

THE YIN AND YANG OF NEUROTROPHIN ACTION

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Abstract | Neurotrophins have diverse functions in the CNS. Initially synthesized as precursors (proneurotrophins), they are cleaved to produce mature proteins, which promote neuronal survival and enhance synaptic plasticity by activating Trk receptor tyrosine kinases. Recent studies indicate that proneurotrophins serve as signalling molecules by interacting with the p75 neurotrophin receptor (p75^{NTR}). Interestingly, proneurotrophins often have biological effects that oppose those of mature neurotrophins. Therefore, the proteolytic cleavage of proneurotrophins represents a mechanism that controls the direction of action of neurotrophins. New insights into the ‘yin and yang’ of neurotrophin activity have profound implications for our understanding of the role of neurotrophins in a wide range of cellular processes.

Neurotrophins are central to many facets of CNS function, from differentiation and neuronal survival to synaptogenesis and activity-dependent forms of synaptic plasticity^{1,2}. In the mammalian brain, four neurotrophins have been identified: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and neurotrophin 4 (NT4). These closely related molecules act by binding to two distinct classes of transmembrane receptor: the p75 neurotrophin receptor (p75^{NTR}) and the Trk family of receptor tyrosine kinases, which includes TrkA, TrkB and TrkC^{3–6}. Unlike the non-selective p75^{NTR} receptor, which has a similar affinity for all neurotrophins, each Trk receptor selectively binds a different neurotrophin (FIG. 1).

Like other secreted proteins, neurotrophins arise from precursors, proneurotrophins (30–35 kDa), which are proteolytically cleaved to produce mature proteins (12–13 kDa)⁷. In 2001, Hempstead and colleagues shattered the traditional view that these precursors are functionally inactive by showing that they promote cell death⁸. This provocative study made three main findings. First, proneurotrophins bind with high affinity to p75^{NTR}, which for years was considered to be a ‘low-affinity’ neurotrophin receptor. By contrast, mature

neurotrophins bind preferentially to Trk receptors. So, pro- and mature neurotrophins have their own preferred cognate receptors. Second, interaction of mature neurotrophins with Trk receptors leads to cell survival, whereas binding of proNGF to p75^{NTR} leads to apoptosis. A recent study showed that proBDNF also induces neuronal apoptosis by activating p75^{NTR} (REF. 13). Finally, proNGF and proBDNF can be cleaved *in vitro* by extracellular proteases, such as matrix metalloproteinase 7 (MMP7) and plasmin, to form mature NGF or BDNF. These results, coupled with those of several other recent studies, indicate that proneurotrophins are secreted^{9–13} and that they may serve as signalling molecules. More importantly, they imply that the cleavage of proneurotrophins is an important mechanism that governs neurotrophin action^{14–16}.

The propensity of neurotrophins to produce diametrically opposing effects on cell survival has led us to propose a ‘yin and yang’ model of neurotrophin action (FIG. 1). In this simple model, the binary actions of neurotrophins depend on both the form of the neurotrophin (pro- versus mature) and the class of receptor that is activated. This conceptual model of neurotrophin function has recently been extended to the bidirectional control of synaptic plasticity in

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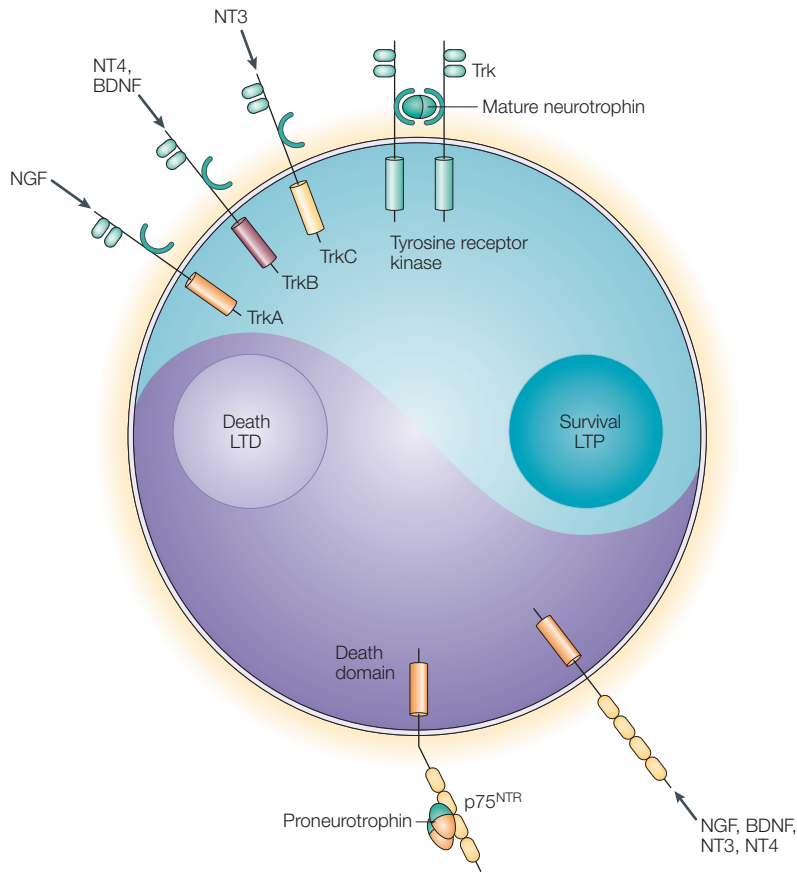


Figure 1 | The yin and yang of neurotrophin receptors and neurotrophin function. The actions of neurotrophins are mediated by two principal transmembrane-receptor signalling systems. Each neurotrophin receptor — TrkA, TrkB, TrkC and the p75 neurotrophin receptor (p75^{NTR}) — is characterized by specific affinities for the neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and NT4. An emerging concept is that the two distinct receptor classes, Trk (top) and p75^{NTR} (bottom), preferentially bind mature and proneurotrophins (neurotrophin precursors), respectively, to elicit opposing biological responses. LTD, long-term depression; LTP, long-term potentiation.

the hippocampus. Although considerable evidence supports the ‘yang’ action of neurotrophins, few studies have addressed the ‘yin’ aspect of the model, in which proneurotrophins, acting through p75^{NTR}, have effects opposite to those of the mature form. It therefore remains to be established whether the yin and yang actions of neurotrophins are equally prevalent. Neurotrophins are known to have diverse and complex actions, and it will be interesting to determine whether the yin–yang model can be extended to other functions. It should also be noted that the effect of mature neurotrophins is not limited to one direction. For example, in addition to promoting long-term potentiation (LTP), mature BDNF has been shown to inhibit long-term depression (LTD) in the hippocampus and visual cortex^{17–20}.

Here, we highlight recent studies that support the yin–yang model, focusing on the regulation of cell survival and synaptic plasticity by pro- and mature neurotrophins acting through two distinct receptor systems. We discuss the functional implications of

changes in the synthesis, trafficking, secretion and proteolytic processing of proneurotrophins, and consider the exciting prospect that the yin–yang model might be extended to include other cellular processes.

