NEUROTROPHINS AND THEIR RECEPTORS: A CONVERGENCE POINT FOR MANY SIGNALLING PATHWAYS

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The neurotrophins are a family of proteins that are essential for the development of the vertebrate nervous system. Each neurotrophin can signal through two different types of cell surface receptor — the Trk receptor tyrosine kinases and the p75 neurotrophin receptor. Given the wide range of activities that are now associated with neurotrophins, it is probable that additional regulatory events and signalling systems are involved. Here, I review recent findings that neurotrophins, in addition to promoting survival and differentiation, exert various effects through surprising interactions with other receptors and ion channels.

LONG-TERM POTENTIATION (LTP). An enduring increase in the amplitude of excitatory postsynaptic potentials as a result of high-frequency (tetanic) stimulation of afferent pathways. It is measured both as the amplitude of excitatory postsynaptic potentials and as the magnitude of the postsynaptic-cell population spike. LTP is most often studied in the hippocampus and is often considered to be the cellular basis of learning and memory in vertebrates.

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The era of growth factor research began fifty years ago with the discovery of nerve growth factor (NGF). Since then, the momentum to study the NGF — or neurotrophin — family has never abated because of their continuous capacity to provide new insights into neural function; the influence of neurotrophins spans from developmental neurobiology to neurodegenerative and psychiatric disorders. In addition to their classic effects on neuronal cell survival, neurotrophins can also regulate axonal and dendritic growth and guidance, synaptic structure and connections, neurotransmitter release, LONG-TERM POTENTIATION (LTP) and synaptic plasticity[1,2].

The surprising discovery that neurotrophins and their receptors do not exist in *Drosophila melanogaster* or *Caenorhabditis elegans* reinforced the idea that these proteins are not absolutely necessary for the development of neuronal circuits *per se*, but are involved in 'higher-order' activities. For example, neurotrophins and their receptors influence many aspects of neuronal activity that result in the generation of new synaptic connections, which can be long lasting[3]. Alterations in neurotrophin levels have profound effects on a wide variety of phenomena, including myelination, regeneration, pain, aggression, depression and substance abuse.

The actions of neurotrophins depend on two different transmembrane-receptor signalling systems[4] — the Trk receptor tyrosine kinases and the p75 neurotrophin receptor[5,6]. Despite considerable progress in understanding the roles of these receptors, additional mechanisms are needed to explain the many cellular and synaptic interactions that occur between neurons. An emerging view is that neurotrophin receptors act as sensors for various extracellular and intracellular inputs, and several new mechanisms have recently been put forward. Here, I will consider several ways in which Trk and p75 receptors might account for the unique effects of neurotrophins on behaviour and higher-order activities.

The levels of neurotrophins are important

It is well established that the overall levels of neurotrophins determine the balance between cell survival and APOPTOSIS during development. Neural activity has profound effects on the levels of neurotrophins. Indeed, the idea that neurotrophins are crucial for synaptic plasticity came from observations that they are synthesized and released in an activity-dependent manner[7–9]. NGF and brain-derived neurotrophic factor (BDNF) messenger RNAs (mRNAs) are highly regulated by electrical stimulation and epileptic activity[10], and BDNF in particular is rapidly released by neuronal activity during periods of activity-dependent synaptic remodelling[11–14].

Studies of mice that express reduced levels of neurotrophins have shown surprising effects on adult brain function and behaviour. Mice that completely

Box 1 | Haploinsufficiency of neurotrophins

***NGF*$^{+/-}$ mice**
- **Decreased cholinergic innervation of the hippocampus**[15]
- **Deficiency in memory acquisition and retention**[15]
- **Loss of neurons of the peripheral nervous system**[121]

***BDNF*$^{+/-}$ mice**
- **Hyperphagia, obesity**[16–18]
- **Impairment of long-term potentiation**[19,20,122]
- **Elevated striatal dopamine levels**[123]
- **Loss of mechanosensitivity**[124]
- **Loss of neurons of the peripheral nervous system**[125,126]

***NT3*$^{+/-}$ mice**
- **Deficient amygdala KINDLING activity**[127]
- **Cardiovascular defects**[128]
- **Reduced mechanoreceptors**[129]
- **Loss of neurons of the peripheral nervous system**[130]

lack neurotrophins die during the first few weeks following birth. Heterozygous mice in which neurotrophin levels are reduced by half are viable but, strikingly, they show other unanticipated deficits (BOX 1). For example, lowering the level of NGF leads to several deficits in memory acquisition and retention[15]. In the absence of normal levels of BDNF, mice show enhanced aggressiveness, hyperactivity and hyperphagia[16–18]. Intracerebroventricular infusion of BDNF or neurotrophin 4 (NT4) reverses the hyperphagic phenotype[17]. In *BDNF*$^{+/-}$ heterozygous mice, 5-HT (5-hydroxytryptamine, serotonin)-mediated neuronal function is abnormal in the forebrain, cortex, hippocampus and hypothalamus, and administration of the selective 5-HT-reuptake inhibitor fluoxetine reduces the aggressive behaviour, hyperphagia and hyperlocomotor activity[16]. A conditional deletion of BDNF in the brains of postnatal mice also leads to hyperphagia and hyperactivity, as well as to higher levels of anxiety as measured by a LIGHT/DARK EXPLORATION TEST[18]. Therefore, the feeding phenotype and the other behavioural abnormalities are mediated by the action of BDNF in the central nervous system (CNS), not in the periphery. Abnormal behaviours, indicative of impulse-control disorders, are also elicited by partial deletion of BDNF.

Lack of BDNF also causes deficits in memory tasks; for example, *BDNF*$^{+/-}$ mice show impairments in spatial memory. This is consistent with defects in LTP that are found in the hippocampus. Interestingly, *BDNF*$^{-/-}$ and *BDNF*$^{+/-}$ mice show the same deficits in LTP[19,20], indicating that not only the availability of BDNF, but also its levels, can profoundly alter plasticity.

