Small molecule activators of the Trk receptors for neuroprotection
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Abstract

The neurotrophin signaling network is critical to the development and survival of many neuronal populations. Especially sensitive to imbalances in the neurotrophin system, cholinergic neurons in the basal forebrain are progressively lost in Alzheimer’s disease. Therapeutic use of neurotrophins to prevent this loss is hampered, however, by a number of pharmacological challenges. These include a lack of transport across the blood-brain barrier, rapid degradation in the circulation, and difficulty in production. In this review we discuss the evidence supporting the neurotrophin system’s role in preventing neurodegeneration and survey some of the pharmacological strategies being pursued to develop effective therapeutics targeting neurotrophin function.

Background

The neurotrophins and their receptors

Nerve growth factor (NGF) was the first neurotrophin discovered and is the prototypical member of a family of structurally related proteins including brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, and NT-4/5. Neurotrophins have been identified in many vertebrate lineages, including reptiles, amphibians, fish, birds, and mammals [1]. Not all neurotrophins have been found in all species; NT-4/5 has not been found in chicken and NT-6 and -7 are found only in fish. More distantly related genes have been found in the Agnatha species lamprey and hagfish. Phylogenetic analysis indicates that NGF and NT-3 form a subfamily as do BDNF and NT-4/5. The neurotrophins bind two classes of receptor, the common p75NTR, which is a member of the tumor necrosis factor receptor family [2,3], and the tropomyosin-related kinase (Trk) receptors, members of the large tyrosine kinase receptor family [4]. Three Trk receptors have been identified in vertebrates, and more distantly related receptors have been found in lamprey and amphioxus. TrkA and TrkC form a distinct phylogenetic subfamily [1]. All neurotrophins bind p75NTR with similar affinity (Kd~10-9 M), but binding to the Trk receptors is more selective. NGF binds the TrkA receptor, BDNF and NT-4/5 bind the TrkB receptor, and NT-3 primarily binds the TrkC receptor [5]. These binding specificities are consistent with the phylogenetic separation into distinct subfamilies. NT-6 and -7 are more closely related to NGF than the other neurotrophins and bind TrkA [1]. The common p75NTR also enhances binding of NGF to TrkA receptors to create high affinity binding sites (Kd~10-11 M), possibly by binding to
different surfaces on the NGF dimer [6]. p75NTR can also bind precursor forms of the neurotrophins. For example, pro-NGF does not bind TrkA, but causes cell death by binding to p75NTR [7,8].

These neurotrophins function to support the growth and survival of many neuronal populations [5,9-11]. NGF-deficient mice die shortly after birth and show a decrease in sensory and sympathetic neurons, but basal forebrain cholinergic neurons develop relatively normally. However, this perinatal lethality can be rescued by transgenic expression of NGF under the K14 keratin promoter, which restores the sensory and sympathetic neuronal populations [12]. These rescued mice exhibit reduced cholinergic innervation in the cortex and hippocampus, but this can be restored by intracerebroventricular delivery of NGF [13]. Disruption of a single NGF allele causes deficits in memory acquisition and hippocampal cholinergic innervation, suggesting that NGF is required for the formation and maintenance of correct innervations [14]. BDNF-deficient mice also die shortly after birth due to defects in brain and sensory, but not motor, neuron development [15]. Long-term potentiation and mechanosensation are impaired in heterozygous BDNF knockout animals [16,17]. NT-3 deficient mice show severe movement defects and die shortly after birth with complete absence of spinal proprioceptive afferents [18]. The number of muscle spindles was reduced in heterozygous NT-3 knockout mice. NT-3 deficiency also causes a decrease in the number of sympathetic cervical ganglion neurons, lingual innervations, and sensory neuron precursor cells [19,20].

The three Trk receptors show distinct patterns of expression throughout the mammalian brain and peripheral nervous system [4,21-24]. TrkA is expressed exclusively by cholinergic neurons in the basal forebrain. TrkB and TrkC are highly expressed in the hippocampus. In the peripheral nervous system, these receptors are expressed on overlapping sets of sensory and motor neurons. None of the Trk receptors are essential for embryonic development and knockout mice are born in the expected ratios. However, the mice fail to thrive and die shortly after birth. TrkA-deficient mice show a marked loss of cholinergic neurons in the forebrain and sensory and sympathetic neurons in the trigeminal, superior cervical, and dorsal root ganglia [25-28]. Mice lacking TrkB show decreased synaptogenesis and mossy fiber maturation in the hippocampus, as well as severe sensory deficits in the vestibular and cochlear ganglia [29-31]. TrkC-deficient mice also show decreased hippocampal synaptogenesis and display abnormal movements due to loss of proprioception and muscle afferents [30-32]. Heterozygosity for a trk allele permits survival, but structural alterations are apparent in aged mice [14,33,34]. TrkC is also expressed on non-neuronal cells and supports glial development [35]. Multiple alternatively spliced isoforms have been observed for TrkA, TrkB, and TrkC, especially in non-neuronal cells [36-39]. Some of these isoforms lack the cytoplasmic tyrosine kinase domain, but retain selective signaling and may inhibit neurite outgrowth [37,40,41].

