Neurotrophin regulation of neural circuit development and function

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Abstract | Brain-derived neurotrophic factor (BDNF) — a member of a small family of secreted proteins that includes nerve growth factor, neurotrophin 3 and neurotrophin 4 — has emerged as a key regulator of neural circuit development and function. The expression, secretion and actions of BDNF are directly controlled by neural activity, and secreted BDNF is capable of mediating many activity-dependent processes in the mammalian brain, including neuronal differentiation and growth, synapse formation and plasticity, and higher cognitive functions. This Review summarizes some of the recent progress in understanding the cellular and molecular mechanisms underlying neurotrophin regulation of neural circuits. The focus of the article is on BDNF, as this is the most widely expressed and studied neurotrophin in the mammalian brain.

This Review is dedicated to Hans Thoenen (1928–2012), who pioneered and inspired much of the neurotrophin research over the past three decades.

The first member of the neurotrophin family, nerve growth factor (NGF)[1,2], was discovered in the early 1950s as a target-derived protein that promotes the survival and growth of sympathetic and sensory neurons during development. The establishment of the neurotrophin family came with the purification and characterization of brain-derived neurotrophic factor (BDNF) from the pig brain by Thoenen and colleagues[3]. They showed that BDNF has a similar neurotrophic effect on cultured sensory neurons[3], and its amino acid sequence is highly homologous to NGF[4]. Since then, two other neurotrophins have been identified in the mammalian brain: neurotrophin 3 (NT3) and NT4 (REF. 5). Neurotrophins bind to a receptor called p75NTR (also known as TNFRSF16) and to one of the three tropomyosin-related kinase (TRK; also known as NTRK) receptors — NGF binds to TRKA, BDNF and NT4 bind to TRKB, and NT3 binds to TRKC[6]. Through the differential expression and cellular localization of their receptors, neurotrophins can elicit diverse cellular functions in different types of neurons and at different cellular loci (for reviews, see REFS 6,7).

A major advance in our understanding of neurotrophin function came with the discovery that the synthesis of neurotrophins in the brain is increased by both seizure activity and sensory stimulation[8]. This discovery inspired later studies on the involvement of neurotrophins in activity-dependent synaptic plasticity. Indeed, active research over the past two decades has shown that neurotrophins regulate nearly all aspects of neural circuit development and function, including cell proliferation and differentiation, axon and dendrite growth, synaptogenesis, and synaptic function and plasticity[7,9,10]. Here, we summarize some of the recent developments in our understanding of the role of neurotrophins in these diverse processes, with a specific focus on BDNF.

Synthesis, processing and secretion

Knowledge of the synthesis, cellular processing and transport, and the spatiotemporal control of secretion of neurotrophins, as well as the dependence of all these processes on neuronal activity, is crucial for understanding the wide-ranging regulatory functions of neurotrophins in the brain.

Transcriptional regulation. In the mammalian brain, the mRNA levels of both *NGF* and *BDNF* are tightly regulated by neural activity[8]. Membrane depolarization initiates Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs) and Ca^{2+}-permeable glutamate receptors, notably NMDA-type glutamate receptors (NMDARs), which then triggers the binding of transcription factors such as Ca^{2+}-response factor (CaRF; also known as ALS2CR8) and cyclic AMP (cAMP) response element (CRE)-binding protein (CREB) to the corresponding regulatory elements of *Bdnf*[11,12]. Initially, four promoters upstream of the 5′

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untranslated region (UTR) of *Bdnf* were described in rodents (termed I, II, III and IV)[13], but further genomic analyses have identified a total of nine promoters in human and rodent *BDNF*, leading to the new nomenclature of *BDNF* 5′ UTR promoters (termed I–IX)[14,15]. Among them, promoter IV (previously known as promoter III) is highly responsive to neuronal activity. Indeed, promoter IV is regulated by various patterns of stimuli encoded by Ca^{2+} and/or cAMP signals[12,16–18], because it contains multiple Ca^{2+}-responsive sequences and CREs to which CaRF and CREB bind, respectively, and initiate transcription. In mice, a specific knock-in mutation in promoter IV disrupts sensory experience-induced expression of *Bdnf* and causes defective development of cortical inhibitory circuits[17], highlighting the physiological importance of *Bdnf* promoter IV-dependent transcription.

The complex structure of the *Bdnf* promoter region is intriguing because it may explain how BDNF can have multiple roles in diverse brain functions. It is possible that differential activation of alternative *Bdnf* promoters in different cell types and under different physiological conditions may induce alternative splicing and polyadenylation. This would lead to the production of multiple *Bdnf* transcripts, each of which could have a distinct profile of stability and cellular localization, allowing *BDNF* expression with precise spatiotemporal patterns. This assertion is supported by semi-quantitative analyses of *BDNF* transcripts from human and rodent organs, which showed that numerous alternatively spliced *BDNF* mRNAs are differentially expressed in the brain and peripheral tissues[14,15,19], suggesting that these tissues use different promoters to ensure correct BDNF levels for normal development. Moreover, different spatiotemporal patterns of cytoplasmic Ca^{2+} increases, arising, for example, from Ca^{2+} influx via activated VGCCs versus NMDARs[20,21], may activate specific sets of downstream effectors that initiate *Bdnf* transcription through different promoters.

Epigenetic regulation of chromatin structure may alter the efficacy of activity-dependent *Bdnf* transcription. *In vitro* studies showed that depolarization-activated Ca^{2+}/calmodulin kinase II (CaMKII) phosphorylates methyl-CpG-binding protein 2 (MECP2) — a transcriptional repressor that binds to methylated DNA in the *Bdnf* promoter region[22]. This phosphorylation causes the release of MECP2 and its interacting proteins histone deacetylase 1 (HDAC1) and SIN3 transcription regulator homolog A (SIN3A) from promoter IV (previously termed promoter III) and, consequently, an increase in *Bdnf* transcription[23,24]. However, *Mecp2*-null mice (which are a model of Rett syndrome) showed a reduced level of *Bdnf* expression (interestingly, additional deletion of *Bdnf* in these mice resulted in an earlier onset of the disease)[25], suggesting that a mechanism besides transcriptional suppression may be involved in determining BDNF levels (for example, MECP2-dependent post-transcriptional regulation of BDNF expression). Another example of epigenetic regulation of *Bdnf* expression is the finding that chronic social defeat stress in mice increases histone methylation at *Bdnf* promoters IV and VI (previously termed promoters III and IV) and suppresses the transcriptional activity at these sites[26]. However, it seems that epigenetic regulation modifies the permissive state of the genome for activity-dependent *Bdnf* transcription rather than mediating the activity-dependent process itself. For example, in mice, strong neuronal activity that is induced by electroconvulsive treatment can induce DNA demethylation through activation of the immediate early gene *Gadd45* (growth arrest and DNA damage-inducible 45), allowing the transcription of *Bdnf* and adult neurogenesis[27]. Whether epigenetic modifications that are induced by physiological neural activity, such as that involved in memory formation, could trigger activity-dependent transcription of specific neurotrophin genes remains to be examined.

Post-transcriptional regulation. During neurotrophin transcription, multiple differentially spliced transcripts are produced, which share a common coding region but have different segments of the 5′ and 3′ UTRs[14,28,29]. The functional significance of having these multiple variants is only beginning to be explored. Rat neurons generate *Bdnf* transcripts with either a short or a long 3′ UTR, but only transcripts containing the long 3′ UTR seem to be directed to dendrites, where local BDNF synthesis may occur[30,31]. Selective genetic deletion of the long 3′ UTR transcript results in an increased number of dendritic spines with a smaller spine head size in hippocampal and cortical neurons[30,32], as well as a reduction in GABAergic innervation of visual cortical neurons[32]. Together, these findings suggest that BDNF is synthesized in dendrites and has an important function at this location. However, as is the case for activity-regulated cytoskeleton-associated protein (ARC/ARG3.1), which seems to undergo synaptic activity-induced dendritic synthesis[33], direct evidence of local dendritic translation of *Bdnf* mRNAs is yet to be demonstrated. It has also been reported that *Bdnf* transcripts with different 5′ UTR splice variants are segregated to distinct cellular compartments — for example, somal and proximal dendrites versus distal dendrites — before being locally translated and secreted[34]. Dendritic accumulation of *Bdnf* transcripts may be promoted by neuronal activity through the Ca^{2+} influx mediated by VGCCs and NMDARs[35]. As mRNA complexes in dendrites interact with motor protein kinesins (for example, kinesin superfamily protein 5 (KIF5)), Ca^{2+}-dependent phosphorylation of kinesins could regulate cargo–motor interactions[36] and therefore the dendritic targeting of *Bdnf* mRNA.

Two lines of evidence gave credence to the idea that synaptic activity can induce local translation of dendritic mRNAs. First, synaptic activity drives the movement of Golgi structures and ribosomes from the dendrite towards the spine[35]. Second, translation initiation factors, such as eukaryotic translation initiation factor 4 (EIF4), elongation factors, such as eukaryotic elongation factor 2 kinase (EEF2) and S6 kinase, and cytoplasmic polyadenylation element-binding protein

(CPEB) can all be found within the polyribosome complexes in dendrites. These factors could initiate local translation of proteins, including BDNF, upon phosphorylation by metabotropic glutamate receptor- or NMDAR-dependent activation of kinases, such as mitogen-activated protein kinase (MAPK), mammalian target of rapamycin (mTOR) and CaMKII[37]. It is of interest to note that MAPK- and mTOR-dependent mechanisms are also potential targets of BDNF secreted by presynaptic nerve terminals[38]. Thus, activity-driven secretion of BDNF could induce synapse-specific synthesis of other proteins that are necessary for stable synaptic modifications, such as those associated with late-phase long-term potentiation (L-LTP). This idea is supported by the finding that both protein synthesis and BDNF–TRKB signalling are necessary for the stable spine enlargement that is associated with the induction of LTP[39]. However, at the moment, there is no direct evidence demonstrating that synaptic activity induces local BDNF synthesis in dendrites or that locally synthesized BDNF is required for synapse-specific long-term plasticity. Interestingly, a recent study found that granules containing BDNF exist in Schaffer collateral axons but not in CA1 pyramidal cell dendrites[38], suggesting that the source of locally secreted BDNF in the CA1 region is presynaptic.

Post-translational processing. BDNF mRNA and proteins levels are present at extremely low levels in the brain. Indeed, a 10^6-fold purification was required to identify BDNF from the pig brain[3], and quantitative determination of *BDNF* mRNA levels in the developing visual system showed that there is only, on average, 1.2 copies of *BDNF* mRNA in the developing retina[40]. It is important to keep the low levels of BDNF in mind when considering how BDNF is normally processed in the neuron, because the processing mechanism may be quite different when the BDNF level is artificially raised. Furthermore, accurate determination of the mRNA and protein levels of BDNF in the brain is difficult and prone to errors. These problems may be the cause of conflicting results reported in the literature.

Like many other secreted proteins, BDNF is initially synthesized in the endoplasmic reticulum as a precursor protein, pre-pro-BDNF, which is then converted to pro-BDNF by removal of the signal peptide. Pro-BDNF is then cleaved to generate BDNF, but exactly where this cleavage takes place and the nature of the protease (or proteases) involved remains controversial. A study involving immunohistochemistry and immunogold electron microscopy with specific antibodies to the pro- and mature domains of BDNF showed that the pro-peptide is colocalized with mature BDNF in secretory granules in the presynaptic axon terminals[38], suggesting that the cleavage may occur in the secretory granule. Under some experimental conditions, however, the processing of pro-BDNF into mature BDNF was found to occur extracellularly via the actions of metalloproteinases and the extracellular protease plasmin[41,42]. The initial evidence for the presence of extracellular pro-BDNF came from studies involving overexpression of epitope-tagged BDNF in cells in which the levels of tagged pro-BDNF may have saturated the limited intracellular processing capacity. In a later study involving knock-in mice in which *Bdnf* was replaced with *Bdnf–Myc* — leading to physiological levels of the MYC-tagged protein — pro-BDNF–MYC was found to be rapidly converted intracellularly to BDNF–MYC before activity-induced secretion[43]. However, in another study using BDNF–haemagglutinin knock-in mice, a substantial amount of endogenous pro-BDNF was found in neonatal mice and in the supernatant of glia-free hippocampal neuronal cultures that were supplemented with plasmin inhibitor[44]. Whether results obtained in cell cultures reflect physiologically relevant conditions remains to be clarified.

Resolving the controversial issue of the primary site of pro-BDNF processing is important because recombinant pro-BDNF does not activate TRK but instead activates p75NTR to promote cell apoptosis[45]. Thus, the level of extracellular pro-BDNF, if it is indeed secreted by neurons, must be under strict spatiotemporal control, as must be the secretion and action of the proteases in the synaptic cleft that cleave it. Interestingly, several studies[41,46] demonstrated that the binding of pro-BDNF to p75NTR is necessary for long-term depression (LTD) induction at CA3–CA1 hippocampal synapses. By contrast, a different study found that LTD induction at these synapses was normal in conditional *Bdnf*-knockout mice, suggesting that neither pro-BDNF nor mature BDNF is involved in LTD[43], although compensatory effects caused by the expression of other neurotrophic factors in these knockout mice cannot be excluded. Given these conflicting findings, both the processing and extracellular function of pro-neurotrophins remain uncertain. However, there is consensus that the release of pro-NGF under pathological conditions — such as after brain injury in rodents — does occur[47] and probably causes neuronal apoptosis via the activation of p75NTR.