Neurotrophins and their receptors

The neurotrophins are initially synthesized as precursors or pro-neurotrophins, which are cleaved to produce the mature proteins[22]. Pro-neurotrophins are cleaved intracellularly by FURIN or pro-convertases at a highly conserved dibasic amino-acid cleavage site to release carboxy-terminal mature proteins. The mature proteins, which are about 12 kDa in size, form stable, non-covalent dimers, and are normally expressed at very low levels during development. The amino-terminal half (or pro-domain) of the pro-neurotrophin is believed to be important for the proper folding and intracellular sorting of neurotrophins.

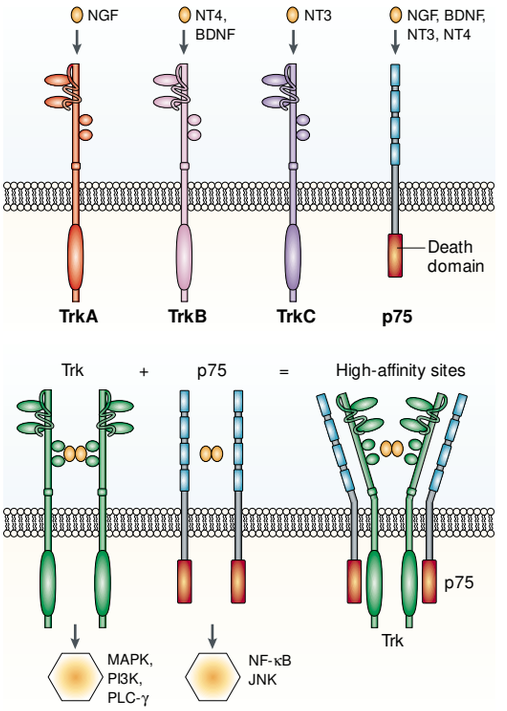


Figure 1 | **Models of Trk and p75 receptor activation.** Neurotrophin binding results in dimerization of each receptor. Neurotrophins bind selectively to specific Trk receptors, whereas all neurotrophins bind to p75. Trk receptors contain extracellular immunoglobulin G (IgG) domains for ligand binding and a catalytic tyrosine kinase sequence in the intracellular domain. Each receptor activates several signal transduction pathways[5,34,35]. The extracellular portion of p75 contains four cysteine-rich repeats, and the intracellular part contains a death domain. Neurotrophin binding to the p75 receptor mediates survival, cell migration and myelination[135] through several signalling pathways[36]. Interactions between Trk and p75 receptors can lead to changes in the binding affinity for neurotrophins[27]. BDNF, brain-derived neurotrophic factor; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; NT, neurotrophin; PI3K, phosphatidylinositol 3-kinase; PLC-γ, phospholipase Cγ.

Receptors encode specificity and responsiveness. Different neurotrophins show binding specificity for particular receptors — NGF binds preferentially to tyrosine receptor kinase A (TrkA); BDNF and NT4 to TrkB; and neurotrophin 3 (NT3) to TrkC (FIG. 1). These interactions have generally been considered to be of high affinity. However, in reality, the binding of NGF to TrkA, and of BDNF to TrkB is of low affinity[23–25], but it can be regulated by receptor dimerization, structural modifications

APOPTOSIS
The process of programmed cell death, characterized by distinctive morphological changes in the nucleus and cytoplasm, chromatin cleavage at regularly spaced sites, and the endonucleolytic cleavage of genomic DNA.

LIGHT/DARK EXPLORATION TEST
This test depends on the natural tendency of rodents to explore the environment in the absence of a threat and to retreat to an enclosed area when fearful. The animals are placed in an apparatus that has a dark and an illuminated compartment. Reduced exploration of the bright compartment and a reduced number of transitions between compartments are commonly interpreted as measures of anxiety.

FURIN
An endopeptidase with specificity for the consensus sequence Arg-X-Lys/Arg-Arg.

KINDLING
An experimental model of epilepsy in which an increased susceptibility to seizures arises after daily focal stimulation of specific brain areas (for example, the amygdala) — stimulation that does not reach the threshold to elicit a seizure by itself.