Due to their ability to protect multiple neuronal cell types from apoptosis, there is considerable interest in whether neurotrophins can stimulate neuronal regeneration in vitro and in model systems [42-45]. If neurotrophins can prevent or reverse neuronal cell loss, they would make good therapeutic targets in neurodegenerative diseases, and in brain or spinal cord injuries [46,47]. One complication, however, is that neurotrophins also bind to p75NTR. Activation of this receptor may cause cell death rather than survival, as p75NTR/-/- mice show reductions in neuronal cell death after pilocarpine-induced seizures compared to wild-type [48,49]. Therefore, the final effect of a neurotrophin is a balance between the cell survival signal derived from the Trk receptor family and the cell death signal from p75NTR. Indeed, neurotrophins cause cell death in approximately 30% of cultured hippocampal neurons expressing p75, but which lack the cognate Trk receptor, potentially limiting the therapeutic utility of the neurotrophins themselves [50].

**Neurotrophins, Trks, and neurodegeneration**

In Alzheimer’s disease (AD), one of the most severely affected systems is the cholinergic neurons projecting from the basal forebrain to the neocortex. There is a strong correlation between the loss of these cholinergic neurons and the loss of memory [51]. Cholinesterase inhibitors can prevent the breakdown of acetylcholine that results in the partial restoration of memory and decreased confusion, but despite this initial improvement, as more cholinergic neurons are lost, there is a continual, gradual loss of function. Many reports have documented the ability of NGF to improve cholinergic function in vitro and to prevent lesion-induced degeneration in rodents and primates, as well as age-associated declines in rats and primates [46,50,51]. NGF is expressed in a number of cell types in the brain, including astrocytes, but not in microglia or oligodendrocytes [52]. Correlative studies have found defects in the NGF system in early stages of AD that may indicate a causative role. NGF mRNA levels are not altered in AD. Total NGF protein is relatively normal, but the ratio of proNGF to mature NGF is increased [53,54]. TrkA expression is reduced, but p75NTR amounts are unchanged in AD [55,56]. The reduction in TrkA suggests a relative deficit in NGF signaling, as NGF positively regulates trkA gene expression. proNGF may increase apoptotic signaling via p75NTR, altering the balance between neuronal survival and death. The importance of NGF is underscored by studies in a transgenic model of neurode-
generation. Mice expressing blocking antibodies to NGF, specifically in the adult brain, showed reduced cholinergic innervations of the cortex and impairments in synaptic plasticity [57]. Similarly, short-term neonatal administration of anti-NGF antibodies by intracerebroventricular (i.c.v.) injection in mice leads to altered exploratory behavior on a hole board [58]. BDNF may play an important role too. In AD, not only are BDNF mRNA and protein levels decreased in basal forebrain, cholinergic neuron target tissues – for example, cortex and hippocampus [59] – but local levels of BDNF in the nucleus basalis are reduced [60]. Hippocampal BDNF levels are increased by exercise, thought to delay decline in patients with AD [61]. Adenoviral expression of BDNF enhances neuronal plasticity, increases long-term potentiation formation, and improves performance on behavioral tests in a rat model with cognitive deficits [62]. NT-3 levels in the motor cortex are also lower in AD patients than controls [63], but other regions appear unaltered [64,65]. Furthermore, the receptors TrkB and TrkC are decreased in cholinergic basal forebrain neurons in AD [55].

Neurotrophins may also be a promising therapy for neuroprotection in traumatic brain injury. In a study of 14 children with severe traumatic brain injury, NGF levels in the cerebrospinal fluid were normal 2 hours post trauma, but increased 5-fold at 24 hours post trauma [66]. In contrast, BDNF levels were elevated >25-fold 2 hours post trauma, but decreased to 3-fold of normal at 24 hours. Higher NGF levels at 24 hours post-trauma correlated with better clinical outcome. These elevations in neurotrophins have also been seen in rodent models of brain injury and are thought to be a protective mechanism to minimize neuronal loss [67-69]. Kainate-induced injury increases BDNF and NGF levels in the hippocampus of Fisher 344 rats [69]. The increase in BDNF was diminished in aged rats, suggesting reduced spontaneous healing with aging [70,71]. Cortical and hippocampal NT-4/5 levels are elevated in rats subjected to a lateral fluid percussion injury, but did not improve performance on behavioral tests [83]. The beneficial effects of neurotrophins may not be limited to neuronal survival, as BDNF reduces blood-spinal cord barrier permeability following spinal cord injury, and reduces leakage of serum proteins [84]. BDNF, and its receptor TrkB, are also required for the anti-depressant effect of imipramine and fluoxetine in rodent models, suggesting that neurotrophin therapy may also have beneficial effects in post-traumatic stress and other depressive disorders [85,86].

