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BDNF and NT-3 widen the scope of neurotrophin activity: pharmacological implications

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Abstract. The neurotrophin gene family comprises four structurally related basic proteins, NGF, BDNF, NT-3 and NT-4/5. Despite high structural homology, these neurotrophins operate via different high-affinity membrane receptors, differ in target specificity, patterns of spatial and temporal distribution and responses to injury of the neuronal tissue. Based on the recent data, some aspects of BDNF and NT-3 neurotrophin activity and their possible role in normal and damaged nervous system are presented. Different effectiveness of exogenously applied neurotrophins in the injured brain as a consequence of responsiveness of particular neuronal populations to these factors, dose requirements, route of delivery, parenchymal penetration and target availability is discussed.

Key words: nerve growth factor, NGF, brain derived neurotrophic factor, BDNF, neurotrophin-3, NT-3, $p75^{NGFR}$, $gp140^{Trk}$, $gp140^{TrkB}$, $gp140^{TrkC}$

INTRODUCTION

Substantial progress has been recently made in research on nerve growth factor (NGF), a prototypic neurotrophic molecule discovered over 40 years ago (Levi-Montalcini 1987), and other members of the gene family of neurotrophic factors. In addition to laying a molecular framework for the neurotrophic hypothesis, the very restricted neuronal specificity of NGF has for many years stimulated the search for related or dissimilar factors that might have specificities for the neuronal types that are not responsive to NGF. A hope to discover the factors which would be used in a potential therapy of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and amyotropic lateral sclerosis (ALS) was also a driving force for this search. As a result, numerous trophic molecules were identified and a family of NGF-related molecules named neurotrophins emerged. Since the NGF discovery several decades of extensive studies had to pass until the next neurotrophin, brain derived neurotrophic factor (BDNF), was identified and characterized (Barde et al. 1982, Leibrock et al. 1989). Shortly afterwards other three family members, namely neurotrophin-3 (NT-3) (Maisonpierre et al. 1990, Rosenthal et al. 1990), neurotrophin-4 (NT-4), and neurotrophin-5 (NT-5) were described; NT-4 is now considered to be a Xenopus homologue to the mammalian NT-5 (Hallböök et al. 1991, Ip et al. 1992). Almost simultaneously tyrosine protein kinase (Trk) family members as high-affinity receptors to these neurotrophins were identified (Martin-Zanca et al. 1989, Squinto et al. 1991, Merlio et al. 1992).

Multiple studies on the role of the NGF, which was discovered as a molecule exerting a trophic influence on the peripheral sensory and sympathetic neurones, revealed its trophic function also in the adult central nervous system (CNS) mainly directed to cholinergic neurones (for reviews see Levi-Montalcini 1987, Whittemore and Seiger 1987, Hefti et al. 1989). Thus, the distribution of this protein and the pattern of its mRNA expression in the brain have been shown to match the topography of target fields for brain cholinergic pathways (Korsching et al. 1985, Ayer-LeLievre et al. 1988). Furthermore, forebrain cholinergic neurones prominently display both low- and high-affinity binding sites to NGF (for review see Chao 1992), observations which are consistent with its postulated trophic and proven reparative effects on these neurones (Hefti et al. 1989, Cuello 1993). Recent studies brought evidence for NGF constitutive and inducible expression in new groups of neurones (Carswell et al. 1993) and also non-neuronal, astrocytic cells (see Oderfeld-Nowak and Bacia, this issue) disclosing its potential in the injured nervous system and changing the view on its function as typical target-derived molecule only.

The picture of recently described BDNF, NT-3 and NT-4/5 neurotrophins, their distribution, mechanism of action and pharmacological potential in experimental therapy is only emerging. In the two years following the identification of their Trk receptors a significant contribution to the understanding of some of the mechanisms of their action has been made and hypotheses were shaped. The paper reviews briefly the attempts to characterize the aforementioned aspects of BDNF and NT-3 in the CNS.

BDNF AND NT-3 NEUROTROPHINS

The data on the regional BDNF and NT-3 distribution within the brain are based mainly on the *in situ* hybridization studies of neurotrophin mRNA expression patterns. In the brain, distinct spatial and temporal patterns of expression of NGF, BDNF and NT-3 mRNAs have been reported. Although all these factors are highly expressed in olfactory bulb and the hippocampal neurones, BDNF mRNA has the most widespread distribution and is very conspicuous in many areas of the cortex as well as in septum, amygdala and the ependymal lining of the lateral ventricles (Phillips et al. 1990, Wetmore et al. 1990), while the expression of NT-3 transcripts in these regions is undetectable (Phillips et al.

1990). Neurones within substantia nigra express both BDNF and NT-3. Curiously, the striatum is among those brain areas where neurotrophin expression is least evident. While it is detectable when monitored by Northern blot analysis (Maisonpierre et al. 1990), to date BDNF, NT-3 and NGF transcripts have not been demonstrated in striatal neurones by *in situ* hybridization. Interestingly, while BDNF and NT-3 appear to be essentially absent from the adult striatum, transient, high levels of expression are apparent for both neurotrophins during the first two weeks of postnatal development. Because this period corresponds to the maturation of the dopaminergic innervation of striatum, it is hypothesized that BDNF and NT-3 may function as classically defined target-derived neurotrophic factors during a critical period of dopamine neurone development, later switching to a paracrine or autocrine mode of support as the system matures (Lindsay et al. 1993). This mode of action has been also suggested by colocalisation studies of both neurotrophins and their receptors. Characterization of the patterns of neurotrophin distribution became possible only recently due to the availability of specific antibodies against BDNF and NT-3. Immunocytochemical localisation of BDNF and NT-3 proteins confirms the pattern of their mRNA expression (Feinstein et al. 1993, Morse et al. 1993).

The restricted number of data available indicates that both neurotrophins are under control of glutamatergic and cholinergic innervation, at least in the hippocampus where their expression is the most abundant. Following nonselective brain injury caused by cerebral ischemia or hypoglycaemic coma, the expression of BDNF mRNA in the hippocampus increases, while that of NT-3 decreases (Lindvall et al. 1992). Similarly, opposite BDNF and NT-3 mRNA responses were reported after induction of long term potentiation (Castrén et al. 1993); in both experiments the response seemed to depend on the enhanced activity of excitatory glutamatergic pathways, up-regulating BDNF and down-regulating NT-3 mRNA levels. Disruption of the main glutamatergic hippocampal input by the entorhinal cortex lesion which leads to a decrease of glutamatergic activity, doesn't influence BDNF and NT-3 mRNA levels (Lapchak et al. 1993a). Recent *in situ* hybridization studies on the BDNF and NT-3 mRNA expression in the hippocampus after experimental removal of the cholinergic input revealed the decrease in mRNA for both neurotrophins in hippocampal granule cells (Lapchak et al. 1993b). Interestingly, in the hippocampus of Alzheimer patients a significant decrease of BDNF and NT-3 mRNA levels in comparison to those in control patients has also been detected (Olson 1993). These data indicate that both factors may be under control of the cholinergic system and that the observed decreases might be a consequence, rather than the cause, of cholinergic degeneration.