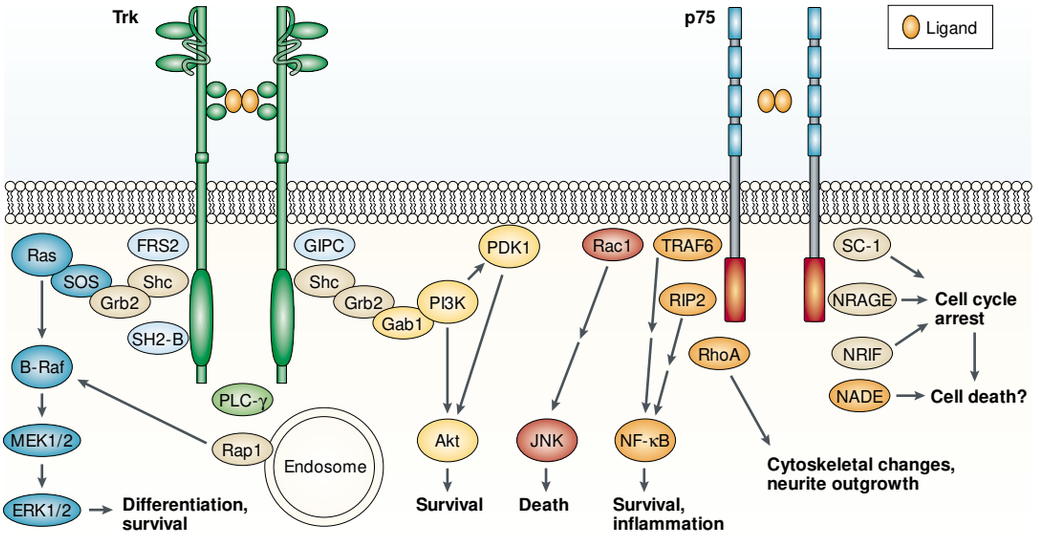


Figure 2 | **Neurotrophin receptor signalling.** Trk receptors mediate differentiation and survival signalling through extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K) and phospholipase Cγ (PLC-γ) pathways[3,5]. Trk family members recruit and increase the phosphorylation of PLC-γ and Src homologous and collagen-like adaptor protein (Shc), which leads to activation of PI3K and ERK. Rap1 exerts its actions from an endosomal location[136]. The p75 receptor predominantly signals to activate NF-κB and Jun N-terminal kinase (JNK), and modulates RhoA activity. These responses are mediated through adaptor proteins that bind to the cytoplasmic domain of p75, including neurotrophin-receptor interacting factor (NRIF), neurotrophin-associated cell death executor (NADE), neurotrophin-receptor-interacting MAGE homologue (NRAGE), Schwann cell 1 (SC1) and receptor-interacting protein 2 (RIP2)[35,36], which can exert effects on apoptosis, survival, neurite elongation and growth arrest. Akt, protein kinase B; FRS2, fibroblast growth factor receptor substrate 2; Gab1, Grb2-associated binder-1; Grb2, growth factor receptor-bound protein 2; GIPC, GAIP interacting protein, C terminus; MEK, mitogen-activated protein kinase (MAPK)/ERK kinase; PDK1, phosphoinositide-dependent kinase 1; SH2B, Src homology 2-B; SOS, Son of Sevenless; TRAF6, tumour necrosis factor receptor-associated factor 6.

or association with the p75 receptor[26,27]. The p75 receptor can bind to each neurotrophin, and also acts as a co-receptor for Trk receptors (FIG. 1). Expression of p75 can increase the affinity of TrkA for NGF and can enhance its specificity for cognate neurotrophins[28–30]. As a result, increased ligand selectivity can be conferred on the Trk receptors by the p75 receptor.

The ability of Trk and p75 receptors to present different binding sites and affinities to particular neurotrophins determines both their responsiveness and specificity. The ratio of receptors is important in dictating the numbers of surviving cells, and interactions between p75 and Trk receptors provide greater discrimination between different neurotrophins. A similar mechanism is also observed in other ligand–receptor systems, such as the glial-derived neurotrophic factor (GDNF)–Ret receptor[31], in which preferential interactions between GDNF ligands and the Ret receptor are facilitated by expression of GDNF family receptor subunits (GFRα) . Not surprisingly, Ret receptors use signalling pathways similar to those used by Trks.

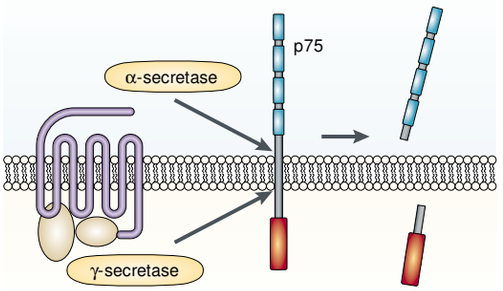
The effects of neurotrophins on axon guidance can also be modulated by the intracellular location of the neurotrophin–receptor complex. During development, neurotrophins are produced and released from the target cells and become internalized into vesicles, which are then transported to the cell body. The biological effects of neurotrophins require that signals be conveyed over long distances, from the nerve terminal to the cell body[32]. Both Trk and p75 receptors undergo retrograde and anterograde transport. Several proteins are associated with the Trk and p75 receptors during transport, and signalling persists after internalization[33]. The proper distribution of these proteins in the growth cone could result from movement of the neurotrophin receptors.

These receptor functions do not necessarily provide an explanation for the numerous phenotypes that are shown by mice that are deficient in neurotrophins. In addition to forming complexes, Trk and p75 receptors show independent signalling properties[5,34–36], and downstream signal transduction pathways significantly contribute to individual physiological responses. Neurotrophins bind as dimers to p75- and Trk-family members. Trk receptor dimerization leads to *trans*-autophosphorylation and to the activation of intracellular signalling cascades. The Src homologous and collagen-like (Shc) adaptor protein links the activated Trk receptor to two separate intracellular signalling pathways (FIG. 2). Neuronal survival requires Shc binding to the Trk receptor, which results in increases in phosphatidylinositol 3-kinase (PI3K) and Akt (protein kinase B) activities (FIG. 2). Phosphorylation of Shc by Trk also leads to increases in the activity of Ras and the extracellular signal-regulated kinase (ERK). These events in turn influence transcriptional events, such as the induction of the cyclic AMP-response element binding (CREB) transcription factor. CREB has effects

Box 2 | Processing of the p75 receptor

The p75 receptor undergoes cleavage by metalloproteinases[131] — such as α-secretase — to produce an ectodomain piece and a fragment containing the transmembrane and cytoplasmic domains (see figure). Unexpectedly, this membrane-spanning region is further cleaved by a presenilin-dependent γ-secretase (REF. 132; T.-W., Kim, unpublished observations). The generation of proteins by regulated intramembrane proteolysis is a universal mechanism[133] that acts on several proteins, including the amyloid precursor protein, Notch and the ErbB4 receptor tyrosine kinase. Whether the proteolytic enzymes involved in these events are regulated is not known. However, the generation of a p75 intracellular domain implies that neurotrophins might use regulated intramembrane proteolysis to transmit an intracellular signal. Analogous to Notch, the p75 intracellular domain might function in the nucleus as a transcriptional modifier. The intracellular domain might be involved in activation or repression of neurotrophin-related genes. Several p75 adaptor proteins, such as neurotrophin-receptor interacting factor (NRIF), tumour necrosis factor receptor-associated factors (TRAFs), receptor-interacting protein 2 (RIP2) and Schwann cell 1 (SC1) (FIG. 2), are candidates for nuclear translocation.

As p75 is expressed after nerve damage, in inflammatory conditions such as multiple sclerosis and in neuronal populations that degenerate in Alzheimer's disease, it is tempting to speculate that the γ-secretase cleavage of p75 reflects an early event in the pathogenesis of neurodegenerative diseases that are characterized by a chronic inflammation reaction. Together with the preponderance of proneurotrophins in Alzheimer's disease-affected tissues[134], proteolytic cleavage of neurotrophins and their receptors represent an intriguing regulatory mechanism for neuronal survival and regeneration during injury and ageing.



on the cell cycle, neurite outgrowth and synaptic plasticity[37]. The small G protein Rap1 accounts for the ability of neurotrophins to signal through ERK for sustained periods[38]. In addition, phospholipase Cγ (PLC-γ) binds to activated Trk receptors and initiates an intracellular signalling cascade, resulting in the release of inositol phosphates and activation of protein kinase C (PKC).