**Neurotrophin therapy**

In preclinical and clinical findings, neurotrophins are thought to be a promising therapy for peripheral neuropathies and neurodegenerative diseases, including AD [87] and Parkinson’s disease [88]. However, neurotrophins do not make good drug candidates due to their poor pharmacokinetic behavior and bioavailability at the desired targets. One of the major hurdles for neurotrophin therapy is the lack of passage of peptide hormones across the blood-brain barrier [89,90]. Peripheral administration of peptide hormones only leads to a small increase in their intracerebral concentration. This has necessitated complicated methods of delivery, such as via the olfactory neural pathway [91-93], *ex vivo* gene therapy by intracranial injection of NGF-expressing fibroblasts [94], or placement of indwelling catheters to allow neurotrophin infusion. Intranasal administration of NGF rescues memory defects in a mouse model of AD [95]. Preliminary trials of i.c.v. infusion of recombinant mNGF for 3 months have shown some benefit in small numbers of AD patients [96]. *Ex vivo* gene therapy using the patients’ own fibroblasts infected with a retrovirus to express human NGF is currently being tested in phase I trials, but recent problems associated with gene therapy make it unlikely that such an approach will succeed in the near future [94].

As a result, considerable effort has been devoted to finding neurotrophin peptidomimetics. These are small molecules that mimic the binding of selective peptides and elicit the desired neuroregenerative responses of neurotrophins. Much of this work is driven by the crystal structures of the neurotrophins and their receptors, and structure-function studies of small peptides [97,98]. NGF
contacts the TrkA receptor through residues in β-hairpin loops 2 and 4, and residues in the amino- and carboxyl termini. A dimeric, cyclized peptide derived from loop 4 activates TrkA and has NGF-like neurotrophic effects [99]. Other β-turn mimetics have also been developed and optimized for neurotrophic activities in vitro [100,101]. NGF contacts p75NTR through residues in loop 1 and peptidomimetics have been developed that block the binding of NGF, preventing apoptotic cell death driven by proNGF activation of p75NTR [102]. Taking a slightly different approach, small peptides have also been developed that prevent the association of the Trk receptors with cellular tyrosine phosphatases. Treatment of PC12 cells with a cell-permeable version of the leukocyte common antigen receptor (a tyrosine phosphatase), expressed in neurons, causes neurite outgrowth in a TrkA-dependent manner, presumably by preventing de-phosphorylation of TrkA [103]. A peptidomimetic, cerebrolysin (N-PEP-12) from Ebewe Pharma, is showing promise in phase II clinical trials [104].

Small molecule, non-peptide neurotrophic factors

The development of small molecules promoting neurotrophic function has been recently reviewed [105]. These are small molecules that can alter neurotrophin function in a manner independent of ligand binding to receptor. The mechanisms can be varied. Some agents modulate the expression of the neurotrophins themselves, while others act by enhancing neurotrophin action. In the former class, a purine-hypoxanthine derivative, leteprinim (Neotrofin), has been shown to elevate neurotrophin levels (including NGF) in animals, and caused increases in acetylcholinesterase labeling in lesioned rats [106]. In humans with mild-to-moderate AD, this molecule improved cognitive scores in memory, executive function, and attention tests [107]. It also increased metabolic rate especially in the cerebellum, and sensory and prefrontal cortices. Leteprinim showed promise in a phase II clinical trial for the treatment of AD but is now being developed for peripheral neuropathy. Similarly, retinoids, AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor agonists, selective serotonin reuptake inhibitors, serotonin/norepinephrine reuptake inhibitors, and tricyclic antidepressants all increase neurotrophin expression [105].

As for the agents acting by enhancing neurotrophin action, xaliproden is a combined NGF potentiator and serotonin 5-HT1A receptor agonist that also reduces chemotherapeutic-induced peripheral sensory neuropathy but has yet to show efficacy in cognitive decline in AD. Its ability to enhance NGF action in vitro is mimicked by tyrosine kinase inhibitors [108]. This is reminiscent of the enhancement of NGF action by inhibitors of mixed-lineage kinases (MLKs), for example, K252a, CEP1347, BMS355249, or L-753,000 [109-112]. For many years, K252a was thought to be a weak TrkA inhibitor, but was subsequently shown to be a potent inhibitor of the MLKs. More specific MLK inhibitors that lack the TrkA inhibitory activity can still enhance neurotrophin action. Nitric oxide donors also contribute to neuronal survival through transactivation of TrkA [113-115]. The protective effect requires cyclic GMP and protein kinase G, but the downstream target is not known. Activation of G-protein coupled receptors by adenosine, or pituitary adenylate cyclase activating peptide (PACAP), also leads to transactivation of TrkA, stimulation of downstream signaling, and neuroprotection [116,117]. These effects may be related to the known redox regulation of TrkA. Antioxidants, such as N-acetylcysteine, blocks NGF-induced neuronal differentiation, whereas buthionine sulfoximine, which reduces cellular glutathione levels, enhances neuronal differentiation [118].