Trafficking and secretion of proneurotrophins
Given that proneurotrophins are signalling molecules rather than inactive precursors, it will be important to determine their distribution in the nervous system if we are to fully understand their functions. Earlier studies used antibodies against the mature regions of processed neurotrophins and, therefore, could not distinguish between pro- and mature neurotrophins. In a preliminary study using an antibody that recognizes the pro-domain of BDNF, Zhou *et al.* showed that proBDNF is expressed in many regions of the rat CNS, including the spinal cord, substantia nigra, amygdala, hypothalamus, cerebellum, hippocampus and cortex²¹. Further work will be necessary to determine whether there are brain regions that selectively express pro- or mature BDNF. Using a Vaccinia virus-based overexpression system, a number of cell types have been shown to preferentially express and secrete the pro- form. For instance, Schwann cells secrete predominantly proNGF and proBDNF, which indicates that the intracellular cleavage of proneurotrophins is less efficient in these cells¹². In addition, the expression of proneurotrophins is dynamically regulated. The pro- form is upregulated in pathological conditions such as Alzheimer’s Disease^{22–24}, brain injury^{25,26} and retinal dystrophy²⁷.

After synthesis in the endoplasmic reticulum, (pro)neurotrophins need to be folded correctly, sorted into the constitutive or regulated secretory pathway, and transported to the appropriate subcellular compartment (FIG. 2). Many non-neuronal cells, such as smooth muscle cells, fibroblasts and astrocytes, may not express molecular components of the regulated secretory pathway and, therefore, secrete neurotrophins only constitutively. REGULATED SECRETION is prevalent in neurons. Neurotrophin-containing secretory granules are transported to dendrites and spines, and are secreted postsynaptically. On the other hand, neurotrophin-containing LARGE DENSE CORE VESICLES (LDCVs) undergo anterograde transport to axonal terminals.

There are three ultimate fates of intracellular proneurotrophins: intracellular cleavage followed by secretion; secretion followed by extracellular cleavage; or secretion without subsequent cleavage. Uncleaved proneurotrophins signal by binding and activating p75^{NTR}. The ligand–receptor complex is presumably transported retrogradely, but, so far, this process has not been investigated. In addition to interacting with p75^{NTR}, secreted proneurotrophins might be degraded extracellularly. The pro-domains of the neurotrophins show substantial sequence homology and are conserved among vertebrate species, which indicates that they have an important role in intracellular processing^{28,29}. Indeed, recent studies support the emerging concept that the pro-domain of neurotrophins is crucial for intracellular trafficking and secretion, and can, therefore, significantly affect neurotrophin function³⁰.

REGULATED SECRETION

A cellular process in which the fusion of vesicles, and secretion of their protein contents, is triggered by extracellular signals.

LARGE DENSE CORE VESICLE (LDCV)

A secretory vesicle that contains protein or peptide. Under the electron microscope, a large dense core can be seen at the centre of the vesicle.

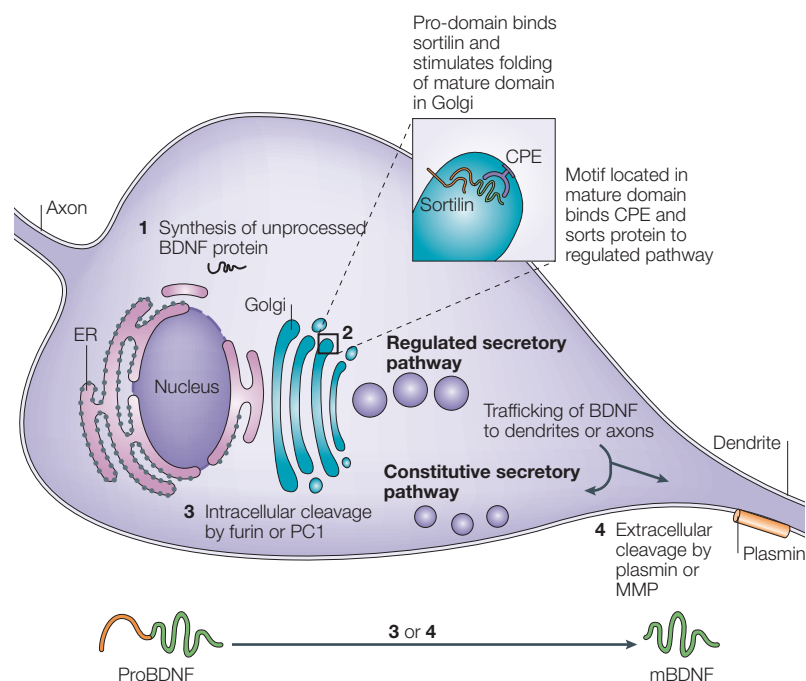


Figure 2 | The synthesis and sorting of BDNF. A schematic showing the synthesis and sorting of brain-derived neurotrophic factor (BDNF) in a typical neuron. First synthesized in the endoplasmic reticulum (ER) (1), proBDNF (precursor of BDNF) binds to intracellular sortilin in the Golgi to facilitate proper folding of the mature domain (2). A motif in the mature domain of BDNF binds to carboxypeptidase E (CPE), an interaction that sorts BDNF into large dense core vesicles, which are a component of the regulated secretory pathway. In the absence of this motif, BDNF is sorted into the constitutive pathway. After the binary decision of sorting, BDNF is transported to the appropriate site of release, either in dendrites or in axons. Because, in some cases, the pro-domain is not cleaved intracellularly by furin or protein convertases (such as protein convertase 1, PC1) (3), proBDNF can be released by neurons. Extracellular proteases, such as metalloproteinases and plasmin, can subsequently cleave the pro-region to yield mature BDNF (mBDNF) (4). MMP, matrix metalloproteinase.

An important advance came from a study showing that a single-nucleotide polymorphism in the pro-domain of the human *BDNF* gene affects the trafficking and secretion of BDNF^{9,31}. This single-nucleotide change occurs at nucleotide 196 (G to A), producing an amino acid substitution (valine to methionine) at codon 66 in the pro-domain. Humans with the Met allele have a selective impairment in hippocampus-dependent episodic memory, lower levels of hippocampal *N*-acetylaspartate (NAA, a putative measure of neuronal integrity and synaptic abundance) and abnormal hippocampal function (as recorded by functional MRI)^{31,32}. How can a single amino acid change in the pro-domain of BDNF have such marked cognitive effects? Cultured hippocampal neurons over-expressing Met-BDNF tagged with green fluorescent protein (GFP) have been shown to have three main phenotypes: impairments in intracellular trafficking, particularly to distal dendrites; failure of synaptic targeting of BDNF-containing vesicles; and impairments in activity-dependent BDNF secretion^{9,31}, possibly due to difficulties in generating BDNF-containing secretory granules. It seems that intracellular trafficking, as well as regulated secretion, of wild-type BDNF can be altered by co-expression with Met-BDNF, which forms

a heterodimer with Val-BDNF⁹. Although these results highlight the importance of the pro-domain, they do not reveal the specific mechanisms by which the pro-domain controls the intracellular sorting of neurotrophins.