The action of neurotrophins is mediated by specific neurotrophin receptors. Despite high structural homology with NGF and similar affinity to the low-affinity NGF receptor ($p75^{NGFR}$, NGFR) (for review see Chao 1992), BDNF and NT-3 seemingly operate via different high-affinity neurotrophin receptors (see Fig.1). Thus, BDNF acts predominantly on TrkB (designated as $gp140^{TrkB}$) (Soppet et al. 1991, Squinto et al. 1991, Chao 1992) while NT-3 acts preferentially on TrkC (designated as $gp140^{TrkC}$) (Chao 1992). It has to be stated here that qualifying Trk molecules as autonomous high-affinity binding sites for neurotrophins - as done above - may be premature in light of data which suggest that Trks may be rather the specific compo-

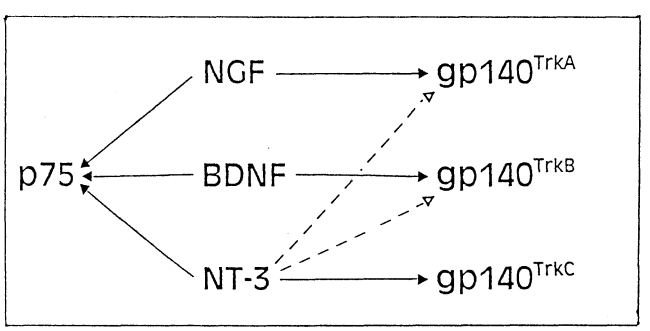

Fig.1. Promiscuity of neurotrophins. Multiple interactions between neurotrophins and their receptor tyrosine kinases are shown. The principal ligand-receptor interactions are denoted by heavy arrows and weaker interactions are shown with dashed arrows.

nents of high-affinity receptors (Hempstead et al. 1991). The distribution of BDNF and TrkB receptors (Phillips et al. 1990, Wetmore et al. 1990, Merlio et al. 1992, Feinstein et al. 1993) suggests that this peptide might exert widespread actions within the CNS, in contrast to the apparently more restricted actions of the NGF and NT-3 molecules. The partial overlap of expression patterns of trkB and trkC transcripts and their ligands reported earlier for substantia nigra, has been also found - with some exceptions, like striatum - in other structures of the adult brain supporting the view of a possible auto- or paracrine function rather than the target-dependent action of NGF.

NEUROTROPHIN RECEPTORS

The p75 neurotrophin receptor

The p75 NGF receptor ($p75^{NGFR}$, NGFR) was originally defined by monoclonal antibodies that interfered with NGF binding and by affinity cross-linking experiments with ^{125}I-labelled NGF. First radioautographic studies with ^{125}I-NGF (Richardson et al. 1986) failed to demonstrate low affinity binding sites over neuronal somata. The production of a monoclonal antibody, 192-IgG, which recognizes the low affinity form of the rat NGFR (Chandler et al. 1984, Taniuchi and Johnson, Jr. 1985) - although its interference both with the low and high affinity forms was also considered (Green and Greene 1986) - brought the tool which allowed to study the detailed distribution of NGFR - immunoreactive (NGFR - IR) cells. Since then numerous laboratories have reported the widespread distribution of NGFR immunoreactivity in the CNS of developing and adult rats using radioimmunoassay (Taniuchi et al. 1986) and immunocytochemical (Dawbarn et al. 1988, Kiss et al. 1988, Yan and Johnson, Jr. 1988, Batchelor et al. 1989, Pioro and Cuello 1990, Pioro et al. 1990, Ferguson et al. 1991, Thomas et al. 1991) techniques. NGFR transcripts (Buck et al. 1988) and NGFR protein are expressed in all NGF-responsive cells, including sympathetic, sensory, and basal forebrain cholinergic neurones (Pioro and Cuello 1990, Pioro et al. 1990) supporting the view of its physiological function in mediating NGF - induced response. However, the data on the lack of the functional responses following ligand binding in the cells expressing solely the low - affinity receptor prompt the hypothesis that NGFR may be a required but not the only condition to elicit signal transduction. Gene transfer and molecular cloning revealed that the $p75^{NGFR}$ gene encodes a transmembrane protein that does not possess a cytoplasmatic tyrosine kinase domain (Chao et al. 1986, Johnson et al. 1986, Radeke et al. 1987) which is an integral constituent of the full lenght Trk proteins described below. The discovery and further characterization of other members of neurotrophin family and their specific binding proteins within last four years led to a change of the view on the $p75^{NGFR}$ specificity and possible role. Recent studies by Rodriguez-Tébar et al. (Rodriguez-Tébar et al. 1990, Rodriguez-Tébar et al. 1992) have revealed that $p75^{NGFR}$ binds not only NGF but also other neurotrophins, namely BDNF and NT-3. Therefore, $p75^{NGFR}$ should be considered more appropriately as a neurotrophin receptor, and not merely an NGF receptor. Binding experiments with NGF, BDNF and NT-3 have demonstrated that each neurotrophin binds to p75 with a low affinity but the rates of association and dissociation vary markedly with NGF having the fastest and BDNF the slowest off rate (Rodriguez-Tébar et al. 1992). These binding variations are believed to reflect differences in the binding between p75 and each neurotrophic factor.

Several functions have been suggested for $p75^{NGFR}$. It may serve as a presentation receptor to concentrate neurotrophins, as it has been proposed for NGF in Schwann cells (Johnson Jr. et al. 1988, Taniuchi et al. 1988). It may be associated with molecules capable of signalling (Ohmichi et al. 1991) or providing substrates for high affinity binding sites. The $p75^{NGFR}$ internalization (Pioro et al. 1990) and involvement in retrograde transport of NGF has been strongly postulated (Johnson Jr. et al. 1988). The striking suggestion has been recently made on the association of p75 with apoptosis, based on the experimental data on the correlation of

$p75^{NGFR}$ occupancy and increased cell death (Rabizadeh et al. 1993). The role of $p75^{NGFR}$ in the formation of high-affinity NGF-binding sites with $gp140^{TrkA}$ glycoprotein (Green and Greene 1986, Hempstead et al. 1989, Hempstead et al. 1991) and possibly also high-affinity binding sites for other neurotrophins with $gp140^{TrkB}$ and $gp140^{TrkC}$, respectively, is strongly supported by recent experimental data. The common coexpression of low- and high- affinity NGF binding sites in NGF-responsive sensory and sympathetic neurones, pheochromocytoma (PC12) cells (Sutter et al. 1979, Schechter and Bothwell 1981, Godfrey and Shooter 1986) as well as more recently described coexpression of mRNA for p75 and gp140 in various cholinergic neuronal nuclei of the rat brain (Vazquez and Ebendal 1991, Figueiredo et al. 1993b) promote the model proposing that coexpression of both p75 and gp140 is required for high affinity binding which determines physiological responsiveness of the cells to neurotrophins. The gene transfer studies performed on cell lines of neuronal origin lacking p75 receptors have indicated, that transfection of the cells with cDNA encoding p75 leads to expression of both low- and high- affinity sites and to responsiveness of transfectants to NGF. Also transient gene transfection of p75 and trk to the cells that normally express neither (like COS cells or human melanoma cells) revealed, that while single transfection with one type of gene leads to low - affinity binding only, introduction of both to the same cell line results in their coexpression and the reconstitution of both high- and low- affinity sites. Other reconstitution studies applying membrane fusion techniques with the use of fibroblast transformant cell lines (rtrk-3T3 expressing gp140 only and PA 317 expressing p75, both binding NGF with low - affinity) indicated that only membrane fusion products bind NGF with high-affinity (Hempstead et al. 1991). High affinity binding appears to require a ratio of p75 to p140 of approximately 10:1, in keeping with the *in vivo* levels of the two receptors in target cells. Interestingly, kinetic measurements of ^{125}I-labelled NGF binding to cells expressing only gp140 have indicated that the on and off rates are both slow ($t_{1/2}$ 10 min), accounting for the lower affinity interaction of NGF with gp140 (Meakin et al. 1992). A second model proposes that high affinity binding sites can be formed in the absence of p75. This hypothesis is supported by experiments performed with fibroblasts expressing only the gp140 receptor, which are responsive to NGF. Furthermore, Barbacid's group reported that high-affinity binding sites can be generated by $gp140^{Trk}$ homodimer formation only (Klein et al. 1990), an additional possibility to that one of heterodimer formation described by Parada's group (Hempstead et al. 1991). Several experimental results have indicated that gp140 can function independently of p75 (Loeb et al. 1991). The use of blocking antibodies directed against p75 (Weskamp and Reichardt 1991) and the biological effects of mutant recombinant NGFs (Loeb et al. 1991, Ibáñez et al. 1992) have supported the conclusion that NGF binding to p140, and not to p75, appears to be sufficient to elicit biological responses. Which model is correct? How much universal it is, in reference to BDNF and NT-3 binding? For now, the role of both types of receptors in the formation of high - affinity binding sites and signal transduction remains controversial.