Other studies have focused on small molecule, direct activators of the TrkA receptor. A cell-based chemical genetic screen for compounds that can protect SN56 neuroblastoma cells against apoptosis in a TrkA-dependent manner identified gambogic amide as a selective agonist for TrkA [119]. Gambogic amide is the major active ingredient of gamboge, a traditional Chinese medicine. This compound binds TrkA, but not TrkB or TrkC, and causes receptor dimerization and phosphorylation. Gambogic amide stimulates neurite outgrowth in PC12 cells, reduces neuronal cell death in a kainate-induced seizure model, and reduces infarct volume in a middle cerebral artery occlusion (MCAO) stroke model [119].

Our studies have focused on the asterriquinone class of compounds. The asterriquinones are naturally occurring bis-indolyl-dihydroxyquinones that were originally identified as activators of the insulin receptor [120,121]. The molecules are small and readily cell-permeable, and act directly on the receptor tyrosine kinase domain, although its mechanism of activation is not known. The original compound, demethylasterriquinone-B1 (DAQ-B1), was demonstrated to cross the blood-brain barrier and activate hypothalamic signaling when given orally [122]. Therefore, we hypothesized that similar compounds could potentially activate signaling in the central nervous system and be used as oral NGF activators for neurotrophin therapy. These compounds would have the very important additional advantage of targeting the kinase domain of TrkA and therefore not activating the p75NTR receptor. To facilitate identification of TrkA activators, we developed a combinatorial library of structurally related asterriquinones and screened them against TrkA [123]. Out of 334 asterriquinones screened, we identified 35 that had agonist activity >50% that of NGF. Dose-dependent toxicity...
was also measured for this library and TrkA activation and toxicity were modeled mathematically using quantitative structure activity relationship (QSAR) models. There was no correlation between activation of TrkA and cellular viability, suggesting that toxicity is not dependent on agonist activity. Based on the library screen, we picked compound 5E5 for further evaluation. This compound is a potent activator of TrkA (200% the activity of NGF) and a partial activator of TrkC (80% the activity of NT-3), but does not appreciably stimulate TrkB or the insulin receptor. Interestingly, this compound has additive effects with NGF and at low doses is able to potentiate the effect of NGF to activate TrkA and downstream signaling. 5E5 enhances neurite outgrowth and neuronal differentiation of PC12 cells in the presence of a low dose of NGF that alone is inefficient at promoting neurite outgrowth.

Conclusion
Progress continues in the development of strategies for neuroprotection in AD. Both the central role for neurotrophins in mediating neuronal survival, and the positive effects using recombinant NGF or peptidomimetics in small clinical trials, suggest that the neurotrophin system would be a good therapeutic target in AD. Multiple approaches are being pursued, though many have their limitations. Peptidomimetics offer greater specificity, but are less readily delivered. Small molecule neurotrophin mimetics are easily delivered, but less selective, and may have non-Trk-mediated side effects. In the end, molecules potentiating the effect of endogenous neurotrophins are particularly appealing as this may avoid the potential complications of systemic neurotrophin stimulation. In this way, neurotrophin action would only be enhanced where endogenous neurotrophins are found. Many of these studies establish proof-of-principle, and it is hoped that they will spur development of second and third generation therapeutics with better selectivity and potency.

List of abbreviations used
AD: Alzheimer’s disease; AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF: brain-derived neurotrophic factor; i.e.v.: intracerebroventricular; MLK: mixed-lineage kinase; NGF: nerve growth factor; NT: neurotrophin; Trk: tropomysin-related kinase.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
NJGW tested the NGF mimics and wrote the manuscript and MCP synthesized the NGF mimics.

