Exp. Ther.* 282: 760-8, 1997.
58. Bowenkamp, K. E., Lapchak, P. A., Hoffer, B. J., and Bickford, P. C.: Glial cell line-derived neurotrophic factor reverses motor impairment in 16-17 month old rats. *Neurosci. Lett.* 211: 81-4, 1996.
59. Emerich, D. F., Plone, M., Francis, J., Frydel, B. R., Winn, S. R., and Lindner, M. D.: Alleviation of behavioral deficits in aged rodents following implantation of encapsulated GDNF-producing fibroblasts. *Brain Res.* 736: 99-110, 1996.
60. Lapchak, P. A., Miller, P. J., and Jiao, S.: Glial cell line-derived neurotrophic factor induces the dopaminergic and cholinergic phenotype and increases locomotor activity in aged Fischer 344 rats. *Neurosci.* 77: 745-52, 1997.
61. Phelps, C. H., Gage, F. H., Growdon, J. H., Hefti, F., Harbaugh, R., Johnston, M. V., Khachaturian, Z. S., Mobley, W. C., Price, D. L., Raskind, M., Simpkins, J., Thal, L. J., and Woodcock, J.: Potential use of nerve growth factor to treat Alzheimer's disease. *Neurobiol. Aging*. 10: 205-207, 1989.
62. Wilcock, G. K., and Harrold, P. L.: Treatment of Alzheimer's disease: future directions. *Acta Neurol. Scand. Suppl.* 165: 128-36, 1996.
63. Zhang, Z., Miyoshi, Y., Lapchak, P. A., Collins, F., Hilt, D., Lebel, C., Kryscio, R., and Gash, D. M.: Dose response to intraventricular glial cell line-derived neurotrophic factor administration in parkinsonian monkeys. *J. Pharmacol. Exp. Ther.* 282: 1396-401, 1997.

64. Olson, L., Nordberg, A., von Holst, H., Backman, L., Ebendal, T., Alafuzoff, I., Amberla, K., Hartvig, P., Herlitz, A., Lilja, A., and et al.: Nerve growth factor affects ¹¹C-nicotine binding, blood flow, EEG, and verbal episodic memory in an Alzheimer patient (case report). *J. Neural. Transm. Park. Dis. Dement. Sect. 4*: 79-95, 1992.
65. Seiger, A., Nordberg, A., von Holst, H., Backman, L., Ebendal, T., Alafuzoff, I., Amberla, K., Hartvig, P., Herlitz, A., Lilja, A., and et al.: Intracranial infusion of purified nerve growth factor to an Alzheimer patient: the first attempt of a possible future treatment strategy. *Behav. Brain Res.* 57: 255-61, 1993.
66. Hoffer, B., and Olson, L.: Treatment strategies for neurodegenerative diseases based on trophic factors and cell transplantation techniques. *J. Neural. Transm. Suppl.* 49: 1-10, 1997.
67. ALS CNTF Treatment Study Group: A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rHCNTF) in amyotrophic lateral sclerosis. *Neurol.* 46: 1244-9, 1996.
68. Williams, L. R.: Hypophagia is induced by intracerebroventricular administration of nerve growth factor. *Exp. Neurol.* 113: 31-37, 1991.
69. Rueff, A., Dawson, A. J., and Mendell, L. M.: Characteristics of nerve growth factor induced hyperalgesia in adult rats: dependence on enhanced bradykinin-1 receptor activity but not neurokinin-1 receptor activation. *Pain.* 66: 359-72, 1996.
70. Della Seta, D., de Acetis, L., Aloe, L., and Alleva, E.: NGF effects on hot plate behaviors in mice. *Pharmacol. Biochem. Behav.* 49: 701-5, 1994.
71. Alleva, E., Aloe, L., and Bigi, S.: An updated role for nerve growth factor in neurobehavioural regulation of adult vertebrates. *Rev. Neurosci.* 4: 41-62, 1993.
72. Levi Montalcini, R., Skaper, S. D., Dal Toso, R., Petrelli, L., and Leon, A.: Nerve growth factor: from neurotrophin to neurokine. *Trends Neurosci.* 19: 514-20, 1996.
73. Winkler, J., Ramirez, G. A., Kuhn, H. G., Peterson, D. A., Day Lollini, P. A., Stewart, G. R., Tuszynski, M. H., Gage, F. H., and Thal, L. J.: Reversible Schwann cell hyperplasia and sprouting of sensory and sympathetic neurites after intraventricular administration of nerve growth factor. *Ann. Neurol.* 41: 82-93, 1997.
74. Lapchak, P. A., Araujo, D. M., Hilt, D. C., Sheng, J., and Jiao, S.: Adenoviral vector-mediated GDNF gene therapy in a rodent lesion model of late stage Parkinson's disease. *Brain Res.* 777: 153-60, 1997.
75. Choi-Lundberg, D. L., Lin, Q., Chang, Y. N., Chiang, Y. L., Hay, C. M., Mohajeri, H., Davidson, B. L., and Bohn, M. C.: Dopaminergic neurons protected from degeneration by GDNF gene therapy. *Science.* 275: 838-41, 1997.
76. Bilang Bleuel, A., Revah, F., Colin, P., Locquet, I., Robert, J. J., Mallet, J., and Horellou, P.: Intrastriatal injection of an adenoviral vector expressing glial-cell-line-derived neurotrophic factor prevents dopaminergic neuron degeneration and behavioral impairment in a rat model of Parkinson disease. *Proc. Natl. Acad. Sci.* 94: 8818-23, 1997.