One of the approaches used to study a role of neurotrophins *in vivo* is the application of the neutralizing antibodies raised against neurotrophins or their receptors. However, limited penetration of the proteins within brain parenchyma restricts the possibility to obtain conclusive data. Until now this method was used only to study the effects of NGF deficiency only; there is no parallel data with the application of anti-BDNF or anti-NT-3 antibodies.

A new approach to address the question about the physiological role of neurotrophin receptors has been to create null mutations by gene targeting. Genetic disruption of the $p75^{NGFR}$ gene in mice has revealed that the p75 molecule plays an essential role in neuronal development (Lee et al. 1992) but the described alterations concerned the peripheral organs only. Homozygous animals lacking $p75^{NGFR}$ are viable but show a pronounced deficit in thermal sensitivity due to a loss of sensory fibres

in the periphery. Dorsal root ganglia (DRG) are smaller than in wild-type mice suggesting that neuronal cell death may have occurred. Surprisingly, most non - neuronal tissues in the null mutant appear normal during development which may indicate that gp140 receptors substitute partially p75 function at this stage. It is of interest to note that severe skin ulcerations and hair loss on distal extremities are prominent in $p75^{NGFR}$ mutant mice. Since this tool became available only recently, there are only preliminary data on mice carrying the "knock - outs" of the genes coding for $gp140^{Trk}$ molecules.

Trk receptors

The relative abundance of p75 and low levels of high affinity receptors had obscured the identity of the latter except with photoaffinity reagents (Sutter et al. 1979, Massague et al. 1981, Hosang and Shooter 1985). First radioautographic studies with ^{125}I-NGF allowed for visualisation of the high affinity NGF receptors over neuronal somata (Richardson et al. 1986, Raivich and Kreutzberg 1987). The early investigations characterised low- and high- affinity binding biochemically revealing that the low-affinity binding (Kd = nM) has a fast dissociation rate while the high-affinity receptors are characterized by a slow rate of dissociation. These studies brought the data suggesting that the major physiological effects of NGF are mediated via the high affinity receptors (Sutter et al. 1979, Hosang and Shooter 1985), however the identity of these receptors has been unknown until recently (Martin-Zanca et al. 1989).

In 1991, to the surprise of many neurobiologists, a flurry of papers were published demonstrating that the tyrosine protein kinase (trk) proto-oncogene encodes a receptor for NGF, while trkB and trkC encode receptors for BDNF and NT-3, namely TrkB and TrkC, respectively (Klein et al. 1991a, Klein et al. 1991b, Lamballe et al. 1991). The trk gene was originally detected as an oncogene isolated from human colon carcinoma biopsy (Martin--Zanca et al. 1986) whose oncogenic activity resulted from genomic rearrangement in which nonmuscle tropomyosine sequences were aberrantly fused onto the transmembrane and cytoplasmic domains of trk (hence, the name "tropomyosin receptor kinase"). *In situ* hybridization revealed that the highest levels of trk expression were confined to embryonic DRG and to other sensory ganglia. This observation promoted the hypothesis that trk may be related to neurotrophin function. In the normal adult brain, the trk transcripts are expressed almost exclusively by neurones, with the exception of truncated forms of trkB which are abundantly expressed by the nonneuronal cells, especially astrocytes and ependyma (Klein et al. 1990). With reference to the postulated role of truncated forms of TrkB receptor ($p95^{TrkB}$) lacking the cytoplasmic tyrosine kinase domain the view of its function as a presentation receptor to concentrate neurotrophins, originally proposed for $p75^{NGFR}$ - NGF interaction, was repeated more recently (see Chao, 1992). Again, the function of Trk receptors is extensively investigated by the analysis of the animals carrying targeted deletions of the gene(s) encoding these proteins. Although teoretically ideal, this approach showed already several limitations. The first data on some functional aspects of these mutations were reported few months ago. It appeared that the viability of the mutated mice lacking intracellular receptor domain is significantly decreased: homozygous trkB (-/-) mice die within the first 48 hours, apparently from starvation and dehydratation (Fagan et al. 1993). Examination of neuronal pathways involved in feeding showed significant defects in trigeminal ganglion and facial motor nucleus. Deficits are also present in DRG and motoneurons of spinal cord, two targets that have been shown to be responsive to BDNF, where 40 -60% of neurones is lost (Fagan et al. 1993, Silos--Santiago et al. 1993). Interestingly, severe skin ulcerations and hair loss, comparable to those ones reported for mice carrying $p75^{NGFR}$ null mutations are reported. Lack of data on the morphological and anatomical abnormalities in the mutant brain, doesn't allow to speculate on the possible role of the deficiency of BDNF and NT-3 Trk receptors in the brain.

THERAPEUTIC POTENTIAL OF BDNF AND NT-3

Effects *in vitro*

BDNF was initially identified as a neurotrophic activity with distinct as well as overlapping actions with NGF toward sensory neurones. Following isolation and purification from the pig brain (Barde et al. 1982), and - several years later - molecular cloning (Leibrock et al. 1989), BDNF is now recognized as a member of the neurotrophin family with over 55% primary sequence homology to NGF. Investigations of its biological activity monitored *in vitro*, followed by extensive studies of its effects in normal and damaged brain, revealed that BDNF acts on a broader spectrum of PNS and CNS neurones than NGF. Apart from the neurones whose survival in primary culture is easily monitored, like neurones of peripheral ganglia, the neurones whose degeneration is characteristic of a particular human neurodegenerative disease, as basal cholinergic neurones in AD, nigral dopaminergic neurones in PD, and motor neurones in ALS are the main subject of the *in vitro* studies. These studies have shown that BDNF promotes the survival and/or differentiation of several classes of neurones responsive also to NGF, like DRG and, less potently, basal forebrain cholinergic neurones. In particular BDNF promotes survival of septal cholinergic cells, upregulates the expression of $p75^{NGFR}$ (Alderson et al. 1990), and elevates choline acetyltransferase (ChAT) activity of developing septal cholinergic neurons (Knüsel et al. 1991). Additionally to common actions with NGF, *in vitro* BDNF acts on many classes of neurones non-responsive to NGF, including neural placode-derived sensory neurones (nodose ganglia neurones, NG) (Lindsay et al. 1985, Maisonpierre et al. 1990), retinal ganglion neurones (Johnson et al. 1986), motor neurones (Henderson et al. 1993, Wong et al. 1993), cerebellar granule neurones (Segal et al. 1992), hippocampal neurones (Ip et al. 1993), and ventral mesencephalic dopaminergic neurones (Hyman et al. 1991, Hyman et al. 1993). In contrast to NGF, BDNF cannot support the survival and growth of neurones of the paravertebral chick sympathetic ganglia (SG) (Barde et al. 1982).