The recent identification of a single-nucleotide polymorphism (SNP) in human *BDNF* — resulting in a methionine substitution for valine at residue 66 (Val66Met)[48] — has revealed the importance of the pro-domain for normal processing of newly synthesized BDNF. Mice expressing Val66Met BDNF displayed impaired sorting of BDNF into secretory granules as well as defective synaptic localization and activity-regulated secretion of BDNF-containing granules. These deficits all contributed to a low level of extracellular BDNF despite the normal level of total BDNF expression and constitutive secretion of BDNF[48,49]. Interestingly, human Val66Met carriers showed smaller volumes of multiple brain regions and impaired episodic memory function[48–50], although the precise neural circuits affected by this polymorphism remain largely unclear. It is plausible that these effects could be due to the reduced levels of BDNF secretion.

Late-phase long-term potentiation
(L-LTP). Transcription- and translation-dependent synaptic potentiation that persists for more than 3 hours, and is typically induced by multiple trains of high-frequency stimulation spaced at intervals of a few minutes.

Activity-induced secretion. Neurotrophin secretion is normally triggered by membrane depolarization[51,52] — although it can also be triggered by extracellular factors, including neurotrophins themselves[53] — through a process that is mediated by an increase in cytoplasmic Ca^{2+} and cAMP levels[52,54]. Secretion of neurotrophins has been monitored mostly by ELISA-mediated detection of neurotrophins from the extracellular medium of cultured neurons or by visualizing changes in fluorescence intensity in cultured neurons transfected with cDNAs that express neurotrophins tagged with a fluorescent protein (for example, green fluorescent protein)[55,56]. Neuronal secretion of BDNF is most pronounced under high-frequency stimulation (HFS; ~100 Hz) or theta-burst stimulation (TBS)[55–58]. These stimuli are capable of inducing LTP at excitatory synapses, which is consistent with the fact that BDNF is required for LTP induction[59]. Moreover, synaptic activation of glutamate receptors (particularly NMDARs) provides additional Ca^{2+} influx that facilitates neurotrophin exocytosis. Despite the caveat that some of these studies were performed on neurons with a high level of exogenous BDNF, these results have contributed to our understanding of the activity pattern and signalling events that are required for triggering BDNF secretion.

During axon–dendrite differentiation, BDNF secretion becomes more localized to the distal end of the putative axon, where secreted BDNF may act in a self-amplifying autocrine manner to stabilize cAMP-dependent axon initiation and growth[54]. After synaptogenesis, neurotrophin secretion seems to be localized mainly to the pre- and postsynaptic regions, where secreted neurotrophins exert their synaptic actions (FIG. 1). In hippocampal organotypic cultures, spontaneous synaptically localized Ca^{2+} transients are observed at postsynapitc sites (as revealed by the punctate immunostaining of postsynaptic density protein 95 (PSD95)) in the presence of tetrodotoxin (a neurotoxin that blocks action potentials), and their frequency can be reduced by blocking BDNF signalling with BDNF-specific antibodies or with the kinase inhibitor K252a[60]. Together, these results suggest that spontaneous BDNF secretion can take place at the synapse. Secretion of neurotrophins from presynaptic nerve terminals also occurs, as indicated by the anterograde transport and axodendritic transfer of neurotrophins that occurs *in vivo*[61–63] and in cultured cortical neurons[64]. Recent studies in cultured hippocampal neurons expressing pH-sensitive fluorescent protein-tagged BDNF, however, showed that axonal secretion of BDNF requires much higher levels of neuronal spiking than dendritic secretion[58]. As discussed below, HFS (>100 Hz) is necessary to trigger BDNF secretion from presynaptic axons that is responsible for inducing a form of hippocampal CA3–CA1 LTP that involves an enhanced presynaptic release mechanism[65]. By contrast, low-frequency stimulation (1–10 Hz) of cultured hippocampal neurons is sufficient to trigger dendritic secretion of BDNF[58]. This asymmetry in the spiking requirement for pre- versus postsynaptic neurotrophin secretion may be attributed to the postsynaptic NMDARs — the source of additional Ca^{2+} — which are more readily activated by neuronal activity than presynaptic ones. Alternatively, or in addition, differences in synaptotagmin isoforms that are associated with axonal versus dendritic neurotrophin-containing granules[66] may confer differential requirements for local Ca^{2+} to trigger exocytotic secretion of neurotrophins.

As all neurotrophins are positively charged at physiological pH and readily bind to cell surfaces and the extracellular matrix, extracellular diffusional spread of secreted neurotrophins is limited. However, secreted neurotrophins that are bound to their surface receptors could be taken up by neurons via receptor-mediated endocytosis. Endosomes containing these neurotrophin–TRK complexes are biochemically active[67] and

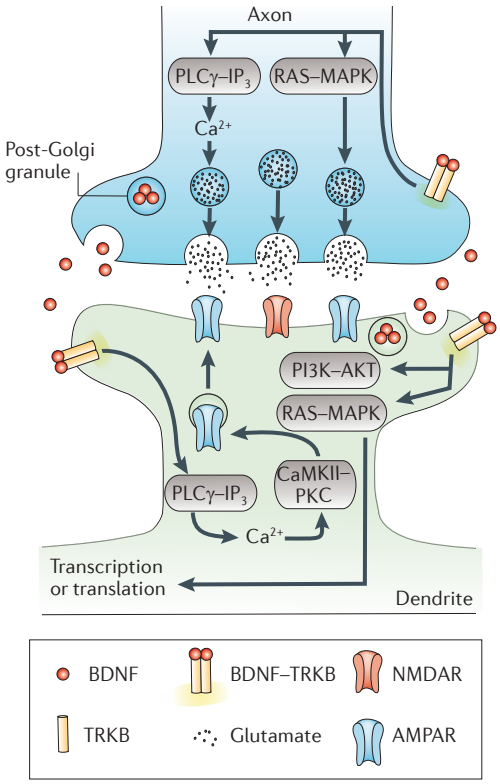

Figure 1 | **Local BDNF effects on synaptogenesis.** Local brain-derived neurotrophic factor (BDNF)–tropomyosin-related kinase B (TRKB) signalling enhances the presynaptic release of neurotransmitter through the following actions: activation of a RAS–mitogen-activated protein kinase (MAPK)-dependent and a phospholipase Cγ (PLCγ)–inositol trisphosphate (IP_3)-dependent signalling mechanism; increased postsynaptic AMPA-type glutamate receptor (AMPAR) insertion by Ca^{2+}-dependent activation of Ca^{2+}/calmodulin kinase II (CaMKII)–protein kinase C (PKC) pathways via PLCγ–IP_3 signalling; and maturation of spine structures and increased expression of synaptic proteins (this is mediated by phosphoinositide 3-kinase (PI3K)–AKT and RAS–MAPK signalling pathways). Secretion of BDNF from post-Golgi BDNF-containing granules may occur either constitutively or in response to electrical activity. NMDAR, NMDA-type glutamate receptor.

Signalling endosomes
Endosomes containing ligand–receptor complexes that remain active, transducing cytoplasmic signals as they are transported within the cell.

are actively transported to other regions of the neuron. Retrograde axonal transport of these signalling endosomes to the soma allows the neurotrophins to exert their transcriptional regulation of activity-dependent genes such as *Arc* in the nucleus[68]. Moreover, neurotrophins that are taken into signalling endosomes could also be secreted in response to neuronal activity. This is suggested by the finding that neuronal depolarization could induce the re-release of false transmitter (acetylcholine)[69], glutamate[70] and the lipid dye FM1-43 (REF. 71) after their endocytic uptake. Neurotrophin secretion via endosomal exocytosis probably differs from that associated with exocytosis of neurotrophin-containing secretory granules, both in its dependence on activity (that is, its Ca^{2+} dependence) and in the cellular functions of the secreted neurotrophins.

In summary, BDNF secretion from both axons and dendrites has been readily demonstrated by the use of cultured hippocampal neurons overexpressing *BDNF*. *In vivo*, BDNF-containing granules have not been found in the postsynaptic dendrites of hippocampal pyramidal cells[38]. However, it is possible that dendrites may secrete BDNF contained in the endosomes that are formed locally at the dendrite or transcellularly transported from the nerve terminals.

Regulation of neural circuit development

The development of neural circuits involves many stages, including neural stem cell survival and differentiation, axon–dendrite differentiation, axonal growth and guidance, synapse formation and maturation, and refinement of developing circuits. Neurotrophins have pivotal roles in nearly all of these stages of circuit development.

Neural stem cell survival and differentiation. Before neuronal differentiation, neurotrophins may already serve a function in regulating the survival and differentiation of neural stem cells, as embryonic neural stem cells express TRKs and p75NTR[72,73]. Indeed, treatment of cultured cortical and hippocampal neural stem cells from mouse embryos with recombinant BDNF and NGF during the first 3 days *in vitro* (DIV) promoted the cells' survival and differentiation into neurons by 10–14 DIV. By contrast, NT3 exerted no such effect[72,74]. After neuronal differentiation (~21 DIV), however, BDNF was found to be unnecessary for neuronal survival, but it was needed for neurite outgrowth[72]. Further supporting a role for BDNF in neuronal differentiation, deletion of *Bdnf* in mice inhibits the differentiation of interneurons[75]. Taken together, these results suggest that neurotrophins serve regulatory functions from the earliest developmental stages of neuronal differentiation in the developing brain.

Neurotrophins also regulate the survival and differentiation of adult neural stem cells in the dentate gyrus of the hippocampus and in the subventricular zone (SVZ). In rats, direct infusion of BDNF into the dentate gyrus or SVZ or adenoviral expression of *Bdnf* in these areas increased the number of adult-born neurons[76,77]. By contrast, BDNF- or TRKB-deficient mice showed reduced survival of newly generated hippocampal granule cells[78,79]. In line with this finding, adult mice with impaired activity-dependent BDNF secretion (which was due to the Val66Met polymorphism in *Bdnf*) showed reduced survival of newly generated neurons in the SVZ[80]. This beneficial effect of neurotrophins on adult neural stem cells seems to be mediated by TRKs, because reduced *Trkb* expression in these stem cells impaired their proliferation and neurogenesis in adult mice[80,81].

Survival of differentiated neurons. NGF was first identified as the target-derived factor that is required for the survival of developing sympathetic and sensory neurons[2], and BDNF and NT3 were later shown to promote the survival of some subpopulations of cultured sensory and CNS neurons[82]. It has generally been thought that these survival effects are mediated by the binding of neurotrophins to TRKs and subsequent activation of downstream pathways that upregulate pro-survival gene expression[6,7]. By contrast, a negative signal for cell survival could be induced through neurotrophin-mediated p75NTR signalling[83], which activates caspase-dependent apoptotic pathways[6,7].

The notion that each neurotrophin promotes the survival of specific populations of CNS neurons had largely been based on findings from cell culture studies[84] and from experiments involving axotomy of retinal ganglion cells (RGCs) and motor neurons[85]. Later studies involving conditional *Bdnf*-knockout mice[86] showed that the absence of BNDF did not cause a significant reduction in neuronal cell number but did lead to a region-specific reduction in dendritic complexity and spine density. These findings suggest that BDNF is not a survival factor but a differentiation factor for most CNS neurons.

A recent study[87] further supports this suggestion and has provided important new insights into the mechanism by which neurotrophins regulate neuronal survival. Through the use of embryonic stem cells that express only one type of TRK, this study demonstrated that cells expressing TRKA or TRKC readily die in the absence of the neurotrophin-binding partner of the TRK, whereas the survival of TRKB-expressing cells is not affected by the absence of BDNF[87]. These results imply that in developing parasympathetic nervous system (PNS) neurons, TRKA itself can trigger cell death when NGF is not available, as was observed in earlier studies[2]. However, BDNF — the most widely expressed neurotrophin in the CNS — does not seem to be essential for the survival of CNS neurons because TRKB does not have a death-activating capability[87].