Through comparative homology modelling of mature BDNF to the crystal structure of PRO-OPIOMELANOCORTIN (POMC), Lou *et al.*¹⁰ identified a putative sorting motif in the mature domain that consists of four crucial amino acids: Ile16, Glu18, Ile105 and Asp106. Substituting the two acidic residues with alanine resulted in attenuation of the regulated secretion of BDNF. This decrease in regulated secretion is accompanied by an increase in CONSTITUTIVE SECRETION. Replacing Val20 with Glu20 in a similar motif in NGF converted its constitutive secretion to regulated secretion. Modelling and binding studies point to a critical interaction between the acidic residues in the BDNF motif and two basic residues in the sorting receptor carboxypeptidase E (CPE)³³. Deletion of the *Cpe* gene in mice eliminated the activity-dependent secretion of BDNF from hippocampal neurons. Therefore, the mature domain of BDNF contains a motif that is essential for the binary decision of sorting to the regulated secretory pathway.

Given the importance of the sorting motif in the mature domain, what is the role of the pro-domain in secretion? The results of recent experiments indicate that the interaction of the pro-domain of BDNF with **sortilin** (BOX 1), a receptor that is localized mainly intracellularly^{34,35}, controls the mode of BDNF secretion³⁶. Sortilin is co-localized with BDNF in secretory granules in neurons, and interacts with two sub-regions, 'box 2' (which contains Val66) and 'box 3', both of which are in the pro-domain of BDNF. Deletion of box 2 and/or box 3 reduced regulated BDNF secretion, and the Val–Met mutation reduced co-localization of BDNF with sortilin or secretory granules. When a truncated DOMINANT-NEGATIVE form of sortilin (tSort) was expressed, there was a significant decrease in the co-localization of BDNF and the secretory granule marker, and in regulated secretion³⁶. Although the Val–Met mutation selectively impaired the regulated secretion of BDNF without a compensatory increase in constitutive secretion^{9,31}, disrupting the interaction between sortilin with the pro-domain of BDNF resulted in an increase in this form of release³⁶. These results indicate that the pro-domain, particularly box 2 and box 3, is also important for the regulated secretion of BDNF.

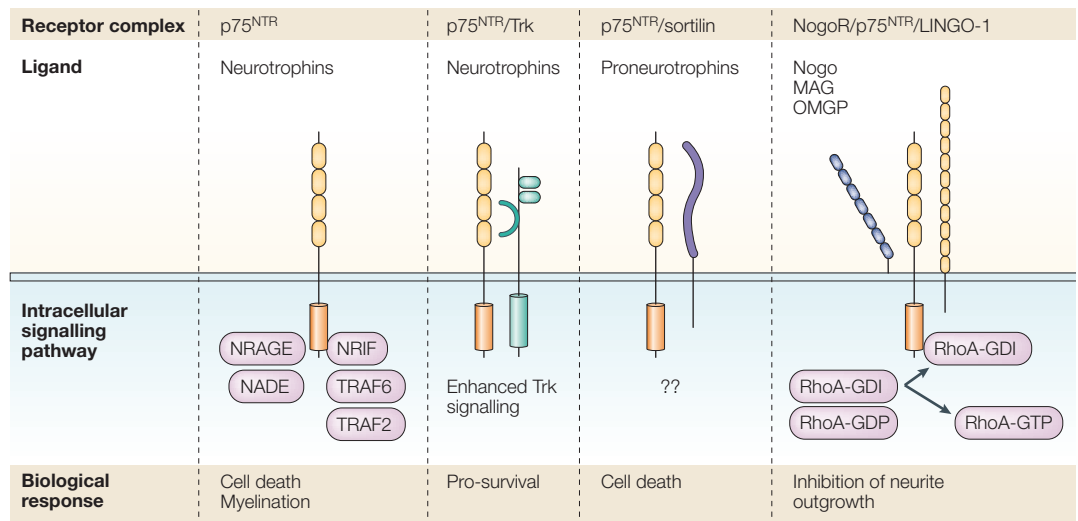
How can we reconcile these two sets of seemingly contradictory results, one pointing to a crucial role of CPE interaction with the mature domain, the other stressing the importance of sortilin and the pro-domain in sorting BDNF to the regulated secretory pathway? If a process is crucial for sorting a protein to the regulated pathway, disruption of that process should increase constitutive secretion and diminish regulated secretion. The Val–Met mutation selectively impaired the regulated secretion of BDNF without a compensatory increase in constitutive secretion^{9,31}. Therefore, the pro-domain — at least the region that surrounds Val66 — might not be important for the binary decision of sorting neurotrophins to the constitutive or regulated

PRO-OPIOMELANOCORTIN (POMC). A peptide precursor that gives rise to various neuropeptides through protease cleavage. Pro-opiomelanocortin is known to contain a three-dimensional sorting motif that interacts with the sorting receptor CPE to direct it to the regulated secretory pathway.

CONSTITUTIVE SECRETION
A cellular process in which vesicles spontaneously fuse with the plasma membrane to release their protein contents into the extracellular space.

DOMINANT-NEGATIVE
A mutant molecule that can form a heteromeric complex with the normal molecule, knocking out the activity of the entire complex.

Box 1 | Multiple partners of p75^{NTR}



Historically, the p75 neurotrophin receptor (p75^{NTR}) has been referred to as a ‘low-affinity’ neurotrophin receptor, but this definition should be avoided because proNGF binds p75^{NTR} with an affinity similar to that of nerve growth factor (NGF) binding to TrkA. Although it lacks a kinase domain, p75^{NTR} can cooperate with many different protein partners and form multimeric receptor complexes to produce a number of cellular responses, including apoptosis, neurite outgrowth and myelination^{111–113}. So far, sortilin⁶¹, LINGO-1, Nogo-66 (NgR)¹¹⁴ and Trk receptors¹¹⁵ have been identified as co-receptors. In addition to extracellular interactions that yield multimeric receptor complexes, the intracellular domain of p75^{NTR} can also interact with many different adaptor and signalling proteins. These include neurotrophin-receptor-interacting MAGE (melanoma-associated antigen) homologue (NRAGE)¹¹⁶, neurotrophin-associated cell death executor (NADE)¹¹⁷, TNF (tumour necrosis factor)-receptor-associated factors 2 and 6 (TRAF2 and TRAF6)^{118,119}, and neurotrophin-receptor-interacting factor (NRIF)^{120,121}. GDI, guanine-nucleotide dissociation inhibitor; MAG, myelin-associated glycoprotein; OMGP, oligodendrocyte myelin glycoprotein; RhoA, small G protein.

pathway. In addition, sortilin interacts better with box 3 (REF 36), a region with high sequence homology between NGF and BDNF^{15,28}. This implies that if sortilin were the crucial factor for sorting, NGF would be sorted to the regulated secretory pathway. However, NGF seems to be secreted mainly constitutively^{10,12} (for contradictory evidence see REFS 37,38). Finally, there is evidence that the pro-domain facilitates the proper folding of NGF^{29,39}. It is conceivable that incorrect folding due to pro-domain deletion or mutation leads to an accumulation of the protein in the endoplasmic reticulum and/or Golgi network, which results in an inability to target BDNF-containing vesicles to neuronal processes and synapses. In parallel, misfolded BDNF cannot generate the three-dimensional sorting motif, leading to failure of the sorting of BDNF to the regulated secretory pathway.