The use of cloning strategy led to the description of a gene encoding the third member of neurotrophin family, sharing over 50% homology with NGF and BDNF, designated neurotrophin-3 (Maisonpierre et al. 1990, Rosenthal et al. 1990). This protein displays different specificity of biological activity from those of NGF and BDNF. *In vitro* NT-3 induces profuse neurite outgrowth from the DRG, NG and SG explants, causing maximal response in NG cultures greater than that evoked by BDNF (Maisonpierre et al. 1990). NT-3 has similar to BDNF effects on survival and differentiation of hippocampal neurones (Ip et al. 1993), dopaminergic neurones, and motoneurones (Henderson et al. 1993, Wong et al. 1993), up-regulating cholinergic phenotype in the latters but does not support the survival of basal forebrain cholinergic neurones (Alderson et al. 1990).

Although *in vitro* studies may be indicative of the potential target cell types for neurotrophins, the final proof may be obtained only in the *in vivo* investigations. The neuronal cells in culture are of embryonic origin and therefore their responsiveness is more characteristic for the developmental than adult neurones.

Effects *in vivo*

In the search for effective and selective factors in the experimental therapeutics of degenerative neuronal processes in the CNS the biological activity of BDNF and NT-3 neurotrophins is investigated in various brain injury models. The *in vitro* responsiveness of dopaminergic and cholinergic cells to these neurotrophins focused the studies on these two types of neurones due to the involvement of dopaminergic and cholinergic systems in neurodegenerative disorders, PD and AD, respectively.

Dopamine neurones appear to be activated when exogenous BDNF, and to a lesser extent, NT-3 are provided to the substantia nigra of both intact and

dopamine-denervated animals by supranigral infusion but not intracortical one (Altar et al. 1992). In intact rats BDNF and NT-3 infusion elevates dopamine metabolism in the ipsilateral neostriatum causing various behavioural responses (Lindsay et al. 1993) but remains without influence on striatal cholinergic interneurones (Altar et al. 1992). The investigations of trophic activity on injured dopaminergic system carried out on the model of striatal injury caused by chronic delivery of 6-OHDA neurotoxin revealed that BDNF and, to a lesser extent again, NT-3 can ameliorate behavioural and neurochemical deficits (Lindsay et al. 1993). The positive effects reported here stand in contrast to studies wherein BDNF induced a "hypofunction" of intact dopamine neurones and failed to attenuate reduction in cell numbers, neurochemistry, or behavioural output after disruption of nigro-striatal pathways involving medial forebrain bundle transection (Knüsel et al. 1992, Lapchak et al. 1993c). This negative result has been discussed in terms of insufficient BDNF dose which can be an important limitation in a treatment due to already known restricted ability of BDNF and NT-3 to penetrate brain tissue.

The most common models of injury for studying trophic effects on brain cholinergic systems are: the disruption of cholinergic septo-hippocampal projections (see Oderfeld-Nowak and Bacia, this issue) and basalo-cortical model of retrograde degeneration of cholinergic neurones of the nucleus basalis magnocellularis (nbm) after partial, cortical devascularization (Fig.2). In the former, the changes are caused by electrocoagulative or mechanical (tissue aspiration or transection) lesion which results in degeneration and death of part of the cholinergic neurones. In the latter, obliteration of superficial pial vessels supplying the selected area of the cerebral cortex causes a gradual infarction resulting in cortical necrosis which affects the terminal network of nbm cholinergic neurones (Cuello 1993), causes their shrinkage but does not result in a cell death. Several studies showed BDNF potency in protecting cholinergic neurones of the septo-hippocampal system against lesion-induced degeneration, although its effectiveness was weaker than that found for NGF in several previous investigations (Knüsel et al. 1992, Morse et al. 1993). We aimed to characterize the effects of exogenously administered BDNF and NT-3 on the basalo-cortical pathway of

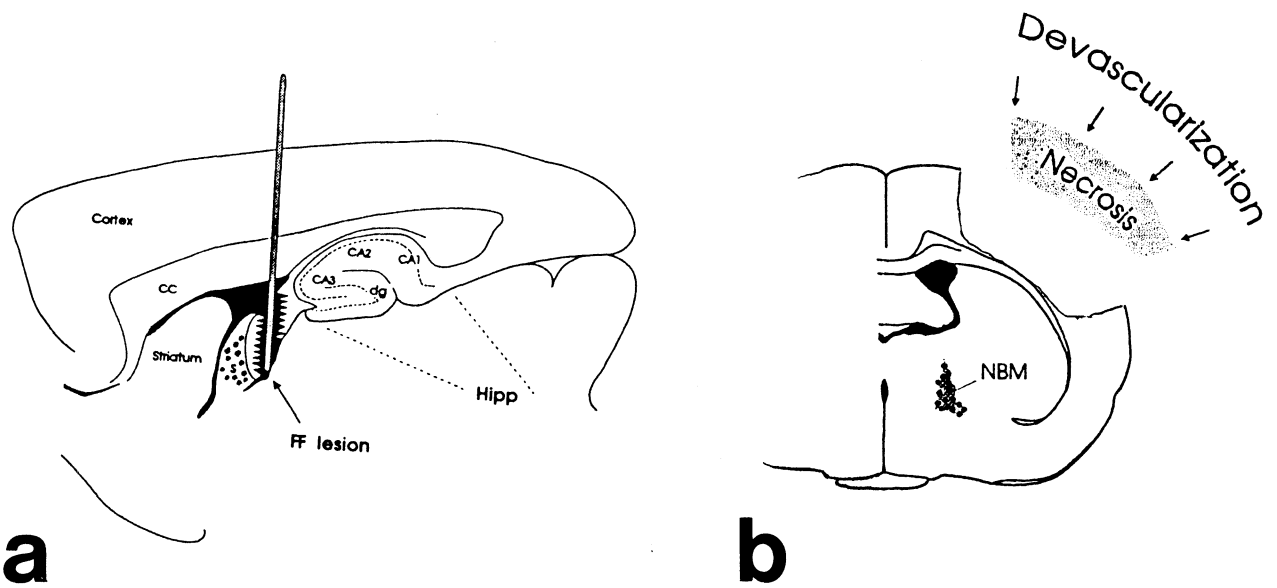

Fig. 2. Schematic representation of two models of injury to the brain cholinergic system: (a) disruption of the septo-hippocampal cholinergic pathway by partial transection of the fimbria-fornix projection (b) partial, unilateral devascularisation of the cerebral cortex.

the forebrain cholinergic system (Skup et al. 1993, Skup et al. 1994). The lesions of the target tissue and nerve terminal network cause well-characterized retrograde changes in the nucleus basalis magnocellularis, where the actions of these two novel neurotrophins can be accurately assessed in relation to the prototypical actions. Previous studies demonstrated that the intracerebroventricular infusion of NGF (Cuello 1993) or aFGF (Figueiredo et al. 1993a) to the lesioned animals provided protection of nbm cholinergic cell bodies and their neuritic network when observed one month after lesioning and 3 weeks after cessation of treatment. In our morphometric studies the cholinergic cell bodies were immunostained with monoclonal antibodies against NGFR and choline acetyltransferase (ChAT), acetylcholine synthesizing enzyme, as cholinergic markers (Fig. 3). The cell size was measured and the density of neuritic network within nbm was analysed. We demonstrated that BDNF and NT-3, when administered in the same experimental paradigm at doses previously found to be effective for NGF or aFGF, failed to prevent forebrain nbm cholinergic neurones from undergoing cell body shrinkage and retraction of neuritic networks (Skup et al. 1993, Skup et al. 1994).