Through a different set of adaptor proteins (FIG. 2), p75 produces increases in Jun N-terminal kinase (JNK), NF-κB and ceramide[36]. One established function of p75 is to promote cell death[35]. This might provide a means for the refinement of correct target innervation during development and eliminate cells during periods of developmental cell death[39]. Apoptosis by p75 is also manifested after seizure or inflammation[40,41]. Injury to the spinal cord leads to oligodendrocyte death that is p75-dependent — a phenomenon that has also been observed in culture[42]. This apoptotic function is accompanied by an increase of Rac and JNK activities (FIG. 2), which are essential for NGF-dependent death[43]. Another function of p75 might be to mediate a non-apoptotic or survival response[44,45], similar to the behaviour of other tumour necrosis factor receptors.

Surprisingly, pro-neurotrophins are more selective ligands for the p75 receptor than mature forms[46], and are more effective at inducing p75-dependent apoptosis[42,46]. This indicates that the biological actions of neurotrophins can be regulated by proteolytic cleavage, with pro-forms preferentially activating p75 to mediate apoptosis and mature forms selectively activating Trk receptors to promote survival. Like the neurotrophins, the p75 receptor can also undergo cleavage (BOX 2).

Many of the components of the pathways that mediate neurotrophin signalling, such as ERK, Akt, PLC, PKC, Ras, JNK and NF-κB (FIG. 2), are not unique to neurotrophins. Each signalling component is used in many different contexts and by other growth factors and cytokines. This complicates the problem of ascribing specific mechanisms to a particular response[47]. Clearly, the effects of neurotrophins depend on various factors — their levels, their affinity of binding to transmembrane receptors, and the duration and intensity of downstream signalling cascades that are stimulated after receptor activation. From these considerations alone, it is still not evident how changes in behaviour and neuronal activity can be explained simply by a 50% reduction in levels of neurotrophins or their signalling components.

Neurotrophin-mediated plasticity

Many observations have indicated that neurotrophins influence both the frequency and amplitude of synaptic currents. Neurotrophins such as BDNF and NT3 produce rapid increases in synaptic strength in nerve–muscle synapses, as well as increases in excitatory postsynaptic currents in hippocampal neurons[48–50]. BDNF and NT3 also induce rapid and long-lasting enhancement of synaptic strength through LTP in hippocampal slices. These effects are not due to the nonspecific effects of using large amounts of proteins in electrophysiological recordings *in vitro*, as mice deficient in BDNF or NT4 show a notable impairment of LTP in hippocampal slices[19,51]. Similarly, the effects on LTP are not due to a developmental or structural alteration created by gene targeting, as normal LTP can be rescued by addition of exogenous BDNF[20,52].

Despite considerable evidence for the effects of neurotrophins on synaptic strength[53,54], there are few molecular and signalling mechanisms that could explain these effects. The use of protein kinase inhibitors has indicated that intracellular protein phosphorylation is important, as well as phosphatidylinositol lipids and inositol-1,4,5-triphosphate ($Ins(1,4,5)P_3$) receptors[55]. A CONDITIONAL MUTATION of the *TrkB* gene results in deficits in memory acquisition and consolidation in several hippocampus-dependent learning tasks[56]. These studies provide convincing evidence that signalling by TrkB receptors is directly responsible for promoting hippocampal LTP[57]. Mutagenesis of the Shc and PLC-γ binding sites in the *TrkB* gene shows that downstream activation of CREB and calcium/calmodulin-dependent kinase II is responsible for the ability of TrkB to modulate LTP. Although Trk receptors are implicated in many forms of neuronal plasticity, there are reports that p75 signalling might also exert some effects on behaviour. Analysis of mice deficient in the full-length p75 receptor

CONDITIONAL MUTATION
A mutation that can be selectively targeted to specific organs (or cell types within an organ) or induced at a specific developmental stage.

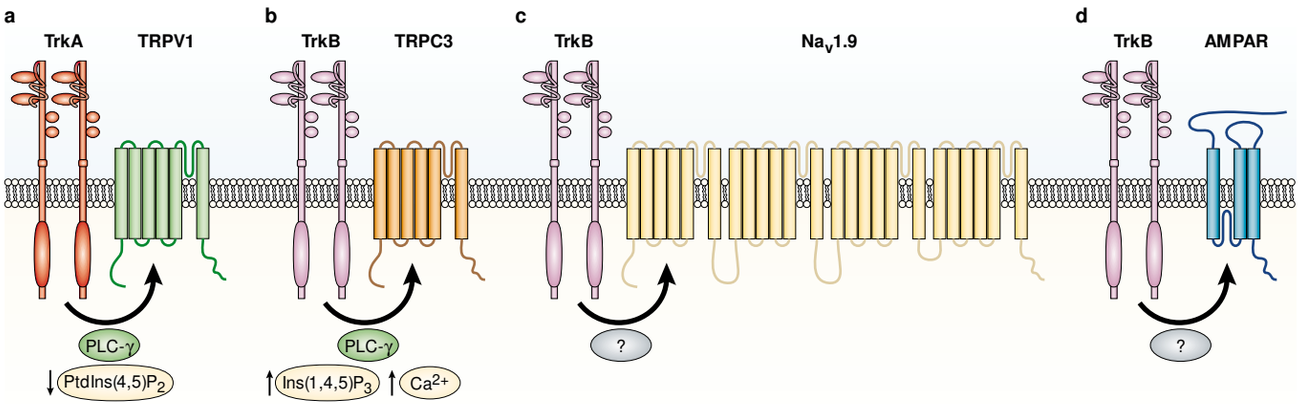


Figure 3 | **Examples of ion channel interactions with Trk neurotrophin receptors.** Several examples of interactions between Trk receptors and ion channels are known. **a** | TrkA with the transient receptor potential (TRP) channel TRPV1 (or VR1)[63]. **b** | TrkB with TRPC3 (REF. 65). **c** | TrkB with $Na_v1.9$ (REF. 77). **d** | α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) activity can be modified by brain-derived neurotrophic factor (BDNF) binding and activation of TrkB receptors[77]. TrkA-mediated PLC-γ activation decreases levels of cellular PIP2, which leads to the opening of TRPV1 channels. In the case of TrkB and TRPC3, TrkB-mediated PLC-γ activity leads to the opening of TRPC3 through inositol-1,4,5-triphosphate ($Ins(1,4,5)P_3$) generation and store-operated calcium release. These associations have been confirmed by co-immunoprecipitation experiments. $PtdIns(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate.