These results support a two-step model (FIG. 2): the interaction between the pro-domain and sortilin promotes an appropriate configuration of proBDNF. This, in turn, allows the sorting motif in the mature domain of BDNF to interact with CPE, and, therefore, to sort BDNF into the regulated secretory pathway. The pro-domain may be necessary, but not sufficient, for sorting neurotrophins into the regulated pathway, and the final mode of secretion is determined by the successful interaction of the sorting motif in the mature domain

of neurotrophins with the sorting receptor CPE. In the case of NGF, although interactions of its pro-domain with sortilin might allow correct folding, the absence of a sorting motif on its mature domain prevents it from entering the regulated secretory pathway.

Intracellular versus extracellular cleavage

The next important consideration is the form of neurotrophin that is secreted. Proneurotrophins can be processed either intracellularly or extracellularly, using distinct families of proteases (FIG. 2). In addition to being secreted through the constitutive pathway, NGF also differs from BDNF in that it is secreted mainly in its mature form^{12,40}. This indicates that under physiological conditions, proNGF synthesized in the endoplasmic reticulum is cleaved mainly intracellularly. Given that NGF is not sorted in the regulated secretory pathway¹², the pro-domain of NGF is probably removed in the *trans*-Golgi network by the serine protease furin, rather than in secretory granules by the prohormone convertases (PC1 or PC2)⁴⁰. By contrast, the 32-kDa proBDNF is the main form secreted from endothelial cells⁸ and cultured neurons^{9,11,12}. These findings indicate that mature BDNF is derived primarily from the cleavage of proBDNF by extracellular proteases, and that if uncleaved, the extracellular proBDNF might serve as an endogenous ligand.

Given that proneurotrophins are secreted, it becomes important to identify the specific extracellular proteases that cleave proneurotrophins, and to understand how these proteases are regulated. Several matrix metalloproteinases (MMP), including MMP3 and MMP7, have been shown to cleave proNGF and proBDNF⁸, and extracellular zinc can activate metalloproteinases and allow the conversion of pro- to mature BDNF in cultured cortical neurons⁴¹. However, the most significant protease that cleaves proneurotrophins is the serine protease plasmin^{8,42}. This enzyme is generally expressed as an inactive zymogen, plasminogen. Its activation requires proteolytic cleavage by tissue plasminogen activator (tPA)⁴³. Both tPA and plasminogen are widely expressed in the nervous system. In the brain, plasminogen is exclusively expressed in neurons, and is present in the extracellular space, particularly at the synaptic cleft⁴⁴. tPA is secreted from axon terminals into the extracellular space⁴⁵, and this secretion depends on high-frequency neuronal activity⁴⁶. It is therefore conceivable that tPA is the key trigger for the tPA–plasmin–proneurotrophin cascade.

If pro- and mature neurotrophins can produce opposing cellular responses — yin and yang effects — the proteolytic processing of proneurotrophins provides a powerful means to control the direction of action of neurotrophins. Because matrix metalloproteinases and plasmin are suitably positioned to cleave proneurotrophins as they are secreted or bound to the recipient cells, regulation of their expression or activation could regulate neurotrophin signalling in a spatially and temporally controlled manner. It will be of great interest to investigate whether neuronal activity and/or extracellular signals can switch the extracellular cleavage of proneurotrophins on or off. For instance, activation of plasmin by tPA is greatly enhanced (more than 100-fold) when it interacts with Annexin II, which is a Ca²⁺-dependent phospholipid-binding protein on the cell surface⁴⁷. Annexin II is expressed in the brain, secreted from neurons by tyrosine kinase signals, and enriched in LIPID RAFT regions of dendritic membranes after secretion⁴⁸. Both neuronal depolarization and spatial learning can facilitate the formation of an Annexin II heterotetramer, which is required for the activation of tPA/plasmin⁴⁸. Future experiments are warranted to determine whether Annexin II is involved in controlling the conversion of pro- to mature neurotrophins by the tPA/plasmin system.

Neurotrophins and cell survival

The regulation of cell survival and cell death is a key aspect of the establishment of functional neuronal circuits. Neurotrophins were first identified as promoters of neuronal survival, particularly in the PNS, and for decades researchers focused largely on the pro-survival effects of neurotrophins on various neuronal populations. However, in the early 1990s, several studies showed that by activating p75^{NTR}, neurotrophins could induce apoptosis in several

cell populations^{49–52}. The puzzling fact that pro- and mature neurotrophins have opposing effects on cell survival was finally understood when it was shown that pro- and mature forms bind to different receptors⁸. In the following sections we describe the yin and yang effects of neurotrophins on cell survival.

Cell survival mediated by mature neurotrophins. Because p75^{NTR} and Trk receptors are expressed in the nervous system and are sometimes present in the same cell, it is important to establish under what circumstances a given neurotrophin has a survival or apoptotic effect. It is becoming apparent that the outcome depends on the cellular context of the receptors and the selective secretion of pro- or mature neurotrophins. Central to the proposed yin–yang model of neurotrophin function is the observation that mature neurotrophins bind preferentially to Trk receptors to enhance cell survival, whereas unprocessed proneurotrophins bind to p75^{NTR} to induce cell death.

The pro-survival effect of mature neurotrophins is well established^{1,2}. This is due, at least in part, to the availability of recombinant mature neurotrophins. In many cell culture systems, the survival of neurons depends on the presence of optimal amounts of neurotrophins. Administration of mature neurotrophins to specific brain regions has also been shown to rescue neuronal loss associated with ageing or induced by chemical or mechanical insults. Conversely, deletion of specific neurotrophin genes results in the selective loss of certain populations of neurons that depend on the neurotrophin for survival⁵³. Although gene knockout should eliminate both pro- and mature forms of a neurotrophin, relatively few studies have reported increases in a given population of neurons⁵⁴ or reduced cell death⁵⁵. One possibility for this paucity of reports is that proneurotrophin–p75^{NTR}-mediated cell death occurs under conditions of elevated proneurotrophin secretion, such as during neuronal degeneration or injury.