Lack of effect of either of the neurotrophins used in that study should be considered in terms of responsiveness of nbm cholinergic neurones to these factors but also in terms of dose requirements and parenchymal penetration. Lack of data on the postlesion regulation of trkB and trkC mRNA expression in nbm cholinergic neurons restricts the possibility to address confidently the issue of expected cellular responsiveness. Although both trkB and trkC transcripts are detectable in the nbm region, their levels appear to be much lower than that of trkA (Merlio et al. 1992). It can be speculated only, that if their postlesion levels are up-regulated by the exogenously applied ligands, as it transiently occurs for p75 and trkA after NGF administration (Figueiredo et al. 1993b), they increase following BDNF or NT-3 administration. If it results in an increase in the levels of their translation products remains an opened question. Evidence of the presence of functional BDNF receptors on nbm cholinergic cells comes from studies demonstrating TrkB immunopositivity (Feinstein et al. 1993) and BDNF retrograde transport to the nbm after intracortical or amygdalar administration of that factor (Kroin et al. 1993). However, it has been shown only recently that intraventricular BDNF (Morse et al. 1993) administration results in very limited diffusion from the ventricles into the adjacent neural tissue, thus seriously limiting its availability to the nbm neurones. Highly abundant TrkB truncated receptor in the brain, and in particular in the paraventricular area, is probably the cause of this limitation; it was suggested that this molecule acts as a scavenger for BDNF, thus regulating its availability in the parenchyma. Thus, if experimental therapy is to be further considered with respect to the application of BDNF and NT-3 neurotrophins, the obstacle of their limited distribution within the brain has to be overcome.

The absence of a demonstrable effect of BDNF on cholinergic nbm neurones may be compared to the other negative data. The apparent lack of BDNF-mediated effects on lesion size or cholinergic cell sparing in the striatum following excitotoxic injury has been reported (Frim et al. 1993). Continuous intrastriatal BDNF administration in doses equivalent to those applied by us, also failed to modify striatal ChAT activity and high-affinity choline uptake (HAChU) (Altar et al. 1992). Although cell culture studies on embryonic basal forebrain neurones revealed their responsiveness to porcine and rhBDNF (Alderson et al. 1990, Knüsel et al. 1991, Knüsel et al. 1992), they showed that rhBDNF is 90 times less potent than NGF in affecting cell survival and elevation of ChAT activity (Knüsel et al. 1992) and that NT-3 was ineffective in that assay (Knüsel et al. 1991). Recent *in vivo* morphological studies revealed some protection from atrophy of axotomized septal cholinergic neurones with BDNF treatment (Knüsel et al. 1992, Morse et al. 1993). Up to the present moment there have been no *in vivo* studies in the cholinergic system reporting NT-3 effectiveness in neural repair. As already mentioned, data obtained in cell culture

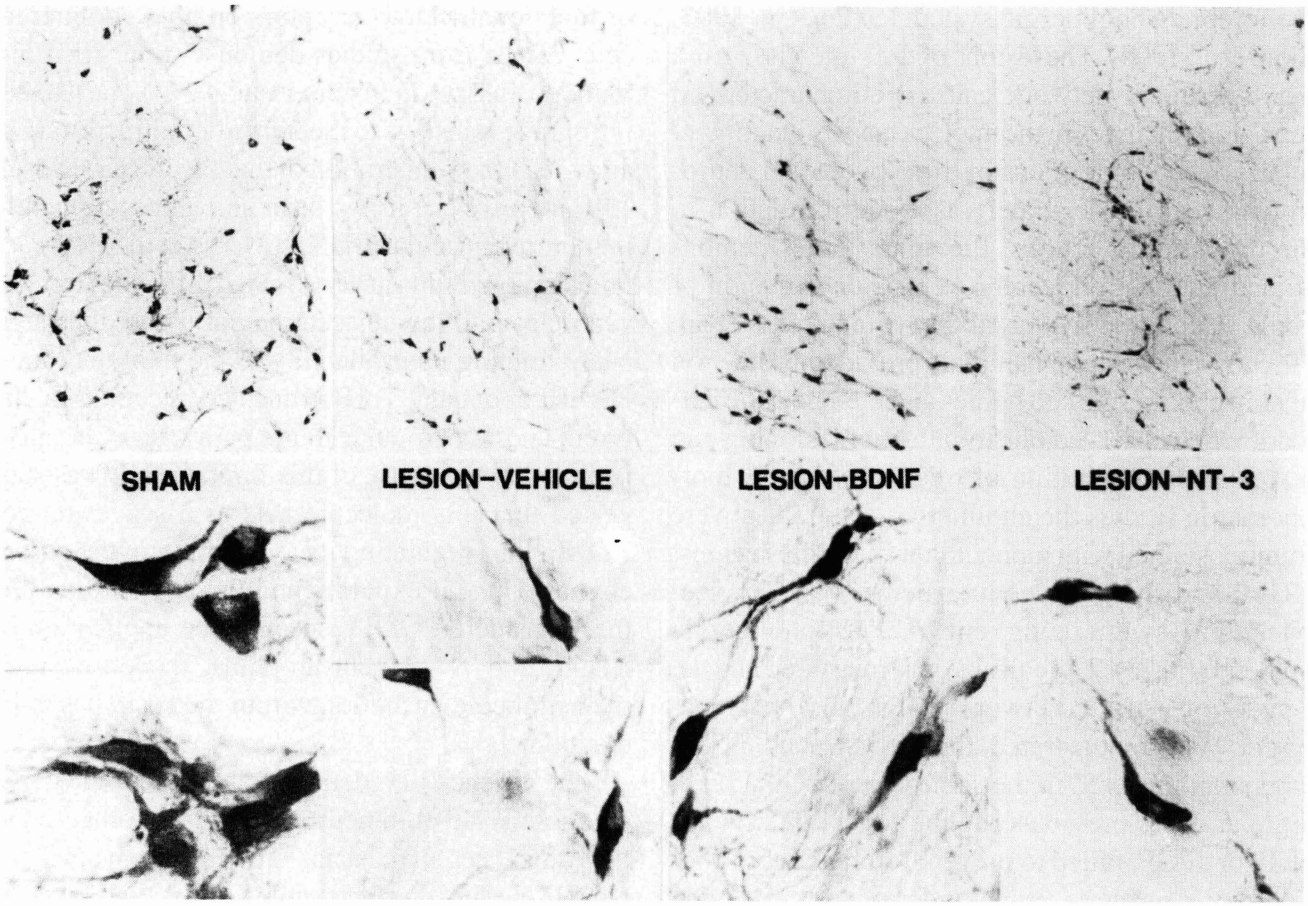

Fig. 3. Morphological appearance of ChAT - immunoreactive neurones in the intermediate nucleus basalis magnocellularis. The bottom panel represents the areas chosen from the respective top photomicrographs at higher magnification. High - power photomicrographs show cell shrinkage and the loss of neuritic processes in all lesioned groups.

studies can be roughly extrapolated to *in vivo* systems but they imply much lower potency of BDNF and NT-3 than of NGF on cholinergic cells. In our study we used a dose (12 μg/day) of BDNF and NT-3 equal to the maximal dose of NGF and aFGF applied in our previous studies (Cuello 1993, Figueiredo et al. 1993). This dose was 120 times higher than the NGF ED50 established for the maintenance of control nbm ChAT activity in devascularized rats and supramaximal to the NGF dose causing full protection of nbm cholinergic neurones from retrograde shrinkage (Cuello 1993). It was also approximately 3 times higher than the BDNF dose previously shown to protect axotomized septal neurones from degeneration after a 3-week administration period (Knüsel et al. 1992, Widmer et al. 1993). The latter treatment resulted in the protection of a limited number of cholinergic neurones, which contrasts to the more complete response to NGF, and showed that the reaction of ChAT-immunopositive cells was weaker than $p75^{NGFR}$-immunoreactive cells. Based on this observation a speculation could be made that BDNF up-regulates $p75^{NGFR}$ mRNA but not ChAT mRNA in septal cholinergic neurones. Furthermore, the BDNF-induced rescue of a part of the septal cholinergic neurones from axotomy-caused cell loss is not paralleled by its effects on biochemical correlates of functional recovery (Hefti et al. 1993, Lapchak et al. 1993b). BDNF treatment does not affect the diminished ChAT activity and HAChU levels in synaptosomal preparations from the septal area ipsilateral to the lesion (Hefti et al. 1993), nor does it attenuate postlesion decrease of ACh synaptosomal content

(Hefti et al. 1993) and ACh release from hippocampal slices (Lapchak et al. 1993b).