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References
1. Lanave C, Colangelo AM, Saccone C, Alberghina L: Molecular evolution of the neurotrophin family members and their Trk receptors. Gene 2000, 249:11-12.
2. Dechant G, Barde YA: The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. Nat Neurosci 2002, 5:131-136.
3. Bothwell M: Functional interactions of neurotrophins and neurotrophin receptors. Annu Rev Neurosci 1995, 18:223-25.
4. Lewin GR, Barde YA: Physiology of the neurotrophins. Annu Rev Neurosci 1996, 19:289-317.
5. Patapoutian A, Reichardt LF: Trk receptors: mediators of neurotrophin action. Curr Opin Neurobiol 2001, 11:272-280.
6. Hennebost EL, Martin-Zanca D, Kaplan DR, Paradis LF, Chao MY: High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. Nature 1991, 350:678-683.
7. Podlesny P, Kichev A, Pedraza C, Saurat J, Encinas M, Perez B, Ferrer I, Espinet C: Pro-NGF from Alzheimer’s disease and normal human brain displays distinctive abilities to induce processing and nuclear translocation of intracellular domain of p75NTR and apoptosis. Am J Pathol 2006, 169:119-131.
8. Pedraza CE, Podlesny P, Vitali N, Arévalo JC, Lee R, Hempstead B, Ferrer I, Iglesias M, Espinet C: Pro-NGF isolated from the human brain affected by Alzheimer’s disease induces neuronal apoptosis mediated by p75NTR. Am J Pathol 2006, 166:533-543.
9. Friedman WJ, Greene LA: Neurotrophin signaling via Trks and p75S. Exp Cell Res 1999, 254:131-142.
10. Kaplan DR, Miller FD: Neurotrophin signal transduction in the nervous system. Curr Opin Neurobiol 2000, 10:381-391.
11. Kirstein M, Farinas I: Sensing life: regulation of sensory neuron survival by neurotrophins. Cell Mol Life Sci 2002, 59:1787-1802.
12. Harrison SM, Davis BH, Nishimura M, Albers KM, Jones MG, Phillips HS: Rescue of NGF-deficient mice I: transgenic expression of NGF in skin rescues mice lacking endogenous NGF. Brain Res Mol Brain Res 2004, 122:116-125.
13. Phillips HS, Nishimura M, Armanini MP, Chen K, Albers KM, Davis BH: Rescue of NGF-deficient mice II: basal forebrain cholinergic projections require NGF for target innervation but not guidance. Brain Res Mol Brain Res 2004, 124:1-11.
14. Chen KS, Nishimura MC, Armanini MP, Crowley C, Spencer SD, Phillips HS: Disruption of a single allele of the nerve growth factor gene results in atrophy of basal forebrain cholinergic neurons and memory deficits. J Neurosci 1997, 17:7288-7296.
15. Jones KR, Farinas I, Backus C, Reichardt LF: Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. Cell 1994, 98:999-999.
16. Carroll P, Lewin GR, Koltenburg M, Toyka KV, Thoenen H: A role for BDNF in mechanosensation. Nat Neurosci 1998, 1:42-46.
17. Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T: Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc Natl Acad Sci USA 1995, 92:8856-8860.
18. Ernfors P, Lee KF, Kucera J, Jaenisch R: Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. Cell 1994, 77:503-512.
19. El-Shamy WM, Linnarsson S, Lee KF, Jaenisch R, Ernfors P: Prenatal and postnatal requirements of NT-3 for sympathetic neuroblast survival and innervation of specific targets. Development 1996, 122:491-500.
20. Nostrat IV, Lindskog S, Seiger A, Nostrat CA: Lingual BDNF and NT-3 mRNA expression patterns and their relation to innervation in the human tongue: similarities and differences compared with rodents. J Comp Neurol 2000, 417:133-152.
21. Ockel M, von Schack D, Schropel A, Dechant G, Lewin GR, Barde YA: Roles of neurotrophin-3 during early development of the peripheral nervous system. Philos Trans R Soc Lond B Biol Sci 1996, 351:383-387.
22. Quartu M, Serra MP, Manca A, Follesa P, Ambro R, Del Fiacco M: High affinity neurotrophin receptors in the human pre-term newborn, infant, and adult cerebellum. Int J Dev Neurosci 2003, 21:309-320.

23. Aoki C, Wu K, Elste A, Len G, Lin S, McAuliffe G, Black IB: Localization of brain-derived neurotrophic factor and TrkB receptors to postsynaptic densities of adult rat cerebral cortex. J Neurosci Res 2000, 59:454-463.

24. Yan Q, Radeke MJ, Matheson CR, Talvenheimo J, Welcher AA, Feinstein SC: Immunocytochemical localization of TrkB in the central nervous system of the adult rat. J Comp Neurol 1997, 378:135-157.

25. Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S, Armanini MP, Ling LH, McMahon SB, Shelton DL, Levinson AD, et al.: Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 1994, 76:1001-1011.

26. Smeyne RJ, Klein R, Schnapp A, Long LK, Bryant S, Lewin A, Lira SA, Barbacid M: Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature 1994, 368:246-249.