It is clear that the survival effects of neurotrophins are mediated by Trk receptors⁴, because the pro-survival effects of neurotrophins are eliminated in cells that lack their respective Trk receptors, and specific cell populations are lost in *Trk* gene knockouts. Two principal signalling pathways that are important for cell survival have been identified. The primary pathway is the phosphatidylinositol 3-kinase (PI3K)–Akt (v-akt murine thymoma viral oncogene homologue, also known as protein kinase B) pathway, which suppresses cell death by inhibiting the apoptotic activities of forkhead⁵⁶ and BCL2 (B-cell leukaemia/lymphoma 2)-associated death protein (BAD)^{57,58}. The other pathway is the mitogen-activated protein kinase (MAPK)–MEK (MAPK/ERK (extracellular signal-regulated kinase) kinase) signalling cascade, which stimulates the activity and/or expression of anti-apoptotic proteins, including BCL2 (REF. 59) and the transcription factor cyclic AMP responsive element-binding protein (CREB)⁶⁰.

LIPID RAFT

A dynamic assembly of cholesterol and sphingolipids in the plasma membrane.

Cell death mediated by proneurotrophins. Although p75^{NTR} was the first neurotrophin receptor to be isolated, our understanding of the functional role and underlying signalling of p75^{NTR} has lagged behind that of the Trk receptors. A breakthrough came when it was discovered that p75^{NTR} could signal independently and promote the death of several cell types, including developing neurons^{51,52}. Subsequently, Hempstead and colleagues showed that a high-affinity ligand for p75^{NTR} was none other than the unprocessed form of the neurotrophins. They found that the binding of proneurotrophins to p75^{NTR} elicits cell death, an effect opposite to that of mature neurotrophins binding to Trk receptors⁸. Several studies have now verified the death-promoting activity of proneurotrophins in various cell types, including oligodendrocytes²⁵, Schwann cells¹³, corticospinal neurons²⁶, photoreceptors²⁷, smooth muscle cells^{8,61} and superior cervical ganglion cells^{8,13,61}.

p75^{NTR} has many binding partners that can produce unique, multimeric receptor complexes (BOX 1). One such binding partner is sortilin^{13,61}, which specifically binds the pro-domain of NGF or BDNF and serves as a co-receptor with p75^{NTR} in mediating cell death. Inhibiting or expressing sortilin in neurons that normally express p75^{NTR} suppressed or enhanced proNGF-mediated cell death, respectively. Furthermore, when the interaction between proBDNF and sortilin/p75^{NTR} was blocked by neurotensin, the apoptotic actions of proBDNF on cultured sympathetic neurons were abolished¹³. So, the presence or absence of sortilin in a cell determines whether or not p75^{NTR} functions as a death receptor.

Because p75^{NTR} can be dynamically regulated after insult or injury in the nervous system, a plausible function of proneurotrophins is to eliminate damaged cells that express p75^{NTR}. In support of this, spinal cord injury induces the expression of p75^{NTR} in oligodendrocytes, which leads to apoptosis of these cells²⁵. Moreover, proNGF extracted from injured spinal cord induced the apoptosis of cultured oligodendrocytes, and this effect was blocked by a proNGF-specific antibody and was absent in cultures prepared from p75^{NTR}-knockout mice. In a related study, axotomy by internal-capsule lesion resulted in p75^{NTR}-mediated apoptosis of corticospinal neurons *in vivo*²⁶. Endogenous proNGF was detected in the cerebrospinal fluid after the lesion was introduced, but it should be noted that proNGF was not detected before the lesion. In this study, proNGF was shown to bind p75^{NTR} *in vivo*, and when this interaction was disrupted by a proNGF antibody, proNGF-mediated apoptosis was largely attenuated. In another model system, microglia-derived proNGF promoted photoreceptor cell death through p75^{NTR} (REF. 27). It was proposed that proNGF might underlie the loss of photoreceptor cells that is observed in retinal dystrophy. These results identify proneurotrophins as death-inducing endogenous ligands that are secreted and initiate the cell death programme through the activation of p75^{NTR}.

TETANIC STIMULATION

A train of stimuli in which afferent axons are briefly activated at high frequency. In LTP experiments, a 1-s train of pulses delivered at a frequency of 100 Hz is commonly used to potentiate transmission.

Neurotrophins and synaptic plasticity

In the adult brain, neurotrophins have a key role in synaptic plasticity^{30,62}. Of all the neurotrophins, BDNF is by far the best characterized for its role in regulating LTP, particularly the early phase of LTP (E-LTP). Acute application of mature BDNF facilitates E-LTP in the hippocampus^{63–65} and visual cortex^{19,66}. Conversely, inhibition of BDNF activity, by gene knockout or functional blocking using BDNF antibody or TrkB-immunoglobulin G (IgG), attenuates hippocampal E-LTP^{63,65,67,68}. The impairment in *Bdnf*-knockout mice was rescued by acute application of recombinant BDNF^{64,65,69} or by virus-mediated gene transfer of BDNF⁷⁰. This argues for an acute rather than a long-term developmental effect of BDNF. Further evidence for the role of BDNF in LTP stems from studies showing that robust LTP can be induced by pairing weak stimulation that would not normally induce E-LTP with BDNF application^{64,71}. It is thought that the potentiating effect of BDNF on E-LTP is achieved, at least in part, by enhancing synaptic responses to TETANIC STIMULATION and facilitating synaptic vesicle docking^{68,69,72–74}. But it is unclear how different patterns of synaptic activity are translated into various synaptic functional and structural changes through BDNF-modulating pathways. Here, we discuss the latest findings on the cellular mechanisms of the activity-dependent conversion of proBDNF to mature BDNF and how they contribute to bidirectional synaptic plasticity and structural modifications.

Regulation of LTP by mature BDNF. Because proBDNF can be cleaved by the extracellular protease plasmin⁸, and the plasmin-activating enzyme tPA is known to be involved in late-phase LTP (L-LTP)^{75–77}, it is reasonable to propose that an important function of the tPA/plasmin system is to convert proBDNF to mature BDNF at hippocampal synapses, and that this conversion is involved in the expression of L-LTP. Indeed, proBDNF is expressed in the hippocampal CA1 area and its expression is increased in mice that lack tPA and plasminogen⁴². tPA, through the activation of plasmin, converts proBDNF to mature BDNF *in vitro*. Furthermore, treatment of hippocampal slices derived from tPA^{-/-} and plasminogen^{-/-} mice with mature BDNF, but not cleavage-resistant proBDNF, rescued L-LTP at CA1 synapses. Therefore, the conversion of proBDNF to mature BDNF by the tPA/plasmin system seems to be crucial for the expression of L-LTP at hippocampal synapses⁴² (FIG. 3a).