The findings summarized here suggest a lower potency of BDNF and NT-3 in comparison to NGF towards brain cholinergic systems, however further studies applying other administration routes and doses of novel neurotrophins may disclose their effectiveness for protection of nbm and other cholinergic neurones after injury and prove their potency toward other neurotransmitter systems.

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REFERENCES

Alderson R.F., Alterman A.L., Barde Y.-A., Lindsay R.M. (1990) Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. Neuron 5: 297-306.

Altar C.A., Boylan C.B., Jackson C., Hershenson S., Miller J., Wiegand S.J., Lindsay R.M., Hyman C. (1992) Brain-derived neurotrophic factor augments rotational behavior and nigrostriatal dopamine turnover *in vivo.* Proc. Natl. Acad. Sci. USA 89: 11347-11351.

Ayer-LeLievre C., Olson L., Ebendal T., Seiger T., Persson H. (1988) Expression of β-nerve growth factor gene in hippocampal neurons. Science 240: 1339-1341.

Barde Y.-A., Edgar D., Thoenen H. (1982) Purification of a new neurotrophic factor from mammalian brain. EMBO J. 1: 549-553.

Batchelor P.E., Armstrong D.M., Blaker S.M., Gage F.H. (1989) Nerve growth factor receptor and choline acetyltransferase colocalization in neurons within the rat forebrain: response to fimbria-fornix transection. J. Comp. Neurol. 284: 187-204.

Buck C.R., Martinez H.J., Chao M.V., Black I.B. (1988) Differential expression of the nerve growth factor receptor gene in multiple brain areas. Dev. Brain Res. 44: 259-268.

Carswell S., Lapchak P.A., Wilcox H., Clapton-Hartpence K., Robbins E., Brown E.R., Saporito M.S. (1993) Constitutively expressed and inducible levels of NGF mRNA in the basal forebrain. Soc. Neurosci. Abstr. 19: 5.

Castrén E., Pitkänen M., Sirviö J., Parsadanian A., Lindholm D., Thoenen H., Riekkinen P. (1993) The induction of LTP increases BDNF and NGF mRNA but decreases NT-3 mRNA in the dentate gyrus. NeuroReport 4: 895-898.

Chandler C.E., Parsons L.M., Hosang M., Shooter E.M. (1984) A monoclonal antibody modulates the interaction of nerve growth factor with PC12 cells. J. Biol. Chem. 259: 6882-6889.

Chao M.V. (1992) Neurotrophin receptors: A window into neuronal differentiation. Neuron 9: 583-593.

Chao M.V., Bothwell M.A., Ross H., Koprowski A.A., Lanahan C.R., Buck C., Seghal A. (1986) Gene transfer and molecular cloning of the human NGF receptor. Science 232: 518-521.

Cuello A.C. (1993) Trophic responses of forebrain cholinergic neurons: a discussion. Prog. Brain Res. 92: 265-277.

Dawbarn D., Allen S.J., Semenenko F.M. (1988) Immunohistochemical localisation of β-nerve growth factor receptors in the forebrain of the rat. Brain Res. 440: 185-189.

Fagan A.M., Klein R., Lira S.A., Wallace J.D., Bryant S., Long L., Wurst W., Joyner A.L., Barbacid M., Smeyne R.J. (1993) Neuronal deficiencies in mice lacking the neurotrophin receptor genes trk, trkB and trkC. Soc. Neurosci. Abstr. 19: 1297.

Feinstein S.C., Matheson C.R., Radeke M.J., Welcher A.A., Blumberg D., Talvenheimo J., Yan Q. (1993) Immunocytochemical localization of trkA and trkB in rat central nervous system. Soc. Neurosci. Abstr. 19: 249.

Ferguson I.A., Schweitzer J.B., Bartlett P.F., Johnson E.M., Jr. (1991) Receptor-mediated retrograde transport in CNS neurons after intraventricular administration of NGF and growth factors. J. Comp. Neurol. 313: 680-692.

Figueiredo B.C., Piccardo P., Maysinger D., Clarke P.B.S., Cuello A.C. (1993) Effects of acidic fibroblast growth factor on cholinergic neurons of nucleus basalis magnocellularis and in a spatial memory task following cortical devascularization. Neuroscience 56: 955-963.

Figueiredo B., Tetzlaff W., Skup M., Garofalo L., Cuello A.C. (1993) Differential expression of $p140^{trk}$, $p75^{NGFR}$ and GAP-43 genes in neurons of nucleus basalis magnocellularis following cortical devascularization. Soc. Neurosci. Abstr. 19: 1305.

Frim D.M., Uhler T.A., Short M.P., Ezzedine Z.D., Klagsbrun M., Breakefield X.O., Isacson O. (1993) Effects of biologically delivered NGF, BDNF and bFGF on striatal excitotoxic lesions. NeuroReport 4: 367-370.

Godfrey E.W., Shooter E.M. (1986) Nerve growth factor receptors on chick embryo sympathetic ganglion cells: binding characteristics and development. J. Neurosci. 6: 2543-2550.

Green S.H., Greene L.A. (1986) A single M_r=103,000 ^{125}I-β-nerve growth factor affinity-labeled species represents

both the low and high affinity forms of the nerve growth factor receptor. J. Biol. Chem. 261: 15316-15326.

Hallböök F., Ibáñez C.F., Persson H. (1991) Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. Neuron 6: 845-858.

Hefti F., Hartikka J., Knüsel B. (1989) Function of neurotrophic factors in the adult and aging brain and their possible use in the treatment of neurodegenerative diseases. Neurobiol. Aging 10: 515-533.

Hefti F., Knüsel B., Lapchak P.A. (1993) Protective effects of NGF and BDNF on basal forebrain cholinergic neurons in adult rats with partial fimbrial transections. Prog. Brain Res. 98: 257-263.

Hempstead B.L., Martin-Zanca D., Kaplan D.R., Parada L.F., Chao M.V. (1991) High-affinity NGF binding requires coexpression of the *trk* proto-oncogene and the low affinity NGF receptor. Nature 350: 678-683.

Hempstead B.L., Schleifer L.S., Chao M.V. (1989) Expression of functional nerve growth factor receptors after gene transfection. Science 243: 373-375.

Henderson C.E., Camu W., Mettling C., Gouin A., Poulsen K., Karihaloo M., Rullamas J., Evans T., McMahon S.B., Armanini M.P., Berkemeier L., Phillips H.S., Rosenthal A. (1993) Neurotrophins promote motor neuron survival and are present in embryonic limb bud. Nature 363: 266-270.