27. Fagan AM, Garber M, Barbacid M, Silos-Santiago I, Holtzman DM: A role for Trk receptors in maturation of striatal and basal forebrain cholinergic neurons in vivo. J Neurosci 1997, 17:6744-6754.

28. Schober A, Minichiello L, Keller M, Huber K, Layer PG, Roig-Lopez JL, Garcia-Arraras JE, Klein R, Unsicker K: Reduced acetylcholinesterase (AChE) activity in adrenal medulla and loss of sympathetic preganglionic neurons in TrkA-deficient, but not TrkB-deficient, mice. J Neurosci 1997, 17:891-903.

29. Klein R, Smejne RJ, Wurst W, Long LK, Auerbach BA, Joyner AL, Barbacid M: Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. Cell 1993, 75:13-122.

30. Otal R, Martinez A, Soriano E: Lack of TrkB and TrkC signaling alters the synaptogenesis and maturation of mossy fiber terminals in the hippocampus. Cell Tissue Res 2003, 319:349-358.

31. Martinez A, Alcántara S, Borrell V, Del Rio JA, Blas J, Otal R, Campos N, Boronat A, Barbacid M, Silos-Santiago I, Soriano E: TrkB and TrkC signaling are required for maturation and synaptogenesis of hippocampal connections. J Neurosci 1998, 18:7336-7350.

32. Klein R, Silos-Santiago I, Smejne RJ, Lira SA, Brambilla R, Bryant S, Zhang L, Snider WD, Barbacid M: Disruption of the neurotrophin-3 receptor gene trkC eliminates the muscle afferent and alters behaviour in normal embryos. Nature 1994, 368:249-251.

33. Halbach O von Bohlen und, Minichiello L, Unsicker K: Haploinsufficiency for trkB and trkC receptors induces cell loss and accumulation of alpha-synuclein in the substantia nigra. FASEB J 2005, 19:1740-1742.

34. Halbach O von Bohlen und, Minichiello L, Unsicker K: Haploinsufficiency in trkB and/or trkC neurotrophin receptors causes structural alterations in the aged hippocampus and amygdala. Eur J Neurosci 2003, 19:2319-2325.

35. Kahn MA, Kumar S, Liebl D, Chang R, Parada LF, De Vellis J: Mice lacking NT-3, and its receptor TrkC, exhibit profound deficiencies in CNS glial cells. Glia 1999, 26:133-163.

36. Barker PA, Lomen-Hoerth C, Gensch EM, Mekin O, Glass DJ, Shooter EM: Tissue-specific alternative splicing generates two isoforms of the trkA receptor. J Biol Chem 1993, 268:15130-15136.

37. Tacconelli A, Farina AR, Cappabianca L, Desantis G, Tessitore A, Vescaschi A, Sforza R, Rucci N, Argenti B, Scarpinati I et al.: A new brain-derived neurotrophic factor transcript and decrease in brain-derived neurotrophic factor transcripts 1, 2 and 3 in Alzheimer’s disease: are we focusing on the wrong molecule? J Neural Transm Suppl 2002:241-252.

38. Berchtold NC, Kessler JP, Cotman CW: Hippocampal brain-derived neurotrophic factor gene regulation by exercise and the medial septum. J Neurosci Res 2002, 68:511-521.

39. Ando S, Kobayashi S, Waki H, Kon K, Fukui F, Tadenuma T, Iwamoto M, Takeda Y, Uzumiyama N, Watanabe K, Nakamura H: Animal model of dementia induced by entorhinal synaptic damage and partial restoration of cognitive deficits by BDNF and carbidopa. J Neurosci Res 2002, 70:519-527.

40. Nairnawa-Saito M, Wakabayashi K, Tsuji S, Takahashi H, Nawa H: Regional specificity of alterations in NGF, BDNF and NT-3 levels in Alzheimer’s disease. Neuroreport 1996, 7:2925-2928.