Axon–dendrite differentiation, growth and guidance. During axon–dendrite polarization of cultured hippocampal neurons, exposure of an undifferentiated neurite to extracellular BDNF promotes its differentiation into an axon[88]. This effect is mediated by a TRKB-dependent increase of cAMP and protein kinase A (PKA) activity, which activates downstream enzymes — including liver kinase B1 (LKB1; also known as STK11) and SAD (also known as BRSK)[89,90]

— that drive the cytoskeletal changes that are associated with axon formation. Interestingly, endogenous BDNF secreted by the neuron itself contributes to axon initiation; among cultured hippocampal neurons, those transfected with specific short hairpin RNAs (shRNAs), which downregulated BDNF synthesis, showed markedly impaired axon initiation, whereas neighbouring neurons that were untransfected showed normal axon initiation[54]. Because an increase in cAMP and Ca^{2+} levels induced by TRKB activation can promote neurotrophin secretion[53,54], a local self-amplifying autocrine loop could be established via neurotrophin-induced neurotrophin secretion, resulting in a persistent local rise in cAMP and PKA activity that is sufficient for axon initiation[54]. Such local autocatalytic production of second messengers could be a general mechanism for other cellular polarization processes. In sympathetic neurons, TRKA that is expressed on the neuronal soma surface is endocytosed and anterogradely transported into and exocytosed at axon growth cones, a process that is enhanced by locally applied NGF at distal axons[91]. Thus, transcytosis could rapidly transport available TRK molecules to the growth cone, facilitating a positive-feedback mechanism for neurotrophin action at this site.

In cultured neurons, an extracellular gradient of neurotrophins can cause chemotropic turning of axonal growth cones[92,93]. This guidance function might also occur *in vivo*, as in developing mouse limb buds, sensory axons can be directed towards beads containing NGF, BDNF, NT3 or NT4 (REF. 94). As secreted neurotrophins readily bind to cell surfaces and the extracellular matrix, they are more suited to being local rather than long-range guidance cues. Dendrites may locally secret neurotrophins to attract axons with appropriate TRK molecules. Indeed, the local effect of secreted neurotrophin was demonstrated in ferret brain slices[95], in which local secretion of BDNF by the cell body or single dendrites of BDNF-overexpressing neurons could elicit increased growth and branching of nearby dendrites (located within ~4.5 μm of the site of BDNF secretion). *In vivo* infusion of BDNF into the optic tectum of *Xenopus laevis* tadpoles also promoted the branching of and complexity in RGC axons, whereas infusion of BDNF-specific antibodies had the opposite effects, implicating a role for the endogenous BDNF in regulating axonal growth[96]. However, mice lacking or showing reduced expression of *Bdnf* or *Trkb* had no gross morphological alterations in axon projections[97,98], presumably because of the compensatory effects of other neurotrophins or guidance factors.

In more mature pyramidal cells of developing cortical slices, dendritic growth is increased by treatment with recombinant BDNF but inhibited by NT3 in layer 4. By contrast, in layer 6, the effects of these two neurotrophins are reversed[99]. Interestingly, neuronal activity helps to promote the BDNF effect on dendritic arborization in developing cortical slices[100], which is similar to the effect of neuronal depolarization in facilitating BDNF-induced potentiation of neurotransmitter release at neuromuscular synapses in culture[101]. Electrical stimulation of cultured hippocampal neurons in the presence of BDNF also potentiates TRKB activity and enhances the endocytic internalization of BDNF–TRKB complexes[102]. As biolistic transfection of cortical pyramidal cells and interneurons in organotypic slice cultures with BDNF and NT4 can accelerate dendrite development in the transfected neurons themselves, these neurotrophins may act as autocrine factors to promote dendrite growth[103], which is reminiscent of the autocrine promotion of axon growth during neuronal polarization described above[54]. Finally, exocytic secretion of various neurotrophins and membrane recruitment of TRKs are all regulated by similar cAMP- and Ca^{2+}-mediated mechanisms[104]. Thus, the presence of one type of neurotrophin may trigger the secretion of multiple endogenous neurotrophins and the surface expression of their receptors that are stored in the cell[53], thereby allowing synergistic autocrine actions on the neuron. The synergistic effect of depolarization on BDNF–TRKB signalling described above could also be attributed to the contribution of depolarization-induced Ca^{2+} influx to neurotrophin secretion and TRKB insertion that amplify the autocrine action of neurotrophins. In view of the recent advance in our understanding of cellular processing of pro-neurotrophins, some of the previous findings based on tranfection of neurons with neurotrophin cDNA may need to be re-evaluated, because a high level of expression of exogenous neurotrophins may result in the release of unprocessed neurotrophins at physiologically irrelevant subcellular compartments.

Apart from their local actions on axon and dendrite growth, endocytosed neurotrophin–TRK complexes may be transported within neurons to affect growth at a long distance from their original site of endocytosis. For example, infusion of BDNF into the *X. laevis* optic tectum not only promoted local RGC axon growth but also increased dendritic arborization of RGCs in the retina[105]. Interestingly, BDNF applied directly to RGC dendrites in the retina inhibited their growth[105]. This surprising difference between the local and distant effects of BDNF signalling suggests that signalling of neurotrophin–TRK complexes at the plasma membrane differs from that of neurotrophin–TRK signalling endosomes. Similar long-range modulatory effects of neurotrophin signalling have been reported in the rat visual system: BDNF injected into the eye could travel along the optic nerve and regulate the survival of neurons in the superior colliculus and lateral geniculate nucleus[106] as well as ocular dominance plasticity in the visual cortex[107].

In addition to full-length TRKB, mammalian brains express a C-terminal-truncated *TRKB* gene product (TRKB-T1; also known as glycoprotein 95) at a high level[108]. By forming a nonfunctional heterodimer with TRKB[109] and binding to BDNF with an affinity that is similar to the full-length protein[110], TRKB-T1 can interfere with BDNF–TRKB signalling by sequestering extracellular BDNF without transducing TRKB signals. The endogenous function of TRKB-T1 in neurons remains to be clarified. There is evidence that it may regulate TRKB signalling during development. In TRKB-T1-selective knockout (*Trkb-t1*$^{-/-}$) mice,

Biolistic transfection
A gene transfection technique for injecting DNA-coated subcellular-sized metal particles at high velocity into target cells using an apparatus known as a gene gun.

Ocular dominance plasticity
The relative efficacy of visual inputs from the left and right eye in eliciting responses in the visual cortical neurons can be affected permanently by a brief period of unbalanced visual inputs from the two eyes during postnatal brain development.

dendritic growth is defective in some brain areas (such as the amygdala)[111], possibly resulting from abnormal TRKB signalling. Given that TRKB-T1 can transduce phospholipase Cγ (PLCγ)-dependent Ca^{2+} transients[112] and Rho GTPase signalling-dependent morphological changes[113] in glial cells, its TRKB-independent function in neurons needs to be further explored.

Together, these observations indicate that spatiotemporal-specific expression and trafficking of neurotrophins and their receptors in different neurons, along with differential neurotrophin actions on dendrite morphogenesis, may contribute to the patterning of neural connections.

Synapse formation and maturation. In the developing optic tectum of *X. laevis* tadpoles, infusion of exogenous BDNF increases the density of immunostained puncta of synaptic proteins, suggesting that BDNF promotes synaptogenesis[114]. This BDNF-induced synaptogenesis may be attributed in part to the BDNF-induced increases in axon and dendrite arborization[96,100,115], which raise the probability of physical encounters between axons and dendrites. In addition, neurotrophins also regulate both functional and morphological maturation of synapses. Functional transmission can be established before morphological maturation of synaptic specializations[116]. The synaptic modulatory effect of neurotrophins was first discovered in cultures of dissociated *X. laevis* spinal neurons and myocytes[117], in which application of recombinant BDNF or NT3 markedly increased the synaptic activity within a few minutes. This rapid action of neurotrophins was caused by a greater probability of exocytosis of synaptic vesicles at presynaptic nerve terminals rather than by enhanced postsynaptic responses, because spontaneous excitatory postsynaptic currents (EPSCs) increased in frequency but not in amplitude. Prolonged exposure of immature neuromuscular synapses to exogenous BDNF and NT3 for a few days also led to accelerated maturation of these synapses, which exhibited increases in the amplitudes of spontaneous EPSCs and the clustering of the synaptic vesicle proteins synaptophysin and synapsin-1 (REF. 118). In the absence of exogenously applied neurotrophins, activity-dependent secretion of endogenous NT3 from the muscle cells seems to have a role in synapse maturation[119]. Thus, BDNF and NT3 may be involved in both functional and morphological maturation of neuromuscular synapses.

In cultures of hippocampal neurons, exogenous BDNF promotes the formation of both excitatory and inhibitory synapses, whereas exogenous NT3 only promotes the formation of excitatory synapses[120]. These effects on synaptogenesis and maturation are consistent with the action of neurotrophins in promoting the expression of many synaptic proteins, including enzymes for neurotransmitter synthesis, synaptic vesicle proteins and subunits of postsynaptic neurotransmitter receptors (for a review, see REF. 121). Interestingly, a brief train of presynaptic action potentials rapidly converts nonfunctional contacts between cultured hippocampal neurons into functional synapses, an effect that can be mimicked by applying exogenous BDNF and that can be impeded by interfering with presynaptic BDNF signalling[122]. A similar effect of endogenous BDNF signalling on synapse maturation was also observed in developing hippocampal synapses — blocking BDNF signalling increases activity-dependent presynaptic release probability by downregulating tonic activation of presynaptic kainate receptors[123]. Thus, activity-induced secretion of endogenous BDNF at the contact site could facilitate synapse maturation.

The role of BDNF in regulating the maturation of GABAergic synapses has attracted much attention. In the developing visual cortex, transgenic mice overexpressing BDNF showed accelerated maturation of GABAergic innervation and inhibition, and earlier termination of critical-period plasticity[124,125]. Deprivation of visual experience-associated activity by dark-rearing prolonged the critical period for ocular dominance plasticity and retarded the maturation of GABAergic inhibition in wild-type mice, but these effects were absent in BDNF-overexpressing transgenic mice[107,126]. In addition, pro-BDNF may be required for the development of GABAergic synapses; a recent study showed that GABAergic synaptic activity could be bidirectionally regulated by pro-BDNF–p75NTR signalling through an NMDAR-dependent manner[127].

The balanced development of excitatory and inhibitory synapses is crucial during neural circuit formation[128]. As BDNF can promote maturation of both excitatory and inhibitory synapses, and secreted BDNF probably acts only locally on nearby synapses, activity-induced BDNF secretion at excitatory synapses may exert a facilitatory effect on the maturation of nearby synapses on the same postsynaptic dendrite. Indeed, in the CA3 area of the postnatal hippocampus, glutamatergic activity caused Ca^{2+}-dependent release of BDNF from postsynaptic dendrites in CA3 neurons, and this secreted BDNF was responsible for a persistent potentiation of type A GABA receptor ($GABA_AR$)-mediated synaptic currents[129]. Furthermore, GABAergic synaptic activity in the developing hippocampus also induces BDNF secretion through activation of type B GABA receptors ($GABA_BRs$), and could promote maturation of perisomatic GABAergic synapses[130]. The action of $GABA_BRs$ may be mediated through increasing CaMKII activity[131], which could trigger postsynaptic secretion of BDNF and NT3 (REF. 132). Thus, the BDNF secretion that is associated with developing GABAergic synapses might also promote maturation of nearby excitatory synapses, leading to bidirectional BDNF-mediated crosstalk that coordinates the maturation of adjacent excitatory and inhibitory synapses. Cell-type specificity in the BDNF regulation of synapse development may also be important for homeostatic scaling of synapses. Secreted BDNF seems to be responsible for upregulating and downregulating the quantal size of miniature excitatory synaptic currents of cultured glutamatergic and GABAergic neurons, respectively, when the activity of these cultured neurons is blocked by prolonged treatment with tetrodotoxin[133].

Despite the well-established role of BDNF in developing circuits, it remains unclear whether the main source of BDNF is the pre- or postsynaptic cell, or

both. The idea that BDNF acts as a retrograde factor secreted by the target cells in the CNS, similar to that found for NGF in developing peripheral systems, requires further study.

Refinement of developing circuits. Early functional neural circuits are refined by electrical activity that either spontaneously occurs in the brain or is evoked by sensory or motor activity[134] through selective stabilization of some connections and elimination of others. The involvement of neurotrophins in this refinement process was first shown in the development of the ocular dominance column in the primary visual cortex, where thalamocortical projections to layer 4 cortical neurons undergo segregation into eye-specific patches as a result of non-coincident activities in the inputs representing the two eyes[135]. Increasing neurotrophin signalling, by infusion of exogenous BDNF or NT4 or by interrupting endogenous TRKB signalling with an infusion of TRKB-immunoglobulin G (IgG), impairs the ocular dominance formation in the visual cortex of cats[136,137]. Activity-dependent refinement of the initial rough synaptic connections has been widely found in the developing brain, and the neurotrophic factor hypothesis[138] has long been postulated to account for activity-dependent refinement of developing connections. Neurotrophins fit the bill for the activity-dependent trophic factors: the synthesis, secretion and endocytic uptake of neurotrophins, as well as the neurotrophin-triggered cytoplasmic signalling, can all be activity-dependent[139]. Furthermore, the developmental refinement of synapses was found to depend on NMDARs[140] in a manner similar to activity-induced LTP and LTD, which are linked to structural modifications of synapses that might account for the stabilization and elimination of connections. Given the ubiquitous synaptic modulatory effects of neurotrophins (see below), the neurotrophin hypothesis[135,139] of circuit refinement is probably widely applicable throughout all brain areas.