has revealed slight impairments in several learning tasks[58]. Another p75-mutant mouse that lacks both the full-length and the short isoform[59] shows a more severe phenotype and could provide additional insight into the role of p75 in higher-order functions.

However, full explanation of the ability of neurotophins to regulate synaptic plasticity in the adult brain requires a better understanding of how neurotrophin-receptor signalling is linked to ion channel function. Recent analysis of the role of ephrins at synapses has shown that EphB2 receptors can regulate postsynaptic function through an interaction with NMDA (*N*-methyl-D-aspartate) receptors[60–63]. These studies highlight the possibility that other receptor tyrosine kinase systems might regulate ion channel function.

The study of transient receptor potential (TRP) ion channels has provided new insights into this question. The TRP superfamily includes more than twenty cation channels, some of which have been shown to be sensitive to cold and hot temperatures, and to pheromones[64]. TRPC3 is a non-voltage-gated, store-operated cation channel that is highly expressed in brain regions where TrkB receptors are found. Treatment of pontine neurons with BDNF resulted in a delayed inward current after 30 s (REF. 65). This response was specific to BDNF, as other ligands — such as fibroblast growth factor and insulin-like growth factor — did not elicit an increase in cation current. The BDNF-induced current depended on activation of TrkB and PLC (FIG. 3). The biological consequences of interactions between TrkB and TRPC3 have not been fully defined, but the increase in cation flux implies a unique neurotrophin-specific function. The abundant expression of TRPC3 during neonatal development indicates that it might have a role in neurotrophin-dependent plasticity.

Neurotrophins and pain

Another TRP family member that has intimate ties with the Trk receptor is the TRPV1 (VR1) channel or capsaicin receptor, a non-selective cation channel that is activated by heat, noxious vanilloid compounds such as capsaicin, and extracellular protons[66]. Previously, NGF was shown to potentiate the responses of nociceptive sensory neurons to capsaicin[67]. This indicated that crosstalk between capsaicin and NGF occurred within sensory neurons. The idea that TRPV1 channels are necessary for NGF-induced thermal hypersensitivity was also underscored by observations of mice lacking TRPV1. In contrast to NGF-injected normal mice, which showed a marked decrease in paw withdrawal latency in response to a thermal stimulus, injection of NGF into TRPV1-deficient mice did not produce any sensitization[68].

Strikingly, NGF produced an approximately 30-fold increase in proton-evoked currents in *Xenopus* oocytes that co-expressed TrkA and TRPV1. Diminution of phosphatidylinositol-4,5-bisphosphate levels through antibody sequestration or PLC-mediated hydrolysis mimicked the potentiating effects of NGF at the cellular level (FIG. 3). Moreover, recruitment of PLC-γ to TrkA was essential for NGF-mediated potentiation of channel activity (FIG. 3). Co-immunoprecipitation studies indicated that TRPV1 associates with TrkA and PLC-γ to form a complex. As an interaction was also observed between TrkB and TRPC3 (REF. 65), it is likely that common sequences are required for these interactions.

Neurotrophins have been shown to produce acute pain as a side effect in clinical trials for neuropathy and neurodegeneration[69,70]. NGF is present at high levels after inflammation and promotes nociceptor sensitization. These responses might reflect the same process as potentiation of thermal sensitivity by TRPV1 or related heat-activated ion channels. In NGF-responsive

nociceptive sensory neurons, TrkA and TRPV1 are frequently co-expressed. In other neuronal populations, similar mechanisms might account for the pronounced pain that is observed when high levels of neurotrophins are administered in animal models or in human clinical trials.

Other ion channels

Increasing numbers of interactions between Trk receptors and ion channels are being discovered. Increased tyrosine phosphorylation of NMDA and voltage-gated potassium channels occurs as a result of treatment with BDNF[71,72]. In the hippocampus, Trk receptors are expressed both pre- and postsynaptically, and both pre- and postsynaptic mechanisms have been proposed to account for changes in synaptic activity[50,73–76]. Electrophysiological measurements show that BDNF can actually suppress $K_V1.3$ currents[72] and block postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor-mediated currents[77]. The catalytic activity of TrkB receptors is required for the decrease in AMPA receptor activity, implying that there might be a close association between TrkB and AMPA receptors (FIG. 3). Alternatively, the exo- and endocytosis of AMPA receptors[78], which determine activity-dependent changes of synaptic efficacy, could be influenced by BDNF signalling.

One reason for suspecting a direct interaction between Trk receptors and ion channels comes from recent studies in which the sodium channel $Na_V1.9$ was activated by BDNF[79]. The effects of BDNF on hippocampal neurons of the CA1 region are remarkable for the rapidity of their response — an inward sodium current was detected within milliseconds of BDNF treatment. Curiously, the BDNF-mediated increase was blocked by K-252a, a Trk-specific inhibitor[80]. This requirement for receptor tyrosine kinase activity is difficult to reconcile with the time course of $Na_V1.9$ activation, as phosphorylation takes a considerably longer time than the patterns of activity that are stimulated by BDNF. For example, the earliest tyrosine phosphorylation events require nearly a minute of neurotrophin treatment[81]. Also, it takes up to a minute of exposure to NGF to elicit a change in sodium channel mRNA expression[82], a far longer time interval than is required for BDNF to activate the $Na_V1.9$ channel.