A provocative finding from the study conducted by Pang *et al.* was that mature BDNF seems to be sufficient for the expression of L-LTP. Perfusion of mature BDNF to hippocampal slices after the application of weak tetanus, which normally induces only E-LTP, resulted in robust L-LTP⁴². Moreover, the mature protein rescued L-LTP in hippocampal slices in which protein synthesis was blocked for the entire course of the experiment by the inhibitor anisomycin or emetine. These results indicate that mature BDNF is a key protein-synthesis product that is required for long-term

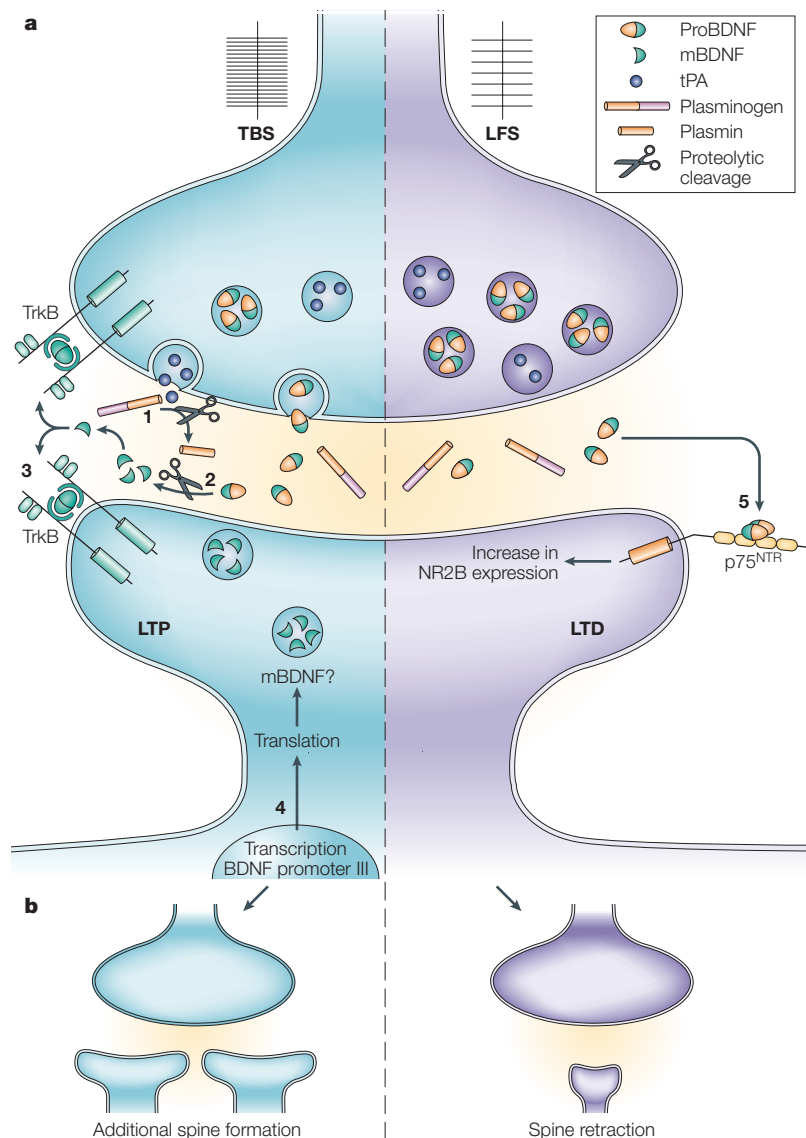


Figure 3 | The yin and yang of long-term synaptic regulation by pro- and mature BDNF.
a | Molecular cascade of brain-derived neurotrophic factor (BDNF) processing in late-phase long-term potentiation (L-LTP). In response to theta-burst stimulation (TBS), tissue plasminogen activator (tPA) is secreted into the synaptic cleft and cleaves the extracellular protease plasminogen to yield plasmin (1). Plasmin then cleaves proBDNF (the precursor of BDNF, which is released in an activity-dependent manner), yielding mature BDNF (mBDNF) (2). mBDNF binds to TrkB and triggers a series of downstream signalling pathways to induce LTP (3). During the maintenance stage of LTP, mBDNF might be generated by intracellular cleavage after postsynaptic transcription and translation (4). By contrast, proBDNF secreted extracellularly remains uncleaved after low-frequency stimulation (LFS). Uncleaved proBDNF binds to the p75 neurotrophin receptor (p75^{NTR}) (5) to facilitate the induction of long-term depression (LTD), possibly through the regulation of NMDA (*N*-methyl-D-aspartate) receptor NR2B subunit expression. **b** | Morphological alterations in synapses induced by pro- and mature BDNF. Left, BDNF–Trk signalling might be an active mechanism that converts activity-induced molecular signals into structural plasticity, contributing to synapse formation. Right, proBDNF–p75^{NTR} signalling might be important in translating activity-dependent signals into negative modulation of structural plasticity, contributing to synapse retraction.

synaptic modifications underlying L-LTP. Although L-LTP and long-term memory have long been believed to require new protein synthesis, the specific product or products that mediate the long-term changes are not known. Given that the synthesis of BDNF is activity

dependent⁷⁸, it is likely that L-LTP-inducing tetanus triggers the synthesis and/or processing of proBDNF, and that the newly generated mature BDNF is responsible for the structural and functional changes that underlie L-LTP at the CA1 synapse.

These findings raise several new questions about BDNF action during L-LTP: when is proBDNF/mature BDNF secreted and when does tPA/plasmin cleavage of proBDNF take place in modulating L-LTP expression? Previous experiments using protein-synthesis inhibitors suggest the presence of two temporally distinct processes that underlie hippocampal long-term memory⁷⁹. Preliminary experiments indicate that L-LTP in hippocampal CA1 synapses can also be divided into two stages: an induction stage (I) and a maintenance stage (II). Both stages require BDNF, but the underlying mechanisms are quite distinct (P. T. Pang, personal communication). Conversion of secreted proBDNF to mature BDNF by the tPA/plasmin protease system is required for stage I, but not stage II. By contrast, mature BDNF might function as a product of activity-dependent translation, responsible for the maintenance of L-LTP at stage II, but not stage I. However, whether the processing of proBDNF during the maintenance stage occurs intracellularly or extracellularly and which protease or proteases are involved in the cleavage remain to be established.

Given the evidence for the local secretion of BDNF at presynaptic terminals shortly after tetanus⁸⁰, and for the upregulation of BDNF mRNA in postsynaptic neurons during the maintenance of L-LTP⁷⁸, it is tempting to speculate that the requirement for BDNF at the induction stage involves presynaptic secretion and extracellular cleavage of proBDNF, whereas postsynaptic transcription and translation of BDNF followed by intracellular and/or extracellular cleavage is required at later stages (FIG. 3a).

Regulation of LTD by proBDNF. Does proBDNF have any functional roles if it remains uncleaved after secretion? As pro- and mature neurotrophins interact with different receptor/signalling systems to have opposing effects on cell survival⁸, proneurotrophin might be expected to facilitate synaptic depression through p75^{NTR}. In contrast to Trk receptors, the role of p75^{NTR} signalling in synaptic plasticity has not been investigated. However, p75^{NTR} mutant mice show impairments in water maze learning^{81,82}, inhibitory avoidance⁸¹ and habituation⁸¹ tasks, which indicates that p75^{NTR} signalling might modulate synaptic plasticity.