41. Hock C, Heese K, Müller-Spahn F, Huber P, Riesen W, Nitsch RM, Otten U: Increased cerebrospinal fluid levels of neurotrophin
3 (NT-3) in elderly patients with major depression. Mol Psychiatry. 2004, 9:510-513.
65. Murase K, Ikeda A, Hayashi K: Neurotrophin-3 (NT-3) levels in the developing rat nervous system and in human samples. Clin Chim Acta 1994, 227:23-36.
66. Chiaretti A, Piastra M, Polidori G, Di Rocco C, Caresta A, Antonelli A, Amendola T, Aloe L: Correlation between neurotrophic factor expression and outcome of children with severe traumatic brain injury. Intensive Care Med 2003, 29:1239-1338.
67. Hicks RR, Martin VB, Zhang L, Seroogy KB: Mild experimental brain injury differentially alters the expression of neurotrophin and neurotrophin receptor mRNAs in the hippocampus. Exp Neurol 1999, 160:469-478.
68. Felderhoff-Mueser U, Sfiringer M, Pedsitschek S, Kuckuck H, Moysisch A, Bittigau P, Ikonomidou C: Pathways leading to apoptotic neurodegeneration following trauma to the developing rat brain. Neurobiol Dis 2002, 11:231-245.
69. Shetty AK, Rao MS, Hattiangady B, Zaman V, Shetty GA: Hippocampal neurotrophin levels after injury: Relationship to the age of the hippocampus at the time of injury. J Neurosci Res 2004, 78:520-532.
70. Ruutger J, Panford-Walsh R, Schimmang T, Tan J, Zimmermann U, Robson M, Kondoh H, Limberger A, Muller M, Fraenzler J, et al.: BDNF mRNA expression and protein localization are changed in age-related hearing loss. Neurobiol Aging 2007, 28:586-601.
71. Silhó M, Bonnichon V, Rage F, Tapie-Arancibia L: Age-related changes in brain-derived neurotrophic factor and tyrosine kinase receptor isoforms in the hippocampus and hypothalamus in male rats. Neuroscience 2005, 132:613-624.
72. Royo NC, Conte V, Saatman KE, Shimiuzzu S, Belford CM, Soltesz KM, Davis JE, Fujimoto ST, McIntosh TK: Hippocampal vulnerability following traumatic brain injury: potential role for neurotrophin-4/5 in pyramidal cell neuroprotection. Eur J Neurosci 2006, 23:1089-1102.
73. Grundy PL, Patel N, Harbuz MS, Lightman SL, Sharpley PM: Glucocorticoids modulate the NGF mRNA response in the rat hippocampus after traumatic brain injury. Brain Res 2001, 892:386-390.
74. Grundy PL, Patel N, Harbuz MS, Lightman SL, Sharpley PM: Glucocorticoids modulate BDNF mRNA expression in the rat hippocampus after traumatic brain injury. Neuroreport 2000, 11:3381-3384.
75. Chang CN, Yang JT, Lee TH, Cheng WC, Hsu YH, Wu JH: Dexamethasone enhances upregulation of nerve growth factor mRNA expression in ischemic rat brain. J Clin Neurosci 2005, 12:60-684.
76. Yang JT, Lee TH, Weng HH, Chang CN, Chen WC, Cheng WC, Wu JH: Dexamethasone enhances NT-3 expression in rat hippocampus after traumatic brain injury. Exp Neurol 2005, 192:437-443.
77. Grundy PL, Patel N, Harbuz MS, Lightman SL, Sharpley PM: Adrenocortectomy further suppresses the NT-3 mRNA response to traumatic brain injury but this effect is not reversed with corticosterone. Brain Res Mol Brain Res 2004, 120:188-192.
78. Ouédraogo M, Hagg T: Nerve growth factor promotes regeneration of sensory axons into adult rat spinal cord. Exp Neurol 1996, 140:218-229.
79. Lu P, Yang H, Jones LL, Filbin MT, Tuszyński MH: Combinatorial therapy with neurotrophins and cAMP promotes axonal regeneration beyond sites of spinal cord injury. J Neurosci Res 2004, 76:640-6409.
80. Pettigrew DB, Li YQ, Kuntz CT, Crutchley KA: Global expression of NGF promotes sympathetic axonal growth in CNS white matter but does not alter its parallel orientation. Exp Neurol 1999, 156:203-109.
81. Dinocoort C, Gallagher SE, Thompson SM: Injury-induced axonal sprouting in the hippocampus is initiated by activation of trkB receptors. Eur J Neurosci 2006, 24:1857-1866.
82. Hattiangady B, Rao MS, Zaman V, Shetty AK: Incorporation of embryonic CA3 cell grafts into the adult hippocampus at 4 months after injury: effects of combined neurotrophic supplementation and caspase inhibition. Neuroscience 2006, 139:1369-1383.
83. Royo NC, Lebold D, Magge SN, Chen I, Hauspurg A, Cohen AS, Watson DJ: Neurotrophin-mediated neuroprotection of hippocampal neurons following traumatic brain injury is not associated with acute recovery of hippocampal function. Neuroreport 2007, 18:671-674.
103. Xie Y, Massa SM, Enslen-Craig SE, Major DL, Yang T, Tisi MA, Der-eyvany VD, Runge WO, Mehta BP, Moore LA, et al. Protein-tyrosine phosphatase (PTP) wedge domain peptides: a novel approach for inhibition of PTP function and augmentation of protein-tyrosine kinase function. *J Biol Chem* 2006, 281:16482-16492.