Although the exact mechanisms for receptor–ion channel interactions are unknown, the considerations outlined in the previous paragraph indicate that the TrkB BDNF receptor might exist in a complex with the $Na_V1.9$ channel (FIG. 3). Conformational changes in the receptor or the channel might account for the ability of sodium channels to be rapidly influenced by the binding of BDNF to TrkB. Previous studies have indicated that conformational changes in the TrkA receptor might account for changes in its NGF-binding properties[25]. In addition, TrkA receptor dimerization and activation might simply result from a point mutation in the extracellular domain of the receptor[83]. This raises the possibility that changes in Trk structure might be transmitted to neighbouring ion channels.

Transactivation through GPCRs

Although ligand-induced dimerization or oligomerization of receptors is a well-established mechanism for growth factor signalling, there is increasing evidence that biological responses can be mediated by two or more receptor systems. For some time, it has been appreciated that heterotrimeric G-protein-coupled receptors (GPCRs) produce similar responses (in terms of cell growth) to other growth factors that use receptor tyrosine kinases[84,85].

Activation of Trk neurotrophin receptors occurs after treatment with adenosine, a neuromodulator that acts through GPCRs. Trk receptor autophosphorylation is increased in hippocampal neurons and PC12 cells after treatment with adenosine. This transactivation requires adenosine A_{2A} receptors[86], and does not result from the production of neurotrophins. The increase in Trk activity was inhibited by protein kinase inhibitors, such as PP1 (4-amino-5-(4-methylphenyl)-7-(*t*-butyl)pyrazolol-[3,4-*d*]-pyrimidine, which is specific for Src family members) or K-252a. The pituitary adenylate cyclase-activating polypeptide (PACAP), a neuropeptide, can also transactivate Trk receptors in a manner similar to transactivation by adenosine[87]. PACAP occurs in two forms, one of 38 and one of 27 amino acids, and is a member of the vasoactive intestinal peptide/secretin/glucagon family. The two PACAP peptides also interact with GPCRs.

The effects of adenosine and PACAP are specific, as other GPCR ligands do not participate in crosstalk with Trk receptors. Bradykinin, carbachol, ATP, apomorphine, quinpirole and angiotensin II do not cause TrkA activation[86], even though receptors for these ligands are expressed on the same cells as those for adenosine and PACAP. By contrast, many of these ligands can stimulate epidermal growth factor (EGF) receptors and other mitogenic growth factor receptors. Conversely, adenosine and its agonists do not activate EGF receptors.

These GPCR transactivation events are unique in other ways. Both adenosine and PACAP require a long period of time (more than 1–2 hours) to activate Trk tyrosine kinase activity. Both ligands produce an activation of PI3K and Akt, which results in enhanced cell survival after withdrawal of NGF. These results provide an explanation for the neuroprotective actions of adenosine and PACAP, and point to a therapeutic use for small-molecule GPCR agonists in neurodegenerative disorders. For example, activation of Trk receptors by PACAP was also observed in primary cultures of basal forebrain cholinergic neurons, and administration of PACAP effectively rescued these neurons after fimbria–fornix lesion *in vivo*[88]. These results are significant, because NGF-responsive cholinergic neurons in the basal forebrain degenerate in Alzheimer's disease[89].

What is the physiological relevance to neurotrophin action of transactivation by GPCR signalling? Transactivation might explain why neuronal survival in the CNS is not adversely affected by the lack of neurotrophins — GPCR ligands might compensate by providing a survival function through a neurotrophin-receptor signalling pathway. Also, other essential activities, such

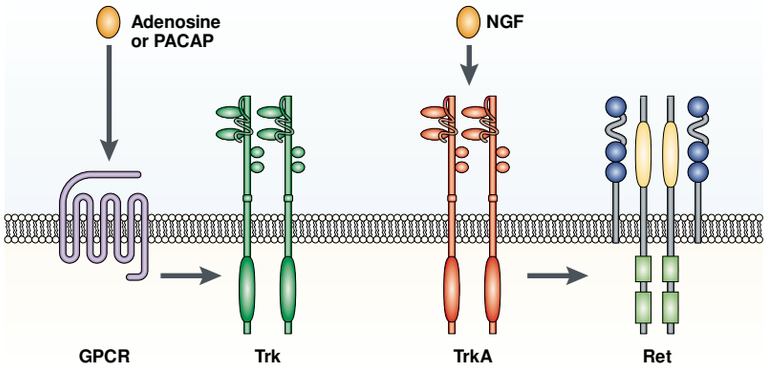


Figure 4 | **Transactivation of receptor tyrosine kinases.** Transactivation of Trk receptor by G-protein-coupled (GPC) ligands — such as adenosine and pituitary adenylate cyclase-activating polypeptide (PACAP) — results in neuroprotection[86,137]. In sympathetic neurons, binding of nerve growth factor (NGF) to TrkA results in the activation of Ret tyrosine kinase receptors[95]. GPCR, G-protein-coupled receptor.

as the regulation of ion channels, might be legitimate actions for transactivated receptor signalling. Indeed, dopamine–GPCR transactivation of platelet-derived growth factor receptors has an acute effect on NMDA ion channel activity in hippocampal neurons[90].

Importantly, mutations in components of the adenosine or PACAP signalling pathways give rise to behavioural problems in learning and memory[91,92] and heightened aggression[93], which are reminiscent of the effects of mutations in the BDNF and TrkB receptor genes[16,17,20,56,75]. These striking similarities imply that adenosine and PACAP signalling might work in parallel or converge with neurotrophin receptor action. These similarities also imply that Trk receptors act as convergence points for signals emanating from other receptor systems. In this manner, Trk receptors act to survey various inputs, in addition to those from neurotrophins.

Crosstalk between different transmembrane receptors might represent a more common signalling mechanism. Further to the influence that GPCRs exert upon Trk receptor activity, Trk receptors can activate other seemingly unrelated receptors. An unusual case is the Ret tyrosine kinase receptor (FIG. 4), which is a common signalling receptor for GDNF-related ligands that also include artemin, neurturin and persephin[94]. These ligands require specific GFRα subunits to confer ligand specificity. However, in postnatal sympathetic neurons, NGF produces an activation of Ret receptors over the course of 1–2 days, which does not require GDNF ligand binding[95]. Activation of Ret signalling provides additional survival advantages during postnatal periods when these sympathetic neurons become independent of NGF. Transactivation of Ret tyrosine kinases by binding of NGF to the TrkA receptor represents a new mechanism for transmitting survival signals within neurons.