The original neurotrophic hypothesis[116,138] for activity-dependent refinement of synaptic connections was based on the findings in sympathetic ganglia and neuromuscular junctions, where competition among co-innervating nerve terminals for a limited amount of target-derived factor (that is, NGF) could account for the stabilization of active terminals and the elimination of the inactive ones. In generalizing this hypothesis to the CNS, where presynaptically derived neurotrophin may provide the source of the secreted factor at the synapse[38], a modified version of the hypothesis could include the presynaptic secretion of neurotrophins. In this modified hypothesis, co-active nerve terminals may promote the stabilization of their synaptic contacts via local presynaptic secretion of neurotrophins that is facilitated by an NMDAR-dependent mechanism, involving, for example, retrograde signalling factors that are activated via postsynaptic NMDAR activity. Inactive terminals become weakened because of the low-level presynaptic neurotrophin secretion, resulting from the absence of NMDAR activation and the deficient postsynaptic retrograde factor. When two spinal neurons innervate one myocyte in *X. laevis* nerve muscle co-cultures, repetitive activation of one neuron results in heterosynaptic suppression of synapses made by the other neuron[141]. A study found that pro-BDNF secreted by the activated myocyte could induce synaptic depression and retraction at nerve terminals[142], whereas neuronal stimulation induces local proteolytic conversion of pro-BDNF to mature BDNF that potentiates and stabilizes active synapses[143]. That BDNF is involved in the refinement of synaptic connections is further supported by the finding that the restoration of normal visual acuity and ocular dominance in amblyopic rats by environmental enrichment was accompanied by increased BDNF expression[144]. It is also supported by the fact that the BDNF mediates a fluoxetine-induced increase in synaptic plasticity and conversion of amygdala fear memory circuits to a more immature state[145].

In addition to the local modulation of synapse maturation by secreted neurotrophins, synaptically released neurotrophins may be taken up via endocytosis by the innervating axonal terminals and retrogradely transported to the soma to regulate gene expression, thus affecting the pattern of all synaptic connections made by the entire neuron. Skin-derived NGF activates TRKA receptors on the peripheral axon terminals of nociceptive sensory neurons. Endocytic uptake of NGF–TRKA and the transport of NGF–TRKA complexes to the cell body of nociceptive sensory neurons are required to induce the expression of the transcription factor RUNX1, which is essential for the lamina-specific targeting of the central afferents of the nociceptive sensory neurons in the dorsal horn[146]. Similarly, NT3–TRKC signals derived from the muscle are necessary for inducing the expression of the transcription factor ER81 in proprioceptive sensory neurons, which is essential for their central afferents to establish synapses with motor neurons in the ventral horn[147,148]. These afferent terminals themselves also seem to be the source of BDNF that is required for the development or maturation of axo-axonal synapses, which are made by GABAergic interneurons onto these afferent terminals[149].

Regulation of mature neural circuits

In addition to their roles in neural circuit development, neurotrophins serve many regulatory functions in mature circuits (FIG. 2; TABLE1). Neurotrophins that are secreted at the synapse can rapidly alter the efficacy of synaptic transmission and the capacity of activity-induced LTP and LTD. Furthermore, besides the well-known synaptic modulatory effects, equally important changes may occur in intrinsic neuronal excitability owing to neurotrophin-induced changes in the expression and function of voltage-gated ion channels. Much progress has been made in understanding the signalling cascade underlying these synaptic and neuronal actions of neurotrophins in mature circuits (TABLE 1). Here, we focus on recent findings as well as issues that remain to be clarified, including the relative contribution of neurotrophin–TRK signalling in pre- versus postsynaptic neurons, the role of BDNF as a modulator or mediator of LTP, the source of synaptically secreted BDNF and the mechanisms regulating its secretion.

Neurotrophic factor hypothesis
Axon terminals from different presynaptic neurons co-innervating the same target cell undergo activity-dependent synaptic competition by competing for a limited supply of a neurotrophic factor secreted by the target cell; terminals that acquire sufficient trophic factor become stabilized, whereas those failing to do so become eliminated. Presumably, axonal activity confers an advantage in the competition.

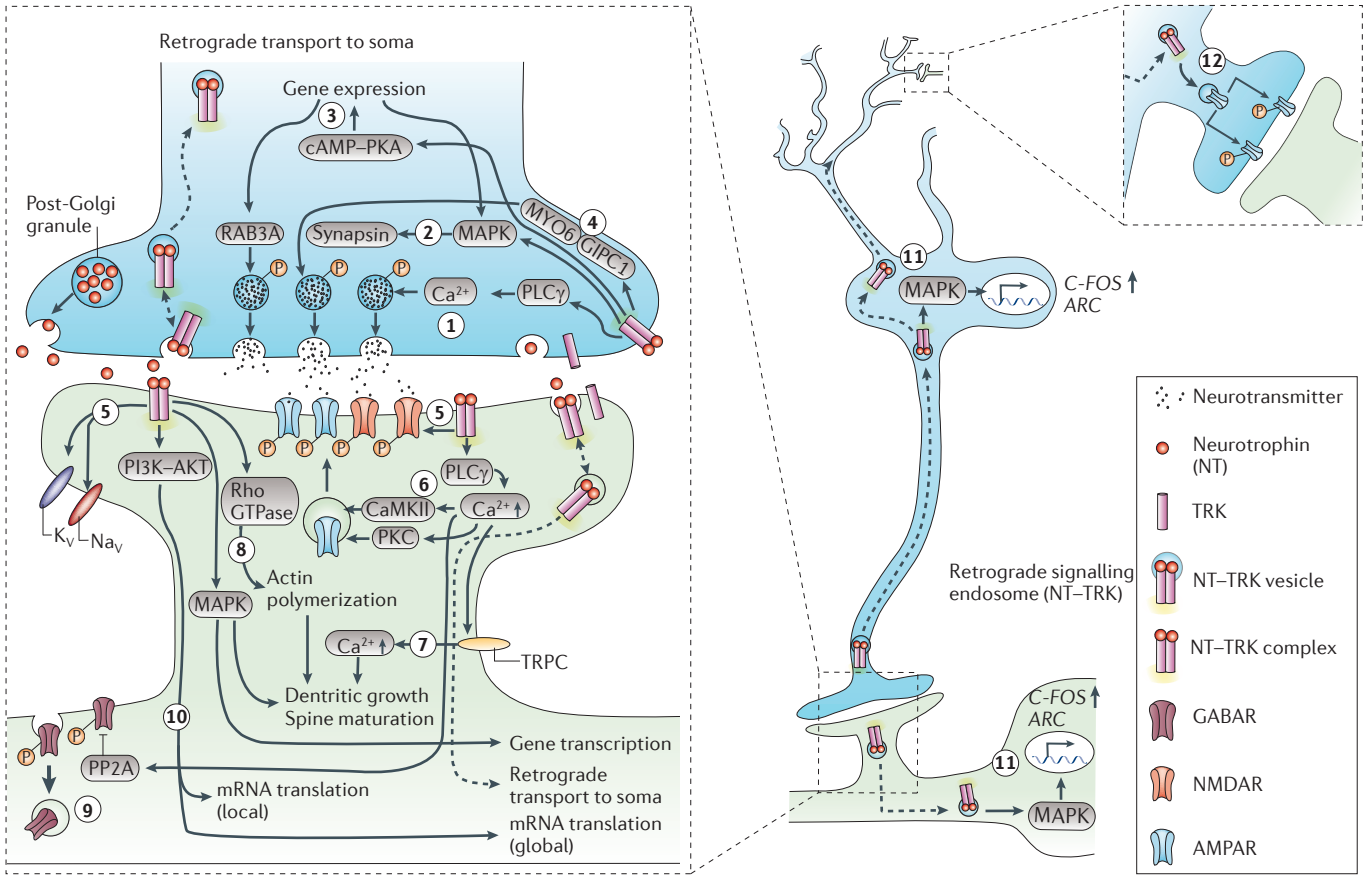

Figure 2 | **Modulation of synaptic functions by neurotrophins.** Neurotrophins (NTs) have diverse pre- and postsynaptic modulatory effects on mature glutamatergic synapses. On the presynaptic side, tropomyosin-related kinase (TRK) signalling could activate the phospholipase Cγ (PLCγ) pathway to increase the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (1), mitogen-activated protein kinase (MAPK) phosphorylation of synapsin (2), and RAB3 expression (3), leading to increased presynaptic neurotransmitter release. TRKB-mediated activation of the motor myosin VI (MYO6)–adaptor protein GIPC1 complex also contributes to the increased presynaptic release (4). On the postsynaptic side, TRK signalling causes tyrosine phosphorylation of voltage-gated Na^+ (Na_V) and voltage-gated K^+ (K_V) channels and NMDA-type glutamate receptor (NMDAR) subunits, modifying the conductance of all these channels (5). TRK signalling also induces activation of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) and protein kinase C (PKC), which phosphorylate AMPA-type glutamate receptor (AMPAR) subunits and increase their synaptic delivery (6). BDNF-induced store-operated activation of transient receptor potential cation channel subfamily C (TRPC) leads to local $[Ca^{2+}]_i$ increase, promoting dendritic growth and spine maturation (7). TRK signalling also activates small Rho GTPases that modulate actin cytoskeleton (8). Postsynaptic GABAergic synapses are suppressed by TRK-dependent activation of protein phosphatase 2A (PP2A) that reduces the surface expression of type A GABA receptors ($GABA_A$Rs) in the dendrite (9). TRK-induced phosphoinositide 3-kinase (PI3K)–AKT signalling enhances both local and global mRNA translation (10). Binding of secreted NTs to their appropriate TRKs leads to endocytic uptake of NT–TRK complexes into 'signalling endosomes', which might be transported retrogradely to the soma of both pre- and postsynaptic neurons, triggering MAPK-dependent transcription of activity-related genes such as *C-FOS* (also known as *FOS*) and activity-regulated cytoskeleton-associated protein (*ARC*), and genes required for the establishment of long-term synaptic plasticity (11). Long-range transport of signalling endosomes from the axon terminal to the dendrite could potentiate upstream synapses by increasing postsynaptic AMPAR density and conductance (12). Note that secretion of NT-containing post-Golgi granules is shown only at the presynaptic nerve terminal, although release of NTs from NT–TRK endosomes from either pre- or postsynaptic cells may also occur, especially at developing synapses. Dashed arrows indicate pathways that remain to be confirmed. cAMP, cyclic AMP.

Modulation of synaptic efficacy. In brain slices and in cultures of dissociated neurons, exposure to recombinant neurotrophins enhances the efficacy of excitatory synapses[150–153] but depresses the efficacy of GABAergic synapses[153,154]. These modulatory effects are mediated by changing either the efficacy of activity-induced presynaptic transmitter release[101,155] or the magnitude of postsynaptic neurotransmitter-induced responses[150], mostly through TRK signalling (TABLE 1). For example, BDNF-induced potentiation of glutamate neurotransmitter release is mediated by TRKB-dependent increases in MAPK phosphorylation of synapsin[156] and RAB3A expression[157,158]. Postsynaptic enhancement of postsynaptic excitatory responses could be caused by increased NMDAR conductance via TRKB-dependent tyrosine phosphorylation of NMDAR subunits[150,158–160].

Table 1 | **Synaptic and neuronal actions of neurotrophins in mature neural circuits**

Target	Neurotrophin	Cellular actions	Suggested mechanisms
Presynaptic neurotransmitter release	BDNF, NT3, NT4	Increased neurotransmitter release	• RAS–MAPK mediated phosphorylation of synapsin[156] • PLCγ-dependent increase of Ca^{2+} (REF. 206) • Increased release sites via the myosin VI–adapter protein GIPC complex[206] • Increased expression of RAB3A[157] • CDC42-dependent increase in actin polymerization[122]
Neurotransmitter receptors	BDNF	Increased postsynaptic response	Postsynaptic tyrosine kinase-dependent mechanism[150]
		Increased NMDA receptor conductance	Tyrosine kinase-mediated phosphorylation of NR1 (REF. 159) and NR2B[160]
		Increased surface expression of AMPA receptors	• CaMKII and PKC phosphorylation at Ser831 in GluR1 (REF. 161) • PKC-mediated interaction between GluR2 and NSF[207]
		Increased synthesis of AMPA receptor subunit	TRKB-dependent protein synthesis[161]
		Decreased surface expression of $GABA_AR$	Reduced phosphorylation of $GABA_AR$ subunit by phosphatase[164]
Ion channels	BDNF	Increased TRPC channel activity	Activation via PLCγ–IP_3 pathway-dependent Ca^{2+} mobilization from the store[208]
	NGF, BDNF, NT4	Increased Na_V channel conductance	Increased MAPK activation and CREB phosphorylation[209,210]
	BDNF	Decreased $Na_V1.2$ channel conductance	FYN tyrosine kinase-mediated phosphorylation[211]
	BDNF	• Decreased $K_V1.3$ channel current (acute) • Increased $K_V1.3$ channel current and accelerated inactivation kinetics (chronic)	TRKB-mediated tyrosine phosphorylation of $K_V1.3$ channel (REF. 212)
	NGF, BDNF, NT4	Increased VGCC conductance	Increased phosphorylation of MAPK and CREB[209,210,213]
Gene expression	BDNF, NT3, NGF	Increased transcription	Increased CREB phosphorylation via CaMKIV and MAPK pathways[177,214–216]
	BDNF	Increased translation	PI3K–AKT–mTOR-mediated phosphorylation of translation-regulating factors[217]
Morphology	BDNF	• Increased dendritic growth and spine density • Altered spine morphology	• Activation of RAS–MAPK pathway[193] • Increased in Ca^{2+} via TRPC activation[194] • Activation of PI3K–AKT–mTOR pathway[195] • Increased actin polymerization[196] • Increased tubulin polymerization[199]

BDNF, brain-derived neurotrophic factor; CaMK, Ca^{2+}/calmodulin kinase; CDC42, cell division cycle 42 (GTP-binding protein, 25kDa); CREB, cyclic AMP response element (CRE)-binding protein; $GABA_AR$, type A GABA receptor; GluR, glutamate receptor (subunit of AMPA receptors); IP_3, inositol trisphosphate; K_V, voltage-gated K^+ channel; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; Na_V, voltage-gated Na^+ channel; NGF, nerve growth factor; NR, NMDA receptor subunit; NSF, *N*-ethylmaleimide-sensitive fusion protein; NT, neurotrophin; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLCγ, phospholipase Cγ; TRKB, tropomyosin-related kinase B; TRPC, transient receptor-potential cation channel subfamily C; VGCC, voltage-gated Ca^{2+} channel.