104. Alvarez XA, Cababelo R, Laredo M, Coueiro V, Sampedro C, Varela M, Corto L, Fernandez-Novoa L, Vargas M, Alexandere M, et al.: A 24-week, double-blind, placebo-controlled study of three dosages of Cerebrolysin in patients with mild to moderate Alzheimer’s disease. *Eur J Neurol* 2006, 13:43-54.

105. Price RD, Milne SA, Sharker J, Matsuoka N: Advances in small molecules promoting neurotrophic function. *Pharmacol Ther* 2007, 115:292-306.

106. Ramirezz JJ, Parakh T, George MN, Freeman L, Thomas AA, White CC, Becton A: The effects of Neotrofin on septodentate sprouting after unilateral entorhinal cortex lesions in rats. *Neuro Res* 2002, 28:51-59.

107. Potkin SG, Alva G, Keator D, Carreon D, Fleming K, Fallon JH: Brain metabolic effects of Neotrofin in patients with Alzheimer’s disease. *Brain Res* 2002, 951:87-95.

108. Pradines A, Magazin M, Schiltz P, Le Fur G, Caput D, Ferrara P: Evidence for nerve growth factor-potentiating activities of the nonpeptidic compound SR 57746A in PC12 cells. *J Neurochem* 1995, 64:1954-1964.

109. Wang LH, Paden AJ, Johnson EM Jr: Mixed-lineage kinase inhibitors require the activation of Trk receptors to maintain long-term neuronal tropism and survival. *J Pharmacol Exp Ther* 2005, 312:1007-1019.

110. Pollack S, Young L, Bilsland J, Wilkie N, Ellis S, Hefy F, Broughton H, Harper S: The staurosporine-like compound L-753,000 (NB-506) potentiates the neurotrophic effects of neurotrophin-3 by acting selectively at the TrkA receptor. *Mol Pharmacol* 1999, 56:185-195.

111. Roux PP, Dorval G, Boudreau M, Angers-Loustau A, Morris SJ, Makker J, Barker PA: K252a and CEP1347 are neuroprotective compounds that inhibit mixed-lineage kinase-3 and induce activation of Akt and ERK. *J Biol Chem* 2002, 277:49473-49480.

112. Lewis MA, Hunihan L, Franco D, Robertson B, Palmer J, Laurent DR, Balastrumman BN, Li Y, Westphal RS: Identification and characterization of compounds that potentiate NT-3-mediated Trk receptor activity. *Mol Pharmacol* 2006, 69:1396-1404.

113. Bennett BM, Reynolds JN, Prusky GT, Douglas RM, Sutherland RJ, Thatcher GR: Cognitive deficits in rats after forebrain cholinergic depletion are reversed by a novel NO mimetic nitrate ester. *Neuropsychopharmacology* 2007, 32:505-513.

114. Culfssse C, Gerlng N, Landshamer S, Rickerts B, Duchstein HJ, Umezawa K, Kluemp S, Kriegstein J: Nitric oxide donors induce neurotrophin-like survival signaling and protect neurons against apoptosis. *Mol Pharmacol* 2005, 68:1006-1017.

115. Akassoglu K: Nerve growth factor-independent neuronal survival: a role for NO donors. *Mol Pharmacol* 2005, 68:952-955.

116. Lee FS, Rajagopal K, Kim AH, Chang PC, Chao MV: Activation of Trk neurotrophin receptor signaling by pituitary adenylate cyclase-activating polypeptides. *J Biol Chem* 2002, 277:9096-9102.

117. Lee FS, Chao MV: Activation of Trk neurotrophin receptors in the absence of neurotrophins. *Proc Natl Acad Sci USA* 2001, 98:3555-3560.

118. Kamata H, Oka S, Shibukawa Y, kakuta J, Hirata H: Redox regulation of nerve growth factor-induced neuronal differentiation of PC12 cells through modulation of the nerve growth factor receptor, TrkA. *Arch Biochem Biophys* 2005, 434:16-25.

119. Jang SW, Okada M, Sayeed I, Xiao G, Stein D, Jin P, Ye K: Gambogic amide, a selective agonist for TrkA receptor that possesses robust neurotrophic activity, prevents neuronal cell death. *Proc Natl Acad Sci USA* 2007, 104:16329-16334.

120. Zhang B, Salituro G, Szalkowski D, Li Z, Zhang Y, Royo I, Vilella D, Dize MT, Palasz F, Ruby C, et al.: Discovery of a small molecule insulin mimetic with antiadipic activity in mice. *Science* 1999, 284:974-977.

121. Liu K, Xu L, Szalkowski D, Li Z, Ding Y, Kwei G, Huskey S, Moller DE, Heck JV, Zhang BB, Jones AB: Discovery of a potent, highly selective, and orally efficacious small-molecule activator of the insulin receptor. *J Med Chem* 2000, 43:3487-3494.