Therefore, there are ways of activating Trk and Ret tyrosine kinase receptors other than direct ligand binding. Activation of the neurotrophin system through other receptor signalling systems is an alternative mechanism of communication in the nervous system, and examples of this crosstalk abound. For example, antidepressant agents that act through monoamine GPCRs can cause increased expression of both neurotrophins and neurotrophin receptors[96]. Notably, only the neurons that express the monoamine GPCRs have the capacity to enhance neurotrophin or Trk receptor levels. The results of studies with GPCR ligands raise the possibility of using small molecules to elicit neurotrophic effects in the treatment of neurodegenerative diseases[70]. This approach would allow selective targeting of neurons that express specific GPCRs and trophic factor receptors.

Regeneration

Proteins that modulate growth cone dynamics have an important role in axonal patterning during development, and in preventing regeneration of axons following injury. Considerable attention has been given to Nogo, myelin-associated glycoprotein (MAG) and semaphorin 3A — proteins that provide potent inhibitory signals for axonal growth. There is increasing evidence that places neurotrophin receptors in the realm of these inhibitory proteins.

Neurotrophins can modulate the response of growth cones to inhibitory axon-guidance molecules. For example, neurotrophins have been shown to affect the extent of the axonal response to MAG[97]. Moreover, semaphorin 3A induces the collapse of dorsal-root ganglion (DRG) and sympathetic growth cones[98], and neurotrophins can rapidly modulate the response of DRG growth cones to semaphorin 3A (REF. 99). The sensitivity of DRG growth cones to semaphorin 3A is influenced by BDNF and NGF in distinct ways — BDNF increases the sensitivity of DRG growth cones to semaphorin 3A, whereas NGF decreases it. These effects depend on Trk signalling, implying that TrkA and TrkB exert differential effects on semaphorin 3A signalling. Furthermore, the effects of NGF in opposing the inhibitory action of semaphorin 3A are highly dependent on ligand concentration and downstream signalling through the activities of protein kinase A and protein kinase G (REF. 100). These observations indicate the operation of a mechanism in which the receptors for neurotrophins and semaphorins are functionally linked.

Like the Trk receptors, p75 interacts with some unlikely partners. Nogo-A is an important inhibitory protein that is expressed in oligodendroyctes. It binds to a glycosylphosphatidylinositol-linked receptor[101] that is recognized by a 66-amino-acid fragment of Nogo (Nogo-66). Unexpectedly, there are other ligands for this Nogo receptor, including oligodendrocyte myelin glycoprotein[102] and MAG[103,104]. The absence of a cytoplasmic domain in the structure of the Nogo receptor implies that other components are involved in signalling, and association of the Nogo receptor with the p75 receptor has proved to be a surprising and intriguing solution to this problem[105,106].

The identification of p75 as a co-receptor of the Nogo receptor was based on several key observations.

POLYMORPHISM
The simultaneous existence in the same population of two or more genotypes in frequencies that cannot be explained by recurrent mutations.

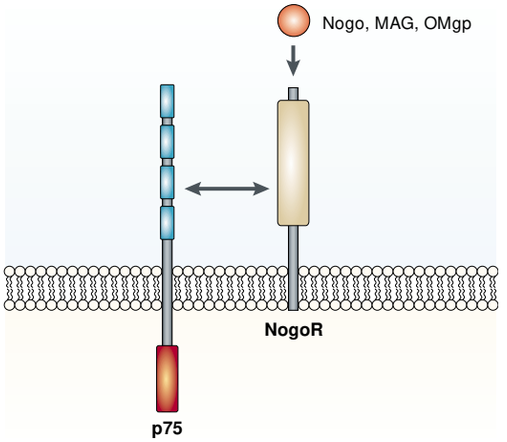


Figure 5 | **Neurotrophins and p75 undergo site-specific cleavages.** The Nogo and p75 receptors are found in a complex. Nogo[138], myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp) are all ligands for the Nogo receptor (NogoR)[102,104]. Inhibition of neurite outgrowth by MAG is mediated by NogoR and the p75 receptor[105,106].

First, MAG serves as a ligand for the Nogo receptor. Second, the inhibitory effects of MAG were found to depend on the presence and action of the p75 receptor[107]. The Nogo receptor is closely associated with p75 through interactions between the extracellular domains of the two proteins (FIG. 5), and this association produces a repulsive effect on axonal growth. Together, these results indicate that myelin-dependent inhibition of axonal regeneration depends on the binding of MAG to a complex containing the Nogo and p75 receptors. Several different proteins bind to the cytoplasmic domain of p75 (FIG. 2); among them, RhoA is the most relevant to the inhibition of neurite outgrowth and growth cone collapse. Indeed, earlier work indicated that binding of p75 to RhoA influences axonal growth[108]. The inhibitory influence of MAG and Nogo might be explained by the recruitment of p75 into a complex that sends a repulsive signal in neurons. The participation of p75 in the prevention of regeneration following injury is plausible because the absence of p75 leads to sprouting and enhanced axonal growth and density[109].

Table 1 | **Induction of p75 receptor expression after injury**

Cell type	Injury
Motor neurons	Axotomy, regeneration[139–141]
Purkinje neurons	Traumatic injury[142]
Entorhinal neurons	Seizure[41]
Hippocampal neurons	Primary culture[143]
Striatal neurons	Ischaemia[144]
Cortical neurons	Zinc, ischaemia[145], Alzheimer's disease[146]
Schwann cells	Axotomy[147,148]
Oligodendrocytes	Spinal cord injury[42], multiple sclerosis lesions[40,149]

p75 expression is also induced in experimental allergic encephalomyelitis[150,151] and Alzheimer's disease[152].