Alternatively, TRKB-mediated CaMKII- or protein kinase C (PKC)-dependent phosphorylation of the subunit glutamate receptor 1 (GluR1) could also enhance postsynaptic responses by inducing membrane insertion of GluR1 subunits[161]. Interestingly, a recent study demonstrated that acute and gradual increases in BDNF concentration could produce opposing effects on the efficacy of excitatory synapses owing to the differential kinetics of TRKB activation and downstream signalling[162], suggesting that temporal aspects of TRKB-mediated signalling is crucial for regulating synaptic efficacy.

In contrast to their facilitatory effect on excitatory synapses, BDNF–TRKB signals suppress the amplitude of inhibitory postsynaptic currents (IPSCs) via acute downregulation of K^+/Cl^- co-transport activity in postsynaptic cells[163] or phosphatase-dependent downregulation of the surface expression of $GABA_AR$ subunits[164,165]. Moreover, some of the actions of neurotrophins can also be mediated indirectly via trans-synaptic factors. For example, postsynaptic BDNF–TRKB signalling could trigger the release of endocannabinoids, which could act as retrograde factors to reduce the frequency of spontaneous presynaptic GABA release[166].

Modulation of LTP and LTD. Extracellular neurotrophins can also modify the capacity of the synapse to undergo activity-induced LTP or LTD, even in the absence of any effect on basal synaptic transmission at some synapses. In neonatal rat hippocampal slices, application of recombinant BDNF facilitated the induction of LTP at CA3–CA1 synapses by enhancing the efficacy of presynaptic transmitter secretion during HFS[167]. By contrast, facilitation of LTP induction by BDNF in dentate granule cells is mediated by a postsynaptic mechanism involving enhanced

Ca^{2+} influx through activated NMDARs and VGCCs[168]. Exogenous BDNF also enhanced tetanus-induced LTP and blocked LTD that was induced by low-frequency stimulation in slices of visual cortex without affecting basal synaptic transmission[169–171].

BDNF as a mediator of LTP. The synaptic modulatory functions of neurotrophins, as described above, are not unique among many neuromodulators that affect synaptic function and plasticity. Neurotrophins, specifically BDNF, are of particular interest to synaptic plasticity because of the possibility that BDNF may serve as a mediator rather than simply as a modulator of activity-induced LTP. Although numerous molecules are involved in LTP[172], few are likely to be true mediators of LTP. Mediators differ from modulators in that their function is directly influenced by the electrical activity that induces LTP, whereas modulators could regulate the processes and mediators of LTP but their level or function is not necessarily regulated by the LTP-inducing activity (FIG. 3). The finding that electrical activity may trigger the secretion of neurotrophins suggests that there is a possibility that neurotrophins may indeed mediate the downstream synaptic changes associated with LTP. However, it is possible that BDNF is not in the pathway that leads to LTP but acts as an activity-dependent modulator of the processes and molecules that mediate LTP (FIG. 3). These two possibilities are difficult to distinguish experimentally.

The excitement surrounding the role of neurotrophins in synaptic plasticity began with the finding that endogenous BDNF is required for LTP induction in hippocampal CA1 pyramidal cells. Deletion of *Bdnf* and *Trkb*[59,173] or functional blockade of BDNF signalling with BDNF-specific antibodies or TRKB-IgG[174] impairs LTP, and this impairment can be rescued by applying exogenous BDNF[175] or by virus-mediated transfer of *Bdnf*[176]. Genetic dissection analysis using specific knock-in mice containing a point mutation in *Trkb* demonstrated that BDNF–TRKB-mediated hippocampal LTP is dependent on PLCγ-dependent induction of CREB and CaMKIV phosphorylation[177]. Moreover, BDNF–TRKB signalling, serving as a synaptic tag, is required for the induction of L-LTP by weak TBS stimulation that is just enough for early-phase LTP (E-LTP)[178], implying that there is an E-LTP-independent pathway to induce L-LTP through BDNF–TRKB signalling. Importantly, LTP-inducing stimulation elicited BDNF secretion that persisted for 5–12 min after the end of the stimulation period[179], and secreted BDNF must be present extracellulary for at least 1 hour after LTP-inducing stimulation for stable LTP to develop[180]. As discussed later, secreted BDNF is required for the structural change (that is, increased volume) in dendritic spines following LTP induction[39]. Thus, existing evidence suggests that activity-triggered secretion of BDNF either modulates the processes that stabilize E-LTP to allow L-LTP or mediates the induction of L-LTP, which involves processes distinct from E-LTP (for example, protein synthesis-dependent synaptic changes). However, to definitively demonstrate the mediator role of BDNF in L-LTP, one needs to show that LTP-inducing activity can trigger localized synaptic release of BDNF at a time and of an amount that allows BDNF to induce L-LTP when applied without activity. Such an experiment has yet to be accomplished.

Pre- and postsynaptic requirement of BDNF action. For the induction of full LTP by the tetanus stimulation paradigm, secreted BDNF must activate TRKB on both pre- and postsynaptic neurons. Illustrating this point, PLCγ, a downstream target of TRKB, had to be inhibited (by overexpressing the pleckstrin homology domain of PLCγ) in both pre- and postsynaptic neurons in order to reduce hippocampal CA3–CA1 LTP to a level comparable to that found in *Trkb*- or *Bdnf*-knockout mice[181]. Similarly, inhibition of both pre- and postsynaptic TRKB activity is required to abolish LTP at *X. laevis* retinotectal synapses that is induced by the application of TBS to the optic nerve[182]. However, depending on the LTP-inducing protocols and the type of synapses, the requirement of pre- or postsynaptic BDNF–TRKB signalling may differ, which is reminiscent of the differences found for the expression mechanisms of LTP[183,184].

At hippocampal CA3–CA1 synapses, tetanic stimulation of Schaffer collaterals at 50 Hz resulted in LTP that expressed only enhancement of postsynaptic responses, whereas tetanus at 200 Hz or TBS induced LTP that expressed both the postsynaptic effect and presynaptic enhancement of neurotransmitter release[183–185]. Interestingly, only the latter induction (200 Hz tetanus or TBS) required BDNF signalling[65,180]. In addition, a general reduction of TRKB expression in the hippocampus affected presynaptic function and reduced the ability of tetanic stimulation to induce LTP but had no effect on LTP induction by low-frequency pairing of presynaptic stimuli with postsynaptic depolarizations[186]. Furthermore, the requirement for pre- versus postsynaptic BDNF–TRKB signalling may differ among various synapse types. In the rodent mesolimbic circuit, BDNF expression levels in the ventral tegmental area (VTA), nucleus accumbens and medial prefrontal cortex (mPFC) are all increased during the withdrawal period following chronic cocaine exposure. However, raised BDNF levels result in the facilitation of LTP induction at excitatory synapses on both VTA dopamine neurons and mPFC layer 5 pyramidal cells via different mechanisms — BDNF enhances presynaptic neurotransmitter secretion at VTA synapses[187] but downregulates surface expression of postsynaptic $GABA_A$Rs at mPFC synapses[165]. Thus, depending on the stimulation pattern and the synapse type, LTP could involve neurotrophin–TRK signalling in pre- or postsynaptic neurons, or both.

The source of synaptic BDNF. Whether the BDNF that is responsible for modulating or mediating LTP is released from the presynaptic or postsynaptic neuron remains an unresolved issue. Evidence exists that, at least in hippocampal Schaffer collateral–CA1 pyramidal cell synapses, presynaptic BDNF secretion is required for LTP induction. A comparison of LTP induction (with 200Hz HFS or TBS) in mice with *Bdnf* deleted in the whole forebrain (including both CA1 and CA3) with that in

Early-phase LTP
(E-LTP). A transcription- and translation-independent synaptic potentiation that lasts for 1–3 hours, typically induced by a single train of high-frequency stimulation.

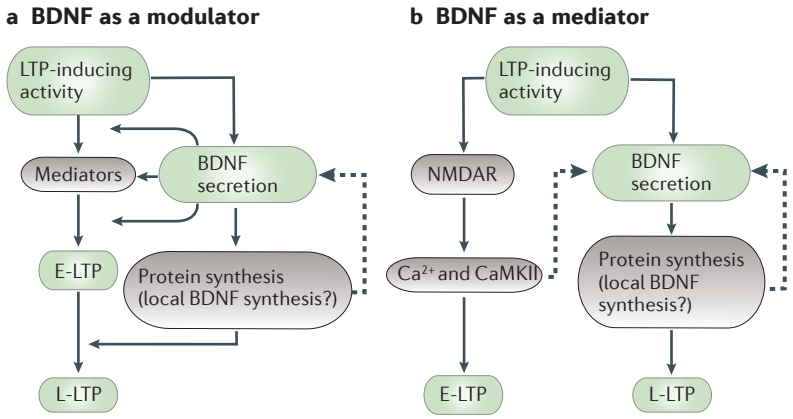

Figure 3 | **Two models of BDNF actions in LTP of central excitatory synapses.** These two models are largely based on studies of Schaffer collateral–CA1 synapses in the hippocampus. **a** | Brain-derived neurotrophic factor (BDNF) as a modulator of long-term potentiation (LTP). Action of a certain level of extracellular BDNF, which is either constitutively released or induced by activity, is required to permit normal cellular events that mediate either early-phase LTP (E-LTP) or late-phase LTP (L-LTP). **b** | BDNF as a mediator of LTP. For activities that are capable of inducing L-LTP, E-LTP could be mediated by the activation of NMDA-type glutamate receptor (NMDAR) and subsequent downstream pathways depending on Ca^{2+} and Ca^{2+}/calmodulin kinase II (CaMKII) signalling, whereas activity-triggered release of BDNF directly mediates the events that lead to L-LTP through, for example, triggering local protein synthesis that is required for structural modification of the synapse. Dashed lines indicate pathways that remain to be demonstrated.

mice with *Bdnf* selectively deleted in CA1 showed that the source of the BDNF required for LTP is located in presynaptic CA3 neurons[65]. This result is supported by a recent report on the localization of endogenous BDNF protein in adult hippocampal synapses, using electron microscopy and immunohistochemical analysis of *Bdnf–Myc* knock-in mice. The mature BDNF was found to be exclusively stored in presynaptic secretory granules in both mossy fibres and Schaffer collaterals[38], suggesting that presynaptic secretory granules that store BDNF are the main source of secreted BDNF required for regulating LTP in the hippocampus. As discussed above, axonal secretion of neurotrophins requires a pattern of stimulation that is effective in creating a sufficiently high axonal Ca^{2+} increase, as is provided by 100–200 Hz HFS or TBS. More recent studies on LTP induced by HFS at corticostriatal synapses also implicate a dependence on presynaptic BDNF secretion, because postsynaptic striatal neurons show negligible endogenous BDNF expression[188]. Together, these studies imply that BDNF stored in presynaptic sites is probably released by LTP-inducing HFS. As BDNF secretion 30–60 min after stimulation is crucial for the development of L-LTP[180], and HFS or contextual conditioning selectively increases postsynaptic expression of *Bdnf* mRNA at hippocampal CA3–CA1 synapses[189,190], it is possible that stabilization of L-LTP requires the continuous expression and release of BDNF from the postsynaptic dendrites. Nevertheless, the timing and relative contribution of BDNF secretion from the pre- versus postsynaptic neuron remains unclear.