Hosang M., Shooter E.M. (1985) Molecular characteristics of nerve growth factor receptors in PC12 cells. J. Biol. Chem. 260: 655-662.

Hyman C., Hofer M., Barde Y.-A., Juhasz M., Yancopoulos G.D., Squinto S.P., Lindsay R.M. (1991) BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 350: 230-232.

Hyman C., Juhasz M., Jackson C., Wright P., Ip N.Y., Lindsay R.M. (1994) Overlapping and distinct actions of the neurotrophins, BDNF, NT-3 and NT-4/5, on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. J. Neurosci. 14: 335-347.

Ibáñez C.F., Ebendal T., Barbany G., Murray-Rust J., Blundell T.L., Persson H. (1992) Disruption of the low affinity receptor-binding site in NGF allows neuronal survival and differentiation by binding to the *trk* gene product. Cell 69: 329-341.

Ip N.Y., Ibáñez C.F., Nye S.H., McClain J., Jones P.F., Gies D.R., Belluscio L., Le Beau M.M., Espinosa R., 3-rd, Squinto S.P., Persson H., Yancopoulos G.D. (1992) Mammalian neurotrophin-4: Structure, chromosomal localization, tissue distribution, and receptor specificity. Proc. Natl. Acad. Sci. USA 89: 3060-3064.

Ip N.Y., Li Y., Yancopoulos G.D., Lindsay R.M. (1993) Cultured hippocampal neurons show responses to BDNF, NT-3, and NT-4, but not NGF. J. Neurosci. 13: 3394-3405.

Johnson J.E., Barde Y.-A., Schwab M., Thoenen H. (1986) Brain-derived neurotrophic factor (BDNF) supports the survival of cultured rat retinal ganglion cells. J. Neurosci. 6: 3031-3038.

Johnson D., Lanahan A., Buck C.R., Sehgal A., Morgan C., Mercer E., Bothwell M., Chao M. (1986) Expression and structure of the human NGF receptor. Cell 47: 545-554.

Johnson E.M., Jr., Taniuchi M., DiStefano P.S. (1988) Expression and possible function of nerve growth factor receptors on Schwann cells. Trends Neurosci. 11: 299-304.

Kiss J., McGovern J., Patel A.J. (1988) Immunohistochemical localization of cells containing nerve growth factor receptors in the different regions of the adult rat forebrain. Neuroscience 27: 731-748.

Klein R., Conway D., Parada L.F., Barbacid M. (1990) The trkB tyrosine protein kinase gene codes for a second neurogenic receptor that lacks the catalytic kinase domain. Cell 61: 647-656.

Klein R., Jing S., Nanduri V., O'Rourke E., Barbacid M. (1991a) The *trk* proto-oncogene encodes a receptor for nerve growth factor. Cell 65: 189-197.

Klein R., Nanduri V., Jing S., Lamballe F., Tapley P., Bryant S., Cordon-Cardo C., Jones K.R., Reichardt L.F., Barbacid M. (1991b) The *trk*B tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. Cell 66: 395-403.

Knüsel B., Beck K.D., Winslow J.W., Rosenthal A., Burton L.E., Widmer H.R., Nickolics K., Hefti F. (1992) Brain-derived neurotrophic factor administration protects basal forebrain cholinergic but not nigral dopaminergic neurons from degnerative changes after axotomy in the adult rat brain. J. Neurosci. 12: 4391-4402.

Knüsel B., Winslow J.W., Rosenthal A., Burton L.E., Seid D.P., Nikolics K., Hefti F. (1991) Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neurotrophic factor but not neurotrophin 3. Proc. Natl. Acad. Sci. USA 88: 961-965.

Korsching S., Auburger G., Heumann R., Scott J., Thoenen H. (1985) Levels of nerve growth factor and its mRNA in the central nervous system of the rat correlate with cholinergic innervation. EMBO J. 4: 1389-1393.

Kroin J.S., Sobreviela T., Penn R.D., Mufson E.J. (1993) Retrograde transport of rhBDNF following cortical, amygdaloid, hippocampal and striatal infusion in rat. Soc. Neurosci. Abstr. 19: 260.

Lamballe F., Klein R., Barbacid M. (1991) *Trk*C, a new member of the *trk* family of protein kinases, is a receptor for neurotrophin-3. Cell 66: 967-979.

Lapchak P.A., Araujo D.M., Hefti F. (1993a) BDNF and trkB mRNA expression in the rat hippocampus following entorhinal cortex lesions. NeuroReport 4: 191-194.

Lapchak P.A., Araujo D.M., Hefti F. (1993b) Cholinergic regulation of hippocampal brain-derived neurotrophic factor mRNA expression: evidence from lesion and chronic cholinergic drug treatment studies. Neuroscience 52: 575-585.

Lapchak P.A., Beck K.D., Araujo D.M., Irwin I., Langston J.W., Hefti F. (1993c) Chronic intranigral administration of brain-derived neurotrophic factor produces striatal dopaminergic hypofunction in unlesioned adult rats and fails to attenuate the decline of striatal dopaminergic function following medial forebrain bundle transection. Neuroscience 53: 639-650.

Lee K.-F., Huber J., Landis S.C., Sharpe A.H., Chao M.V., Jaenisch R. (1992) Target mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral nervous system. Cell 69: 737-749.

Leibrock J., Lottspeich F., Hohn A., Hofer M., Hengerer B., Masiakowski P., Thoenen H., Barde Y.-A. (1989) Molecular cloning and expression of brain-derived neurotrophic factor. Nature 341: 149-152.

Levi-Montalcini R. (1987) The nerve growth factor 35 years later. Science 237: 1154-1162.

Lindsay R.M., Altar C.A., Cedarbaum J.M., Hyman C., Wiegand S.J. (1993) The therapeutic potential of neurotrophic factors in the treatment of Parkinson's disease. Exp. Neurol. 124: 103-118.

Lindsay R.M., Thoenen H., Barde Y.-A. (1985) Placode and neural crest-derived sensory neurons are responsive at early developmental stages to brain-derived neurotropic factor (BDNF). Dev. Biol. 112: 319-328.

Lindvall O., Ernfors P., Bengzon J., Kokaia Z., Smith M.-L., Siesjö B.K., Persson H. (1992) Differential regulation of mRNAs for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3 in the adult rat brain following cerebral ischemia and hypoglycemic coma. Proc. Natl. Acad. Sci. USA 89: 648-652.

Loeb D.M., Maragos J., Martin-Zanca D., Chao M., Parada L.F., Greene L.A. (1991) The trk proto-oncogene rescues NGF responsiveness in mutant NGF-nonresponsive PC12 cell lines. Cell 66: 961-966.

Maisonpierre P.C., Belluscio L., Squinto S., Ip N.Y., Furth M.E., Lindsay R.M., Yancopoulos G.D. (1990) Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. Science 247: 1446-1451.

Martin-Zanca D., Hughes S.H., Barbacid M. (1986) A human oncogene formed by the fusion of truncated tropomyosin and protein kinase sequences. Nature 319: 743-748.

Martin-Zanca D., Oskam R., Mitra G., Copeland T., Barbacid M. (1989) Molecular and biochemical characterization of the human *trk* proto-oncogene. Mol. Cell Biol. 9: 24-33.

Massague J., Guilette B.J., Czech M.P., Morgan C.J., Bradshaw R.A. (1981) Identification of a nerve growth factor receptor protein in sympathetic ganglia membranes by affinity labeling. J. Biol. Chem. 256: 9419-9424.