The participation of p75 receptors in the axonal regeneration process provides further insight into the function of these receptors. There are many examples of elevated p75 expression in the adult brain and spinal cord after injury, inflammation and stress[6,110]. Interestingly, many cell types — including hippocampal and cortical neurons, oligodendrocytes and microglial cells — ordinarily show low levels of p75. However, after ischaemia, seizure, axotomy or other forms of stress, the expression of this receptor is considerably elevated in these types of cells (TABLE 1). Also, many cell types express p75 in culture, presumably owing to the change in environmental conditions. In fact, the magnitude of NF-κB signalling through p75 is highly dependent on whether cells have experienced stress, such as changes in serum, temperature or cell–cell contact[111].

Neurotrophins and disease

Few associations have been found between neurotrophin genes and neurological or psychiatric disorders, although a recent series of studies has linked a POLYMORPHISM in the pro-domain of BDNF with depression, bipolar disorders and schizophrenia. This polymorphism — which was identified from a single nucleotide polymorphism screen — is caused by a single amino-acid change, from valine (Val) to methionine (Met), at position 66 in the pro-domain of the BDNF protein[112–114]. In patients with bipolar disorder or depression, the *Val* allele seems to confer greater risk for the disease, whereas in patients with schizophrenia, the *Met* allele seems to be associated with impaired memory functions. The existence of mutations in BDNF — a highly conserved protein — implicates neurotrophins in the complex pathophysiology of psychiatric diseases[115], as well as neurodegenerative diseases such as Alzheimer's disease[116]. Cleavage of the p75 receptor has also been implicated in the pathogenesis of Alzheimer's disease (BOX 2).

An analysis of the Val→Met change in the pro-BDNF protein indicated that this alteration is responsible for abnormal sorting and secretion of BDNF[115]. The impact of this BDNF genotype was followed in human subjects who were examined for alterations in episodic memory. Individuals in which the pro-domain of BDNF has Met at position 66 performed relatively poorly in verbal episodic memory tests, and functional magnetic resonance imaging of hippocampal function showed an abnormal pattern of activation during cognitive tests. These effects of this polymorphism in BDNF indicate that neurotrophins can participate in hippocampal function and memory through a mechanism that relies on correct BDNF secretion. So, activity-dependent secretion of BDNF, and its subsequent effects on LTP and synaptic plasticity now have an important correlate in the human population.

An unexpected example of the involvement of neurotrophins in psychiatric disorders has come from the pathophysiology of depression, especially when depression is associated with stress. Several lines of evidence have implicated neurotrophins in depression. First, in animal models, restraint stress leads to decreased expression of BDNF in the hippocampus[117,118]. Second, the

LEARNED HELPLESSNESS
A commonly used model of depression in which animals are exposed to inescapable shock and subsequently tested for deficits in learning a shock-avoidance task. Learned helplessness is a rare example in which, rather than working from the psychiatric disorder to the model, the behavioural effect was originally discovered in experimental animals (dogs) and later invoked to explain depression.

administration of BDNF to the midbrain or hippocampus results in antidepressant effects in animal models of depression — forced swim and LEARNED HELPLESSNESS. This effect is comparable to chronic treatment with pharmacological antidepressants[119]. Third, BDNF has been shown to have trophic effects on 5-HT and noradrenergic neurons. Mutant mice with decreased levels of BDNF show a selective decrement in the function of 5-HT neurons and behavioural dysfunctions that are consistent with serotonergic abnormalities.

Many functions of the neurotrophic factors in the adult nervous system — other than their effects on neuronal survival — have now been elucidated. These functions include the maintenance of differentiated neuronal phenotypes, regulation of synaptic connections, activity-dependent synaptic plasticity, and neurotransmission. These additional functions show that neurotrophin receptors act as a point of convergence that might be involved in the integration of many environmental inputs. This can lead to alterations in neuronal circuitry and, ultimately, in behaviour. In particular, it has become clear that neurotrophins can produce long-term changes in the functionality of adult neurons through changes in transcription. As several psychotropic drugs affect neurotrophin signalling, this ability might help to explain the delay in therapeutic action of many psychiatric treatments.

Perspectives

To explain the complex behavioural effects that are related to the function of neurotrophins, an understanding of how local circuits and signal transduction pathways are integrated is required. Neurotrophins show both rapid and slow effects that are breathtaking in their scope and duration, but need to be further differentiated and defined. Cell-surface receptors are generally represented as isolated integral membrane proteins that span the lipid bilayer, with closely associated receptor components and with signal transduction proceeding in a linear stepwise fashion. This view of receptor function will undoubtedly be modified in the future. Neurotrophins provide an excellent example of how receptors can act not only in a linear manner, but can also influence the activity of other transmembrane molecules, either directly or through signalling intermediates. The description of these actions will require new methods of computational analysis, such as the effort to describe activity-dependent neurotrophic interactions by mathematical modelling[120].

Cell–cell communication represents the combined effects of many growth factors. Unlike studies that have been carried out *in vitro*, in which cell lines are treated with single factors, the growth and survival of cells *in vivo* are under the influence of the simultaneous actions of many polypeptide factors. Cooperativity between just two different transmembrane proteins implies that the possibilities for extracellular signalling are greatly expanded. In reality, the regulation of trophic activities is probably determined by the additive effects of many receptors and the duration of signalling events, as well as by protein cleavage events. The lack of a detectable effect on cell numbers in mice that are deficient in key growth factors also indicates that cell growth and survival are supported by multiple proteins. Interactions between neurotrophin receptors and ion channels and other cell-surface proteins provide a powerful mechanism for merging the actions of different ligand–receptor systems to achieve new cellular outcomes.

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Online links

DATABASES
The following terms in this article are linked online to:
Swiss-Prot: http://ca.expasy.org/sprot/
BDNF | CREB | GDNF | JNK | K_v1.3 | MAG | Na_v1.9 | NF-κB | NGF | Nogo | PACAP | Semaphorin 3A | Shc | TrkA | TrkB | TrkC | Tumour necrosis factor
OMIM: http://www.ncbi.nlm.nih.gov/Omim/
Alzheimer disease
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