Studies in cultured hippocampal neurons have shown robust Ca^{2+}-dependent BDNF secretion from both synaptic and extra-synaptic sites along the dendrite[132]. Under low-frequency synaptic activity, BDNF is probably secreted by dendrites rather than by axons[58]. However, *in vivo* presynaptic axon terminals are exposed to substantial neurotransmitter crosstalk owing to limited extracellular diffusion space. Under repetitive burst spiking of axons that is associated with 200 Hz HFS or TBS, secreted glutamate may trigger additional Ca^{2+} influx through presynaptic NMDARs in the adjacent co-activated axon terminals, thereby facilitating the presynaptic BDNF secretion required for LTP induction[65]. Dendritic release of neurotrophins was demonstrated mostly in cultures of dissociated developing neurons. Although prestored BDNF has been identified only at the presynaptic site in the hippocampus of mature mice[38], it is possible that BDNF secretion from postsynaptic dendrites may also occur at developing synapses. Furthermore, the existence of TRKB in both pre- and postsynaptic cells suggests, even in the absence of postsynaptic synthesis of BDNF, that presynaptically released (either constitutive or activity-induced) BDNF may be acquired by postsynaptic dendrites via the formation of endocytic BDNF–TRKB-containing signalling endosomes, which may become an additional source of synaptic BDNF that can be released in response to activity. Indeed, activity-induced secretion of endocytosed recombinant BDNF could be used for the maintenance of L-LTP in the absence of protein synthesis at Schaffer collateral–CA1 synapses[191].

Modulation of synaptic structures. Activity-induced LTP at mature excitatory synapses is also accompanied by persistent postsynaptic structural changes, including an increased number and volume of dendritic spines (for a review, see REF. 192). The role of neurotrophin signalling in dendritic structural changes has been well characterized in hippocampal neurons, in which the application of recombinant BDNF induces structural changes via TRKB-dependent activation of the RAS–MAPK pathway[193], transient receptor-potential cation channel subfamily C (TRPC)[194] and the phosphoinositide 3-kinase (PI3K)–AKT–mTOR pathway[195]. Structural modification of dendritic spines depends on persistent changes in the cytoskeleton. In the adult hippocampus, TBS-induced BDNF–TRKB signalling stimulates small GTPase-dependent actin polymerization via a p21-activated kinase (PAK)–cofilin pathway, resulting in increased actin polymerization in dendritic spines[196], which is required for the development of L-LTP[197]. Increased microtubule stabilization is also required for HFS-induced LTP[198] and promotes BDNF-induced changes in spine morphology[199] and accumulation of the postsynaptic protein PSD95 (REF. 200).

At hippocampal CA3–CA1 synapses, the persistent enlargement of the spine by LTP-inducing synaptic activity depended on the secretion of endogenous BDNF and protein synthesis[39]. Thus, secreted BDNF may act as an autocrine or a paracrine signal to induce protein synthesis-dependent spine morphological changes. Moreover, locally synthesized BDNF in dendrites may also participate in the structural plasticity. In cultured hippocampal neurons, EEF2 phosphorylation-dependent local translation of BDNF in the dendrites could increase both spine

maturation and density[201], although no direct evidence exists to show that LTP-inducing activity is capable of triggering local BDNF synthesis *in vivo*.

Long-range retrograde synaptic potentiation. Within minutes after BDNF was infused into the optic tectum of *X. laevis* tadpoles, retinotectal synapses became highly potentiated[202]. With a delay of ~15 min, light-induced responses recorded in RGCs became enhanced, as a result of the potentiation of the synapses made by bipolar cells onto RGCs. This spread of BDNF's effect from the tectum to the retina is mediated by retrograde signalling through the optic nerve, because no synaptic potentiation was found in the retina when the nerve was transected or when axon transport was inhibited by colchicine[202]. Furthermore, LTP induction at these retinotectal synapses, which requires secretion of endogenous BDNF[203], was also accompanied by a retrograde spread of potentiation to the bipolar cell–RGC synapses, a process that required TRKB signalling in the RGC[182]. This retrograde BDNF effect, which is presumably mediated by signalling endosomes, allows rapid information flow from the output synapses to the input synapses of a neuron. Interestingly, whereas locally applied BDNF in the tectum potentiated neurotransmitter secretion at retinotectal synapses, the long-range retrograde effect at RGC dendrites involved an increase in the density of postsynaptic AMPA-type glutamate receptors[182,202]. Induction of LTD at retinotectal synapses also led to depression of bipolar cell–RGC synapses in the retina via a retrograde signal mediated by nitric oxide[182]. Such transcellular communication of activity-induced synaptic modifications from downstream to upstream synapses is a useful mechanism for the coordinated refinement of developing circuits and for learning and memory processes in mature circuits. Whether endocytic neurotrophin uptake at postsynaptic dendrites can also cause long-range modulation of synaptic efficacy at the axonal terminals is unknown.

Concluding remarks

The past two decades have witnessed a remarkable expansion in our knowledge of the diverse functions of neurotrophins in the nervous system — from the initial focus on neuronal survival and growth to nearly all aspects of neural circuit function in the developing and mature brain. This functional diversity stems from two important features of neurotrophins. First, the expression, secretion and cellular actions of neurotrophins all depend on neuronal activity, thus linking their functions to neuronal signalling in the brain. Second, as secreted extracellular factors, their actions span wide spatial and temporal scales.

Spatially, neurotrophins not only act locally as autocrine or paracrine factors after secretion but also act over long distances throughout neural circuits via transcytosis between axonal and dendritic compartments and trans-synaptic transfer between connected neurons in a neural circuit. Temporally, neurotrophins act within seconds after secretion to modulate synaptic functions, over tens of minutes to modify synaptic structure and axonal or dendritic arbors and over hours and days to alter gene expression and protein synthesis. How these neurotrophin-dependent events, which occur over such wide spatial and temporal scales, are coordinated in neural circuits and how they become integrated into behaviourally relevant circuit regulation remain challenging problems for future studies. Recent advances in optical imaging and optogenetic manipulation of neuronal activity have now made *in vivo* studies of neurotrophin signalling at a subcellular resolution possible. For example, experiments combining two-photon imaging of neuronal activity (via fluorescent Ca^{2+} dye signals) and neurotrophin secretion (via fluorescence protein-tagged neurotrophins), together with optogenetic control of spiking of selective population of neurons, will provide direct information on the spatiotemporal dynamics of neurotrophin signalling in circuit functions and animal behaviours. In view of the importance of physiologically relevant BDNF secretion, future work in elucidating the secretory pathway that is used by endogenous BDNF *in vivo* is essential. We need to know more about the characteristics of the secretory vesicles storing and releasing BDNF in various brain areas. The possibility that biosynthesis of these storage compartments and intracellular transport of BDNF are also activity-dependent needs to be investigated, and the regulatory components responsible for sensing the stimuli that trigger the neurotrophin release need to be further clarified. Some of these issues were raised by Thoenen more than two decades ago[8] but remain outstanding today.

The existence of transcytosis of neurotrophin-containing signalling endosomes and trans-synaptic transfer of neurotrophins in both anterograde and retrograde directions[61,64] also raises several immediate questions. How do neurons control the trafficking of neurotrophin signalling endosomes so that local and global neurotrophin signalling can be properly regulated? Are transcytosis and trans-synaptic transfer of neurotrophins bidirectional and symmetrical? Are neurotrophin-containing endosomes processed differently in neurons to other endosomes derived from receptor-mediated endocytosis? These questions need to be addressed by future subcellular studies on the mechanism of endosome trafficking in axons and dendrites, the role of neuronal activity in regulating both the trafficking and secretion of neurotrophin-containing endosomes, and the cellular processing of neurotrophin-containing endosomes in the nerve terminals and postsynaptic dendrites.

On the basis of genetic association studies in human populations and experimental findings in rodent models of human diseases, there is now accumulating evidence suggesting that defective neurotrophin functions in the brain may underlie many neurological and psychiatric diseases, including severe obesity and eating disorders[204,205]. It is likely that a combination of genetic and environment factors are responsible for the defective expression and cellular processing of neurotrophins and their receptors, as well as activity-dependent secretion of neurotrophins in various brain regions. Further understanding of the dynamics of neurotrophins signalling and their regulatory actions on neural circuits will offer insights into the pathology of many neurotrophin-related brain disorders and potential therapeutic approaches.

Colchicine
A compound isolated from *Colchicum autumnale* (autumn crocus) that disrupts microtubule polymerization by binding to tubulin.