There is now evidence that proBDNF functions as an endogenous ligand for p75^{NTR} in the regulation of LTD⁸³. Immuno-electron microscopy detected the expression of p75^{NTR} in the dendrites and spines of hippocampal CA1 neurons, which raises the possibility that proBDNF–p75^{NTR} signalling could act directly on the postsynaptic CA1 neurons. The NMDA (*N*-methyl-D-aspartate) receptor (NMDAR)-dependent form of LTD, induced by a train of prolonged low-frequency stimulation, was quite robust at hippocampal CA1 synapses in wild-type animals, but was completely

absent in p75^{NTR}-knockout mice^{83,84}. Remarkably, other forms of synaptic plasticity, including NMDAR-dependent LTP^{83,84} and NMDAR-independent LTD⁸³, are normal in p75^{NTR} mutant mice. Moreover, application of exogenous, cleavage-resistant proBDNF to slices derived from wild-type mice enhanced LTD⁸³. The role of p75^{NTR} in synaptic depression seems to involve a specific NMDAR subunit, NR2B, a key molecule that has been implicated in LTD but not in LTP^{85,86}. Using immunostaining and western blot experiments, Woo *et al.* showed that there was a significant reduction in NR2B expressed in the p75^{NTR}^{-/-} hippocampus. Moreover, whole-cell recordings showed a severe reduction in NR2B-mediated synaptic currents in CA1 neurons of p75^{NTR}^{-/-} mice. Finally, application of proBDNF to wild-type slices increased the NR2B-mediated synaptic currents, and the proBDNF-induced enhancement of LTD was reversed by an NR2B-specific antagonist. These results indicate that proBDNF activates p75^{NTR} to promote NMDAR-dependent hippocampal LTD, and that this is achieved primarily by enhancing the expression of the NR2B subunit (FIG. 3a). It remains to be established whether proBDNF facilitates the membrane insertion, trafficking into synapses or channel properties of NR2B, all of which could, in theory, enhance LTD.

BDNF and tPA are perhaps the two best-characterized secretory molecules involved in L-LTP and long-term memory⁷⁷. The recent findings have profound physiological implications because different forms of synaptic plasticity might be regulated by the activity-dependent extracellular cleavage of proBDNF. Given that proBDNF preferentially activates p75^{NTR} over TrkB receptors¹³, and regulates hippocampal LTD, it is possible that inhibition of the proteolytic conversion of proBDNF to mature BDNF could tip the balance of the plasticity in favour of synaptic depression. Because a plausible function of LTD is to increase the signal-to-noise ratio of synaptic transmission, the proteolytic cleavage of proBDNF may also influence network function.

Structural alterations of synapses. Long-term changes in synaptic efficacy (LTP and LTD) are accompanied by, or might even lead to, morphological alterations of synapses, particularly the structure and density of dendritic spines. Two-photon imaging has shown that the induction of LTP — by pairing presynaptic stimulation with postsynaptic depolarization or by high-frequency tetanic stimulation — is followed by the appearance of new spines or the growth of filopodia-like protrusions at hippocampal CA1 dendrites in an NMDAR-dependent manner^{87,88}. Using calcium precipitation to identify tetanized spines in a three-dimensional reconstitution of EM images, Toni and colleagues showed that LTP is associated with the formation of new synapses that are created by perforated spines contacting the same presynaptic terminal^{89,90}. Spine growth is accompanied by rapid delivery and clustering of glutamate receptor subunit 1 (GluR1)⁹¹ and long-lasting enhancement of F-actin content in dendritic spines⁹². In addition, the

uncaging of glutamate by LTP-inducing repetitive stimulation leads to a rapid and selective enlargement of spine heads, and this enlargement is associated with the activation of NMDARs and calcium/calmodulin-dependent protein kinase II (CaMKII), and with actin polymerization⁹³.

In contrast to the spine growth that accompanies L-LTP expression, shrinkage or retraction of dendritic spines seems to be associated with LTD^{94,95}. NMDAR-dependent spine retraction can be induced by LTD-inducing low-frequency stimulation, whereas LTP-inducing theta-burst stimulation leads to the formation of new spines⁹⁴. Moreover, LTD-inducing low-frequency stimulation results in a marked shrinkage of spines, which can be reversed by subsequent high-frequency stimulation that induces LTP⁹⁵. Spine shrinkage depends on calcineurin and is mediated by the actin-depolymerizing factor cofilin, but not by protein phosphatase 1 (PP1), which also mediates LTD. These results indicate that different downstream pathways are involved in spine shrinkage and LTD. This activity-induced spine retraction might contribute to activity-dependent synaptic elimination. These studies support the idea that structural modifications of synapses are associated with the bidirectional expression of long-term synaptic plasticity: LTP is accompanied by new synaptic formation, whereas LTD is accompanied by synapse retraction.

The morphogenetic actions of mature BDNF raise the possibility that bidirectional structural changes of synapses could be mediated by BDNF (FIG. 3b). Exposure to BDNF leads to axonal branching^{96,97}, dendritic growth^{98,99} and the activity-dependent refinement of synapses¹⁰⁰. In cerebellar cultures and cocultured granule cells, BDNF increases the spine density of Purkinje cells without affecting dendritic complexity¹⁰¹. Long-term treatment of hippocampal slice cultures with BDNF increases synapse number and spine density in apical dendrites of CA1 pyramidal neurons without affecting dendritic length and branching⁷⁴. Interestingly, BDNF might act on different types of spine, depending on whether there is spontaneous synaptic transmission¹⁰². Under normal conditions, BDNF increases the proportion of short and stubby spines. When miniature synaptic transmission is blocked, BDNF increases the proportion of long and thin spines. Along the same lines, BDNF regulation of spine formation in the dendrites of hippocampal neurons is controlled by cyclic AMP (cAMP), a signalling molecule that is involved in L-LTP and long-term memory¹⁰³. Therefore, spontaneous synaptic activity and the consequent increase in cAMP might work together with BDNF to promote spinogenesis, favouring the formation of spines that mediate long-term synaptic plasticity. cAMP controls dendritic spine growth through two mechanisms¹⁰³. One is the 'cAMP gating' of BDNF signalling through TrkB phosphorylation, and the other is the cAMP regulation of TrkB trafficking to the postsynaptic density. These findings indicate that BDNF/Trk signalling is an active mechanism that deciphers molecular signals

Table 1 | **The yin–yang regulation of hippocampal plasticity by BDNF**

Condition	Basal	E-LTP* (1T or weak TBS)	L-LTP* (Strong TBS)	LTD (1 Hz, 15 min)
Mature BDNF	No change	Enhancement	No change	Inhibition
BDNF knockout	No change	Absent	Absent	?
ProBDNF	No change	No change	No change	Enhancement

*Brain-derived neurotrophic factor (BDNF) is required for this form of plasticity. Basal, basal synaptic transmission; E-LTP, early long-term potentiation (LTP); L-LTP, late LTP; LTD, long-term depression; TBS, theta-burst stimulation; 1T, tetanic stimulation.

into structural modifications to strengthen synaptic efficacy.