Meakin S.O., Suter U., Drinkwater C.C., Welcher A.A., Shooter E.M. (1992) The rat *trk* protooncogene product exhibits properties characteristic of the slow nerve growth factor receptor. Proc. Natl. Acad. Sci. USA 89: 2374-2378.

Merlio J.-P., Ernfors P., Jaber M., Persson H. (1992) Molecular cloning of rat *trk*C and distribution of cells expressing messenger RNAs for members of the *trk* family in the rat central nervous system. Neuroscience 51: 513-532.

Morse J.K., Wiegand S.J., Anderson K., You Y., Cai N., Carnahan J., Miller J., DiStefano P.S., Altar C.A., Lindsay R.M., Alderson R.F. (1993) Brain-derived neurotrophic factor (BDNF) prevents the degeneration of medial septal cholinergic neurons following fimbria transection. J. Neurosci. 13: 4146-4156.

Ohmichi M., Decker S.J., Pang L., Saltiel A.R. (1991) Nerve growth factor binds to the 140 kd *trk* proto-oncogene product and stimulates its association with the *src* homology domain of phospholipase C gamma1. Biochem. Biophys. Res. Commun. 179: 217-223.

Olson L. (1993) NGF and the treatment of Alzheimer's Disease. Exp. Neurol. 124: 5-15.

Phillips H.S., Hains J.M., Laramee G.R., Rosenthal A., Winslow J.W. (1990) Widespread expression of BDNF but not NT3 by target areas of basal forebrain cholinergic neurons. Science 250: 290-294.

Pioro E.P., Cuello A.C. (1990) Distribution of nerve growth factor receptor-like immunoreactivity in the adult rat central nervous system: Effect of colchicine and correlation with the cholinergic system-I.Forebrain. Neuroscience 24: 57-87.

Pioro E.P., Ribeiro-da-Silva A., Cuello A.C. (1990) Immunoelectron microscopic evidence of nerve growth factor receptor metabolism and internalization in rat nucleus basalis neurons. Brain Res. 527: 109-115.

Rabizadeh S., Oh J., Zhong L.-T., Yang J., Bitler C., Butcher L., Bredesen D. (1993) Induction of apoptosis by the low-affinity NGF receptor. Science 261: 345-348.

Radeke M.J., Misko T.P., Hsu C., Herzenberg L.A., Shooter E.M. (1987) Gene transfer and molecular cloning of the rat nerve growth factor receptor. Nature 325: 593-597.

Raivich G., Kreutzberg G.W. (1987) The localisation and distribution of high affinity -nerve growth factor binding sites in the central nervous sytem of the adult rat. A light microscopic autoradiographic study using [^{125}I] β-nerve growth factor. Neuroscience 20: 23-36.

Richardson P.M., Verge Issa V.M.K., Riopelle R.J. (1986) Distribution of neuronal receptors for nerve growth factor in the rat. J. Neurosci. 6: 2312-2321.

Rodriguez-Tébar A., Dechant G., Barde Y.-A. (1990) Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. Neuron 4: 487-492.

Rodriguez-Tébar A., Dechant G., Götz R., Barde Y.-A. (1992) Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. EMBO J. 11: 917-922.

Rosenthal A., Goeddel D.V., Nguyen T., Lewis M., Shih A., Laramee G.R., Nikolics K., Winslow J.W. (1990) Primary structure and biological activity of a novel human neurotrophic factor. Neuron 4: 767-773.

Schechter A.L., Bothwell M.A. (1981) Nerve growth factor receptors: evidence for two receptor classes with differing cytoskeletal association. Cell 24: 867-874.

Segal R.A., Takahashi H., McKay R.D.G. (1992) Changes in neurotrophin responsiveness during the development of cerebellar granule neurons. Neuron 9: 1041-1052.

Silos-Santiago I., Zhang L., Snider W.D., Klein R., Barbacid M., Smeyne R.J. (1993) Characterisation of the dorsal root afferent projection to spinal cord in the trkB "knock-out" mouse. Soc. Neurosci. Abstr. 19: 1100.

Skup M., Figueiredo B., Albert P., Cuello A.C. (1993) BDNF and NT-3 do not prevent cholinergic neurons of the nucleus basalis magnocellularis from atrophy caused by cortical devascularization. Soc. Neurosci. Abstr. 19: 657.

Skup M., Figueiredo B., Cuello A.C. (1994) Intraventricularly applied BDNF and NT-3 failed to protect nbm cholinergic neurons. NeuroReport 5:

Soppet D., Escandon E., Maragos J., Middlemas D.S., Reid S.W., Blair J., Burton L.E., Stanton B.R., Kaplan D.R., Hunter T., Nikolics K., Parada L.F. (1991) The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the *trk*B tyrosine kinase receptor. Cell 65: 895-903.

Squinto S.P., Stitt T.N., Aldrich T.H., Davis S., Bianco S.M., Radziejewski C., Glass D.J., Masiakowski P., Furth M.E., Valenzuela D.M., DiStefano P.S., Yancopoulos G.D. (1991) *trk*B encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. Cell 65: 885-893.

Sutter A., Riopelle L.F., Harris-Harrik R.M., Shooter E.M. (1979) Nerve growth factor receptors: characterization of two distinct classes of binding sites on chick embryo sensory gnglia cells. J. Biol. Chem. 254: 5972-5982.

Taniuchi M., Clark H.B., Schweitzer J.B., Johnson E.M., Jr. (1988) Expression of nerve growth factor receptors by Schwann cells of axotomized peripheral nerves: ultrastructural location, suppression by axonal contact, and binding properties. J. Neurosci. 8: 664-681.

Taniuchi M., Johnson E.M., Jr. (1985) Characterization of the binding properties and retrograde axonal transport of a monoclonal antibody directed against the nerve growth factor receptor. J. Cell Biol. 101: 1100-1106.

Taniuchi M., Schweitzer J.B., Johnson E.M., Jr. (1986) Nerve growth factor receptor molecules in rat brain. Proc. Natl. Acad. Sci. USA 83: 1950-1954.

Thomas L.B., Book A.A., Schweitzer J.B. (1991) Immunohistochemical detection of a monoclonal antibody directed against the NGF receptor in basal forebrain neurons following intraventricular injection. J. Neurosci. Methods 37: 37-45.

Vazquez M.E., Ebendal T. (1991) Messenger RNAs for *trk* and the low affinity NGF receptor in basal forebrain. NeuroReport 2: 593-596.

Weskamp G., Reichardt L.F. (1991) Evidence that biological activity of NGF is mediated through a novel subclass of high affinity receptors. Neuron 6: 649-663.

Wetmore C., Ernfors P., Persson H., Olson L. (1990) Localization of brain-derived neurotrophic factor mRNA to neurons in the brain by *in situ* hybridization. Exp. Neurol. 109: 141-152.

Whittemore S.R., Seiger . (1987) The expression, localization and functional significance of -nerve growth factor in the central nervous system. Brain Res. Rev. 12: 439-464.

Widmer H.R., Knüsel B., Hefti F. (1993) BDNF protection of basal forebrain cholinergic neurons after axotomy: complete protection of p75NGFR-positive cells. NeuroReport 4: 363-366.

Wong V., Arriaga R., Ip N.Y., Lindsay R.M. (1993) The neurotrophins BDNF, NT-3 and NT-4/5, but not NGF, upregulate the cholinergic phenotype of developing motor neurons. Eur. J. Neurosci. 5: 466-474.

Yan Q., Johnson E.M., Jr. (1988) An immunohistochemical study of nerve growth factor receptor in developing rats. J. Neurosci. 8: 3481-3498.

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