1. Cohen, S., Levimontalcini, R. & Hamburger, V. A. Nerve growth stimulating factor isolated from sarcomas 37 and 180. *Proc. Natl Acad. Sci. USA* **40**, 1014–1018 (1954).
2. Levi-Montalcini, R. The nerve growth factor 35 years later. *Science* **237**, 1154–1162 (1987).
3. Barde, Y. A., Edgar, D. & Thoenen, H. Purification of a new neurotrophic factor from mammalian brain. *EMBO J.* **1**, 549–553 (1982).
A ground-breaking study showing the existence of a NGF-like substance in the brain that is expressed at an extremely low level.
4. Leibrock, J. *et al.* Molecular-cloning and expression of brain-derived neurotrophic factor. *Nature* **341**, 149–152 (1989).
This work shows a high amino acid sequence homology between BDNF and NGF, and thus the existence of a family of proteins now known as neurotrophins.
5. Lewin, G. R. & Barde, Y. A. Physiology of the neurotrophins. *Annu. Rev. Neurosci.* **19**, 289–317 (1996).
6. Chao, M. V. Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nature Rev. Neurosci.* **4**, 299–309 (2003).
7. Reichardt, L. F. Neurotrophin-regulated signalling pathways. *Phil. Trans. R. Soc. B.* **361**, 1545–1564 (2006).
8. Thoenen, H. The changing scene of neurotrophic factors. *Trends Neurosci.* **14**, 165–170 (1991).
This is a landmark review at a crucial juncture of the neurotrophin field, with a specific agenda proposed for the study of activity-dependent regulation and secretion of neurotrophins that is yet to be fully completed.
9. Lu, B., Pang, P. T. & Woo, N. H. The yin and yang of neurotrophin action. *Nature Rev. Neurosci.* **6**, 603–614 (2005).
This is a thorough review of cellular processing and synaptic actions of neurotrophins together with an intriguing proposal on the antagonistic and complementary actions of mature BDNF and pro-BDNF.
10. Minichiello, L. TrkB signalling pathways in LTP and learning. *Nature Rev. Neurosci.* **10**, 850–860 (2009).
A thorough review on the cytoplasmic BDNF–TRKB signalling, much of which is not covered in the present article.
11. Tao, X., Finkbeiner, S., Arnold, D. B., Shaywitz, A. J. & Greenberg, M. E. Ca^{2+} influx regulates *BDNF* transcription by a CREB family transcription factor-dependent mechanism. *Neuron* **20**, 709–726 (1998).
12. Tao, X., West, A. E., Chen, W. G., Corfas, G. & Greenberg, M. E. A calcium-responsive transcription factor, CaRF, that regulates neuronal activity-dependent expression of BDNF. *Neuron* **33**, 383–395 (2002).
13. Timmusk, T. *et al.* Multiple promoters direct tissue-specific expression of the rat BDNF gene. *Neuron* **10**, 475–489 (1993).
14. Aid, T., Kazantseva, A., Piirsoo, M., Palm, K. & Timmusk, T. Mouse and rat *BDNF* gene structure and expression revisited. *J. Neurosci. Res.* **85**, 525–535 (2007).
15. Pruunsild, P., Kazantseva, A., Aid, T., Palm, K. & Timmusk, T. Dissecting the human *BDNF* locus: bidirectional transcription, complex splicing, and multiple promoters. *Genomics* **90**, 397–406 (2007).
This study provides the latest characterization of the human *BDNF* structure, including newly identified promoters for alternative transcripts and the newly proposed nomenclature of BDNF promoters (see reference 14 to make a comparison with the rodent gene).
16. Metsis, M., Timmusk, T., Arenas, E. & Persson, H. Differential usage of multiple brain-derived neurotrophic factor promoters in the rat-brain following neuronal activation. *Proc. Natl Acad. Sci. USA* **90**, 8802–8806 (1993).
17. Hong, E. J., McCord, A. E. & Greenberg, M. E. A. Biological function for the neuronal activity-dependent component of *Bdnf* transcription in the development of cortical inhibition. *Neuron* **60**, 610–624 (2008).
18. Sakata, K. *et al.* Critical role of promoter IV-driven BDNF transcription in GABAergic transmission and synaptic plasticity in the prefrontal cortex. *Proc. Natl Acad. Sci. USA* **106**, 5942–5947 (2009).
19. Brady, R., Zaidi, S. I. A., Mayer, C. & Katz, D. M. BDNF is a target-derived survival factor for arterial baroreceptor and chemoafferent primary sensory neurons. *J. Neurosci.* **19**, 2131–2142 (1999).
20. Dolmetsch, R. E., Pajvani, U., Fife, K., Spotts, J. M. & Greenberg, M. E. Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. *Science* **294**, 333–339 (2001).
21. Takasu, M. A., Dalva, M. B., Zigmond, R. E. & Greenberg, M. E. Modulation of NMDA receptor-dependent calcium influx and gene expression through EphB receptors. *Science* **295**, 491–495 (2002).
22. Zhou, Z. L. *et al.* Brain-specific phosphorylation of MeCP2 regulates activity-dependent *Bdnf* transcription, dendritic growth, and spine maturation. *Neuron* **52**, 255–269 (2006).
23. Chen, W. G. *et al.* Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. *Science* **302**, 885–889 (2003).
24. Martinowich, K. *et al.* DNA methylation-related chromatin remodeling in activity-dependent *Bdnf* gene regulation. *Science* **302**, 890–893 (2003).
25. Chang, Q. A., Khare, G., Dani, V., Nelson, S. & Jaenisch, R. The disease progression mutant mice is affected of *Mecp2* by the level of BDNF expression. *Neuron* **49**, 341–348 (2006).
26. Tsankova, N. M. *et al.* Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nature Neurosci.* **9**, 519–525 (2006).
27. Ma, D. K. *et al.* Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science* **323**, 1074–1077 (2009).
This article shows that *BDNF* transcription could be increased by convulsive activity-induced epigenetic DNA modification, which is mediated by GADD45B, leading to increased neurogenesis of granules cells in the adult hippocampus.
28. Salin, T., Timmusk, T., Lendahl, U. & Metsis, M. Structural and functional characterization of the rat neurotrophin-4 gene. *Mol. Cell. Neurosci.* **9**, 264–275 (1997).
29. Kendall, S., Yeo, M., Henttu, P. & Tomlinson, D. R. Alternative splicing of the neurotrophin-3 gene gives rise to different transcripts in a number of human and rat tissues. *J. Neurochem.* **75**, 41–47 (2000).
30. An, J. J. *et al.* Distinct role of long 3′ UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell* **134**, 175–187 (2008).
Using cultured hippocampal neurons, the authors show that the short 3′ UTR *Bdnf* mRNA is localized at the soma and the long 3′ UTR *Bdnf* mRNA is transported to dendrite; when the latter is deleted in the mice, apical dendrites have denser and thinner spines, suggesting dendritic synthesis of BDNF may be important for spine development.
31. Lau, A. G. *et al.* Distinct 3′ UTRs differentially regulate activity-dependent translation of brain-derived neurotrophic factor (BDNF). *Proc. Natl Acad. Sci. USA* **107**, 15945–15950 (2010).
32. Kaneko, M., Xie, Y. X., An, J. J., Stryker, M. P. & Xu, B. J. Dendritic BDNF synthesis is required for late-phase spine maturation and recovery of cortical responses following sensory deprivation. *J. Neurosci.* **32**, 4790–4802 (2012).
In mice in which the long 3′ UTR *Bdnf* mRNA was deleted (reference 30), the authors found dendrite maturation in the primary visual cortex and the recovery of neuronal responses following monocular deprivation during the critical period were impaired.
33. Bramham, C. R., Worley, P. F., Moore, M. J. & Guzowski, J. F. The immediate early gene *Arc/Arg3.1*: regulation, mechanisms, and function. *J. Neurosci.* **28**, 11760–11767 (2008).
34. Baj, G., Leone, E., Chao, M. V. & Tongiorgi, E. Spatial segregation of BDNF transcripts enables BDNF to differentially shape distinct dendritic compartments. *Proc. Natl Acad. Sci. USA* **108**, 16813–16818 (2011).
35. Tongiorgi, E. Activity-dependent expression of brain-derived neurotrophic factor in dendrites: facts and open questions. *Neurosci. Res.* **61**, 335–346 (2008).
36. Hirokawa, N., Niwa, S. & Tanaka, Y. Molecular motors in neurons: transport mechanisms and roles in brain function, development, and disease. *Neuron* **68**, 610–638 (2010).
37. Besse, F. & Ephrussi, A. Translational control of localized mRNAs: restricting protein synthesis in space and time. *Nature Rev. Mol. Cell. Biol.* **9**, 971–980 (2008).
38. Dieni, S. *et al.* BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. *J. Cell Biol.* **196**, 775–788 (2012).
This study provides the ultrastructural evidence that endogenous BDNF is present exclusively in the secretory granules in the presynaptic axons, together with cleaved pro-peptide, in the adult hippocampus.
39. Tanaka, J. I. *et al.* Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. *Science* **319**, 1683–1687 (2008).
This study provides the best evidence so far that links the action of endogenously secreted BDNF and the structural change (gradual enlargement) of dendritic spines associated with the induction of LTP.
40. Herzog, K. H., Bailey, K. & Barde, Y. A. Expression of the *BDNF* gene in the developing visual-system of the chick. *Development* **120**, 1643–1649 (1994).
41. Pang, P. T. *et al.* Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. *Science* **306**, 487–491 (2004).
42. Nagappan, G. *et al.* Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. *Proc. Natl Acad. Sci. USA* **106**, 1267–1272 (2009).
43. Matsumoto, T. *et al.* Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. *Nature Neurosci.* **11**, 131–133 (2008).
Using knock-in mice and biochemical assays of pro-BDNF and mature BDNF *in vivo* and *in vitro*, the authors argue that pro-BDNF is rapidly processed into BDNF within the cell, rather than in the extracellular space.
44. Yang, J. M. *et al.* Neuronal release of proBDNF. *Nature Neurosci.* **12**, 113–115 (2009).
In response to reference 42, these authors also used knock-in mice to examine the processing of pro-BDNF and reported that substantial amount of pro-BDNF is secreted into extracellular space, and high levels of pro-BDNF exist in the postnatal mouse brain. Unlike in reference 43, a monoclonal antibody made against the pro-domain of BDNF was used in this study.
45. Lee, R., Kermani, P., Teng, K. K. & Hempstead, B. L. Regulation of cell survival by secreted proneurotrophins. *Science* **294**, 1945–1948 (2001).
46. Woo, N. H. *et al.* Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nature Neurosci.* **8**, 1069–1077 (2005).
47. Harrington, A. W. *et al.* Secreted proNGF is a pathophysiological death-inducing ligand after adult CNS injury. *Proc. Natl Acad. Sci. USA* **101**, 6226–6230 (2004).
48. Egan, M. F. *et al.* The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112**, 257–269 (2003).
49. Chen, Z. Y. *et al.* Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J. Neurosci.* **24**, 4401–4411 (2004).
50. Hariri, A. R. *et al.* Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J. Neurosci.* **23**, 6690–6694 (2003).
51. Blochl, A. & Thoenen, H. Localization of cellular storage compartments and sites of constitutive and activity-dependent release of nerve growth factor (NGF) in primary cultures of hippocampal neurons. *Mol. Cell. Neurosci.* **7**, 173–190 (1996).
52. Lessmann, V., Gottmann, K. & Malcangio, M. Neurotrophin secretion: current facts and future prospects. *Prog. Neurobiol.* **69**, 341–374 (2003).
53. Kruttgen, A., Moller, J. C., Heymach, J. V. Jr & Shooter, E. M. Neurotrophins induce release of neurotrophins by the regulated secretory pathway. *Proc. Natl Acad. Sci. USA* **95**, 9614–9619 (1998).
54. Cheng, P. L. *et al.* Self-amplifying autocrine actions of BDNF in axon development. *Proc. Natl Acad. Sci. USA* **108**, 18430–18435 (2011).
This study shows that BDNF secreted at the growth cone could serve as an autocrine factor for axon initiation and growth via positive feedback mechanisms involving BDNF-induced BDNF release and local insertion of TRKB into the membrane.
55. Haubensak, W., Narz, F., Heumann, R. & Lessmann, V. BDNF-GFP containing secretory granules are localized in the vicinity of synaptic junctions of cultured cortical neurons. *J. Cell Sci.* **111**, 1483–1493 (1998).
56. Balkowiec, A. & Katz, D. M. Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA in situ. *J. Neurosci.* **20**, 7417–7423 (2000).