If mature BDNF–TrkB signalling is the ‘yang’ for synaptic growth, could proBDNF be the ‘yin’, promoting synaptic retraction and elimination through p75^{NTR}? A preliminary study indicated that p75^{NTR} can negatively modulate the dendrite morphology and dendritic spine number of hippocampal pyramidal neurons¹⁰⁴. In this study, hippocampal pyramidal neurons were transfected with enhanced green fluorescent protein (EGFP). In wild-type animals, overexpression of full-length p75^{NTR} in pyramidal neurons resulted in a marked reduction in dendritic length and complexity as well as spine density. By contrast, total spine density increased by 100% and dendritic complexity was much greater in EGFP-labelled pyramidal neurons in p75^{NTR}^{-/-} mice than in wild-type mice. These results, together with our finding that LTD expression is enhanced by exogenous proBDNF but impaired by p75^{NTR} mutation⁸³, indicate that proBDNF–p75^{NTR} signalling is important in translating activity-dependent signals into negative modulation of the structural plasticity of hippocampal synapses.

The signalling pathways that mediate the morphological alterations in response to various stimulation paradigms are not well elucidated. However, given that the stimulation paradigms used to induce spine formation and spine retraction are similar to those used to induce L-LTP⁴² and LTD⁸³, respectively, these results support the hypothesis that mature BDNF–TrkB and proBDNF–p75^{NTR} underlie both physiological and morphological changes (FIG. 3a,b). This type of regulation through different receptor systems mirrors that of cell survival and is consistent with the yin–yang nature of neurotrophin function.

Conclusions and future perspectives

The discovery that proneurotrophins can have biological effects opposite to those of mature neurotrophins through a different receptor system has not only changed the landscape of neurotrophin research, but also introduced new ways of looking at synaptic plasticity. To validate the ‘yin and yang’ model, it will be important to determine whether endogenous proneurotrophins are secreted under physiological conditions and how this secretion is regulated. Initial clues to proneurotrophin secretion were provided by transfection studies in culture. Because overexpression could push otherwise constitutively secreted

neurotrophins into a regulated pathway¹⁰⁵, caution is warranted in interpreting these results. However, several recent studies have shown that endogenous proBDNF is secreted from cultured cortical neurons under physiological conditions^{10,13}. Unlike the mature forms of NGF and BDNF, their respective pro- forms could have pro-apoptotic effects. This ‘killing’ effect of proneurotrophins seems to correlate with some pathological conditions of the nervous system, such as injury, Alzheimer’s disease and retinal dystrophy. Whether proneurotrophins contribute to naturally occurring cell death remains to be shown. Further work will be needed to ascertain whether other proneurotrophins (proNT3 and proNT4) have the ‘yin’ effect on the survival of distinct populations of cells and neurons.

In addition to the modulation of cell survival or death, we predict that future studies will show that the yin–yang model applies to other aspects of neurotrophin function, such as neurogenesis, growth cone turning, dendritic and axonal growth, and synapse formation. There is already evidence that p75^{NTR} activates RhoA, a small GTPase that inhibits neurite elongation and elicits growth cone repulsion^{106,107}. It seems likely that proneurotrophins are the endogenous ligands for p75^{NTR} that have these inhibitory effects. The most significant impact of the yin–yang model will be in the area of synaptic plasticity. The finding that proBDNF promotes LTD through p75^{NTR}, whereas mature BDNF facilitates LTP through TrkB at hippocampal CA1 synapses, might be just the tip of the iceberg (TABLE 1).

Opposing effects of pro- and mature BDNF are likely to be found in other models of synaptic plasticity. Indeed, the results of recent studies indicate that ocular dominance plasticity in the visual cortex is regulated by the tPA/plasmin system^{108,109}. Brief monocular deprivation elicited an increase in spine motility and a rapid pruning of dendritic spines, and such changes could be mimicked by tPA/plasmin proteolytic cascade. Future experiments should investigate whether the conversion of pro- to mature BDNF is involved in this process. It is tempting to speculate that pro- and mature BDNF might serve as the ‘punishing’ and ‘rewarding’ signals during activity-dependent synaptic competition and elimination.

The bidirectional regulation of synaptic plasticity by pro- and mature BDNF is an emerging area of research with many important implications, and a number of pressing issues need to be resolved. For example,

innovative imaging approaches will be necessary to show that secreted proBDNF is cleaved extracellularly at the hippocampal synapses by the tPA/plasmin system. Given the new role of proBDNF in LTD, the direction of hippocampal synaptic plasticity seems to depend on the extracellular cleavage of proBDNF. An unavoidable question is how the cleavage of proBDNF is controlled by stimulation frequency. One hypothesis is that high-frequency stimulation induces the secretion and/or activation of tPA, which cleaves proBDNF, leading to L-LTP. Low-frequency stimulation, on the other hand, cannot activate the tPA/plasmin system, allowing proBDNF-p75^{NTR} to control LTD. It will therefore be crucial to demonstrate the frequency-dependence of tPA secretion or activation.

The opposing effects of pro- and mature BDNF on hippocampal plasticity have also posed questions about the cell biology of the neurotrophins. For example, is proBDNF secreted presynaptically (from axonal terminals) or postsynaptically (from dendrites or spines)? BDNF-containing LDCVs have been found in sensory terminals¹¹⁰, but in hippocampal neurons BDNF is stored mainly in non-LDCV vesicles in dendrites³¹. Studying how the axonal and dendritic

secretion of BDNF is regulated could provide important insights into the functional role of BDNF under different physiological conditions. If proBDNF is the main form of BDNF that is secreted from neurons^{9,31}, could the cleavage of proBDNF occur intracellularly so that mature BDNF is secreted instead? It is possible that under particular circumstances, such as stress or sustained elevation of neuronal or synaptic activity, mature BDNF becomes the main form secreted. Finally, it will be important to determine the proportions of proBDNF and mature BDNF that are secreted in the constitutive or regulated secretory pathways under physiological conditions, and how this process is regulated. In this regard, there is an urgent need to develop appropriate techniques, such as enzyme-linked immunosorbant assay (ELISA) or immunohistochemistry, to distinguish between pro- and mature BDNF, and to measure their proportions *in vitro* and *in vivo*. Studying the yin-yang aspects of neurotrophin function represents an exciting new challenge that is likely to generate important advances in our understanding of how neurotrophins regulate bidirectional processes at the cellular level, and how this affects a range of cognitive processes.

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Competing interests statement

The authors declare no competing financial interests.

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