57. Gartner, A. & Staiger, V. Neurotrophin secretion from hippocampal neurons evoked by long-term-potentiation-inducing electrical stimulation patterns. *Proc. Natl Acad. Sci. USA* **99**, 6386–6391 (2002).
58. Matsuda, N. *et al.* Differential activity-dependent secretion of brain-derived neurotrophic factor from axon and dendrite. *J. Neurosci.* **29**, 14185–14198 (2009).
59. Korte, M. *et al.* Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc. Natl Acad. Sci. USA* **92**, 8856–8860 (1995).
The first study showing that endogenous BDNF is essential for activity-induced LTP in the CA1 region of the hippocampus.
60. Lang, S. B., Stein, V., Bonhoeffer, T. & Lohmann, C. Endogenous brain-derived neurotrophic factor triggers fast calcium transients at synapses in developing dendrites. *J. Neurosci.* **27**, 1097–1105 (2007).
61. von Bartheld, C. S., Byers, M. R., Williams, R. & Bothwell, M. Anterograde transport of neurotrophins and axodendritic transfer in the developing visual system. *Nature* **379**, 830–833 (1996).
62. Altar, C. A. *et al.* Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature* **389**, 856–860 (1997).
63. Baquet, Z. C., Gorski, J. A. & Jones, K. R. Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. *J. Neurosci.* **24**, 4250–4258 (2004).
64. Kohara, K., Kitamura, A., Morishima, M. & Tsumoto, T. Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. *Science* **291**, 2419–2423 (2001).
This study provides a direct demonstration of activity-dependent transneuronal transfer of BDNF protein in cultured neurons.
65. Zakharenko, S. S. *et al.* Presynaptic BDNF required for a presynaptic but not postsynaptic component of LTP at hippocampal CA1–CA3 synapses. *Neuron* **39**, 975–990 (2003).
This study demonstrates that presynaptic release of BDNF is the source of the BDNF that is required for the induction of LTP by TBS and other HFS protocols that produce LTP with presynaptic changes.
66. Dean, C. *et al.* Axonal and dendritic synaptotagmin isoforms revealed by a pHluorin-syt functional screen. *Mol. Biol. Cell* **23**, 1715–1727 (2012).
67. Grimes, M. L., Beattie, E. & Mobley, W. C. A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. *Proc. Natl Acad. Sci. USA* **94**, 9909–9914 (1997).
68. Cohen, M. S., Bas Orth, C., Kim, H. J., Jeon, N. L. & Jaffrey, S. R. Neurotrophin-mediated dendrite-to-nucleus signaling revealed by microfluidic compartmentalization of dendrites. *Proc. Natl Acad. Sci. USA* **108**, 11246–11251 (2011).
69. Dan, Y. & Poo, M. M. Quantal transmitter secretion from myocytes loaded with acetylcholine. *Nature* **359**, 733–736 (1992).
70. Dan, Y., Song, H. J. & Poo, M. M. Evoked neuronal secretion of false transmitters. *Neuron* **13**, 909–917 (1994).
71. Betz, W. J. & Bewick, G. S. Optical monitoring of transmitter release and synaptic vesicle recycling at the frog neuromuscular junction. *J. Physiol.* **460**, 287–309 (1993).
72. Ahmed, S., Reynolds, B. A. & Weiss, S. BDNF enhances the differentiation but not the survival of CNS stem cell-derived neuronal precursors. *J. Neurosci.* **15**, 5765–5778 (1995).
73. Lachyankar, M. B. *et al.* Embryonic precursor cells that express Trk receptors: induction of different cell fates by NGF, BDNF, NT-3, and CNTF. *Exp. Neurol.* **144**, 350–360 (1997).
74. Shetty, A. K. & Turner, D. A. *In vitro* survival and differentiation of neurons derived from epidermal growth factor-responsive postnatal hippocampal stem cells: inducing effects of brain-derived neurotrophic factor. *J. Neurobiol.* **35**, 395–425 (1998).
75. Jones, K. R., Farinas, I., Backus, C. & Reichardt, L. F. Targeted disruption of the *BDNF* gene perturbs brain and sensory neuron development but not motor neuron development. *Cell* **76**, 989–999 (1994).
76. Benraiss, A., Chmielnicki, E., Lerner, K., Roh, D. & Goldman, S. A. Adenoviral brain-derived neurotrophic factor induces both neostriatal and olfactory neuronal recruitment from endogenous progenitor cells in the adult forebrain. *J. Neurosci.* **21**, 6718–6731 (2001).
77. Scharfman, H. *et al.* Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Exp. Neurol.* **192**, 348–356 (2005).
78. Sairanen, M., Lucas, G., Ernfors, P., Castren, M. & Castren, E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J. Neurosci.* **25**, 1089–1094 (2005).
79. Bergami, M. *et al.* Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. *Proc. Natl Acad. Sci. USA* **105**, 1 5570–15575 (2008).
80. Bath, K. G. *et al.* Variant brain-derived neurotrophic factor (Val66Met) alters adult olfactory bulb neurogenesis and spontaneous olfactory discrimination. *J. Neurosci.* **28**, 2383–2393 (2008).
81. Li, Y. *et al.* TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. *Neuron* **59**, 399–412 (2008).
82. Barde, Y. A. Trophic factors and neuronal survival. *Neuron* **2**, 1525–1534 (1989).
83. Frade, J. M., Rodriguez-Tebar, A. & Barde, Y. A. Induction of cell death by endogenous nerve growth factor through its p75 receptor. *Nature* **383**, 166–168 (1996).
84. Meyer-Franke, A., Kaplan, M. R., Pfrieger, F. W. & Barres, B. A. Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. *Neuron* **15**, 805–819 (1995).
85. Shen, S., Wiemelt, A. P., McMorris, F. A. & Barres, B. A. Retinal ganglion cells lose trophic responsiveness after axotomy. *Neuron* **23**, 285–295 (1999).
86. Rauskolb, S. *et al.* Global deprivation of brain-derived neurotrophic factor in the CNS reveals an area-specific requirement for dendritic growth. *J. Neurosci.* **30**, 1739–1749 (2010).
This paper provides direct evidence that BDNF is not a major survival factor for CNS neurons but is required for normal dendritic growth in specific brain areas.
87. Nikoletopoulou, V. *et al.* Neurotrophin receptors TrkA and TrkC cause neuronal death whereas TrkB does not. *Nature* **467**, 59–63 (2010).
This study offers new insights into the mechanism of neurotrophin-dependent neuronal survival and explains why neurons in the PNS and CNS showed the differential sensitivity to the specific neurotrophin signalling.
88. Shelly, M., Cancedda, L., Heilshorn, S., Sumbre, G. & Poo, M. M. LKB1/STRAD promotes axon initiation during neuronal polarization. *Cell* **129**, 565–577 (2007).
89. Kishi, M., Pan, Y. A., Crump, J. G. & Sanes, J. R. Mammalian SAD kinases are required for neuronal polarization. *Science* **307**, 929–932 (2005).
90. Barnes, A. P. *et al.* LKB1 and SAD kinases define a pathway required for the polarization of cortical neurons. *Cell* **129**, 549–563 (2007).
91. Ascano, M., Richmond, A., Borden, P. & Kuruvilla, R. Axonal targeting of Trk receptors via transcytosis regulates sensitivity to neurotrophin responses. *J. Neurosci.* **29**, 11674–11685 (2009).
92. Gundersen, R. W. & Barrett, J. N. Neuronal chemotaxis: chick dorsal-root axons turn toward high concentrations of nerve growth factor. *Science* **206**, 1079–1080 (1979).
93. Song, H. J. Ming, G. L. & Poo, M. M. cAMP-induced switching in turning direction of nerve growth cones. *Nature* **388**, 275–279 (1997).
94. Tucker, K. L., Meyer, M. & Barde, Y. A. Neurotrophins are required for nerve growth during development. *Nature Neurosci.* **4**, 29–37 (2001).
95. Horch, H. W. & Katz, L. C. BDNF release from single cells elicits local dendritic growth in nearby neurons. *Nature Neurosci.* **5**, 1177–1184 (2002).
96. Cohen-Cory, S. & Fraser, S. E. Effects of brain-derived neurotrophic factor on optic axon branching and remodelling *in vivo*. *Nature* **378**, 192–196 (1995).
97. Martinez, A. *et al.* TrkB and TrkC signaling are required for maturation and synaptogenesis of hippocampal connections. *J. Neurosci.* **18**, 7336–7350 (1998).
98. Lyckman, A. W., Fan, G., Rios, M., Jaenisch, R. & Sur, M. Normal eye-specific patterning of retinal inputs to murine subcortical visual nuclei in the absence of brain-derived neurotrophic factor. *Vis. Neurosci.* **22**, 27–36 (2005).
99. McAllister, A. K., Katz, L. C. & Lo, D. C. Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. *Neuron* **18**, 767–778 (1997).
100. McAllister, A. K., Katz, L. C. & Lo, D. C. Neurotrophin regulation of cortical dendritic growth requires activity. *Neuron* **17**, 1057–1064 (1996).
101. Boulanger, L. & Poo, M. M. Gating of BDNF-induced synaptic potentiation by cAMP. *Science* **284**, 1982–1984 (1999).
102. Du, J. *et al.* Regulation of TrkB receptor tyrosine kinase and its internalization by neuronal activity and Ca^{2+} influx. *J. Cell Biol.* **163**, 385–395 (2003).
103. Wirth, M. J., Brun, A., Grabert, J., Patz, S. & Wahle, P. Accelerated dendritic development of rat cortical pyramidal cells and interneurons after biolistic transfection with BDNF and NT4/5. *Development* **130**, 5827–5838 (2003).
104. Meyer-Franke, A. *et al.* Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. *Neuron* **21**, 681–693 (1998).
105. Lom, B., Cogen, J., Sanchez, A. L., Vu, T. & Cohen-Cory, S. Local and target-derived brain-derived neurotrophic factor exert opposing effects on the dendritic arborization of retinal ganglion cells *in vivo*. *J. Neurosci.* **22**, 7639–7649 (2002).
106. Caleo, M., Medini, P., von Bartheld, C. S. & Maffei, L. Provision of brain-derived neurotrophic factor via anterograde transport from the eye preserves the physiological responses of axotomized geniculate neurons. *J. Neurosci.* **23**, 287–296 (2003).
107. Mandolesi, G. *et al.* A role for retinal brain-derived neurotrophic factor in ocular dominance plasticity. *Curr. Biol.* **15**, 2119–2124 (2005).
108. Klein, R., Conway, D., Parada, L. F. & Barbacid, M. The *trkB* tyrosine protein kinase gene codes for a second neurogenic receptor that lacks the catalytic kinase domain. *Cell* **61**, 647–656 (1990).
109. Eide, F. F. *et al.* Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling. *J. Neurosci.* **16**, 3123–3129 (1996).
110. Biffo, S., Offenhauser, N., Carter, B. D. & Barde, Y. A. Selective binding and internalisation by truncated receptors restrict the availability of BDNF during development. *Development* **121**, 2461–2470 (1995).
111. Carim-Todd, L. *et al.* Endogenous truncated TrkB.T1 receptor regulates neuronal complexity and TrkB kinase receptor function *in vivo*. *J. Neurosci.* **29**, 678–685 (2009).
112. Rose, C. R. *et al.* Truncated TrkB-T1 mediates neurotrophin-evoked calcium signalling in glia cells. *Nature* **426**, 74–78 (2003).
113. Ohira, K. *et al.* A truncated tropomyosin-related kinase B receptor, T1, regulates glial cell morphology via Rho GDP dissociation inhibitor 1. *J. Neurosci.* **25**, 1343–1353 (2005).
114. Alsina, B., Vu, T. & Cohen-Cory, S. Visualizing synapse formation in arborizing optic axons *in vivo*: dynamics and modulation by BDNF. *Nature Neurosci.* **4**, 1093–1101 (2001).
115. McAllister, A. K., Lo, D. C. & Katz, L. C. Neurotrophins regulate dendritic growth in developing visual cortex. *Neuron* **15**, 791–803 (1995).
116. Sanes, J. R. & Lichtman, J. W. Development of the vertebrate neuromuscular junction. *Annu. Rev. Neurosci.* **22**, 389–442 (1999).
117. Lohof, A. M., Ip, N. Y. & Poo, M. M. Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. *Nature* **363**, 350–353 (1993).
118. Wang, T., Xie, K. & Lu, B. Neurotrophins promote maturation of developing neuromuscular synapses. *J. Neurosci.* **15**, 4796–4805 (1995).
119. Liou, J. C. & Fu, W. M. Regulation of quantal secretion from developing motoneurons by postsynaptic activity-dependent release of NT-3. *J. Neurosci.* **17**, 2459–2468 (1997).
120. Vicario-Abejon, C., Collin, C., McKay, R. D. & Segal, M. Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons. *J. Neurosci.* **18**, 7256–7271 (1998).
121. Vicario-Abejon, C., Owens, D., McKay, R. & Segal, M. Role of neurotrophins in central synapse formation and stabilization. *Nature Rev. Neurosci.* **3**, 965–974 (2002).
122. Shen, W. *et al.* Activity-induced rapid synaptic maturation mediated by presynaptic cdc42 signaling. *Neuron* **50**, 401–414 (2006).
123. Sallert, M. *et al.* Brain-derived neurotrophic factor controls activity-dependent maturation of CA1 synapses by downregulating tonic activation of

presynaptic kainate receptors. *J. Neurosci.* **29**, 11294–11303 (2009).
124. Hanover, J. L., Huang, Z. J., Tonegawa, S. & Stryker, M. P. Brain-derived neurotrophic factor overexpression induces precocious critical period in mouse visual cortex. *J. Neurosci.* **19**, RC40 (1999).
125. Huang, Z. J. *et al.* BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell* **98**, 739–755 (1999).
126. Gianfranceschi, L. *et al.* Visual cortex is rescued from the effects of dark rearing by overexpression of BDNF. *Proc. Natl Acad. Sci. USA* **100**, 12486–12491 (2003).
127. Langlois, A., Diabira, D., Ferrand, N., Porcher, C. & Gaiarsa, J. L. NMDA-dependent switch of proBDNF actions on developing GABAergic synapses. *Cereb. Cortex* 17 Apr 2012 (doi:10.1093/cercor/bhs071).
128. Tao, H. W. & Poo, M. M. Activity-dependent matching of excitatory and inhibitory inputs during refinement of visual receptive fields. *Neuron* **45**, 829–836 (2005).
129. Kuczewski, N. *et al.* Spontaneous glutamatergic activity induces a BDNF-dependent potentiation of GABAergic synapses in the newborn rat hippocampus. *J. Physiol.* **586**, 5119–5128 (2008).
130. Fiorentino, H. *et al.* $GABA_B$ receptor activation triggers BDNF release and promotes the maturation of GABAergic synapses. *J. Neurosci.* **29**, 11650–11661 (2009).
131. Xu, C., Zhao, M. X., Poo, M. M. & Zhang, X. H. $GABA_B$ receptor activation mediates frequency-dependent plasticity of developing GABAergic synapses. *Nature Neurosci.* **11**, 1410–1418 (2008).
132. Kolarow, R., Brigadski, T. & Lessmann, V. Postsynaptic secretion of BDNF and NT-3 from hippocampal neurons depends on calcium–calmodulin kinase II signaling and proceeds via delayed fusion pore opening. *J. Neurosci.* **27**, 10350–10364 (2007).
133. Rutherford, L. C., Nelson, S. B. & Turrigiano, G. G. BDNF has opposite effects on the quantal amplitude of pyramidal neuron and interneuron excitatory synapses. *Neuron* **21**, 521–530 (1998).
134. Zhang, L. I. & Poo, M. M. Electrical activity and development of neural circuits. *Nature Neurosci.* **4**, 1207–1214 (2001).
135. Katz, L. C. & Shatz, C. J. Synaptic activity and the construction of cortical circuits. *Science* **274**, 1133–1138 (1996).
136. Cabelli, R. J., Hohn, A. & Shatz, C. J. Inhibition of ocular dominance column formation by infusion of NT-4/5 or BDNF. *Science* **267**, 1662–1666 (1995).
137. Cabelli, R. J., Shelton, D. L., Segal, R. A. & Shatz, C. J. Blockade of endogenous ligands of trkB inhibits formation of ocular dominance columns. *Neuron* **19**, 63–76 (1997).
138. Purves, D. & Lichtman, J. W. Formation and maintenance of synaptic connections in autonomic ganglia. *Physiol. Rev.* **58**, 821–862 (1978).
139. Schinder, A. F. & Poo, M. The neurotrophin hypothesis for synaptic plasticity. *Trends Neurosci.* **23**, 639–645 (2000).
140. Constantine-Paton, M., Cline, H. T. & Debski, E. Patterned activity, synaptic convergence, and the NMDA receptor in developing visual pathways. *Annu. Rev. Neurosci.* **13**, 129–154 (1990).
141. Lo, Y. J. & Poo, M. M. Activity-dependent synaptic competition *in vitro*: heterosynaptic suppression of developing synapses. *Science* **254**, 1019–1022 (1991).
142. Yang, F. *et al.* Pro-BDNF-induced synaptic depression and retraction at developing neuromuscular synapses. *J. Cell Biol.* **185**, 727–741 (2009).
143. Je, H. S. *et al.* Role of pro-brain-derived neurotrophic factor (proBDNF) to mature BDNF conversion in activity-dependent competition at developing neuromuscular synapses. *Proc. Natl Acad. Sci. USA* **109**, 15924–15929 (2012).
144. Sale, A. *et al.* Environmental enrichment in adulthood promotes amblyopia recovery through a reduction of intracortical inhibition. *Nature Neurosci.* **10**, 679–681 (2007).
145. Karpova, N. N. *et al.* Fear erasure in mice requires synergy between antidepressant drugs and extinction training. *Science* **334**, 1731–1734 (2011).
146. Chen, C. L. *et al.* Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. *Neuron* **49**, 365–377 (2006).
147. Chen, H. H., Tourtellotte, W. G. & Frank, E. Muscle spindle-derived neurotrophin 3 regulates synaptic connectivity between muscle sensory and motor neurons. *J. Neurosci.* **22**, 3512–3519 (2002).
148. Patel, T. D. *et al.* Peripheral NT3 signaling is required for ETS protein expression and central patterning of proprioceptive sensory afferents. *Neuron* **38**, 403–416 (2003).
149. Betley, J. N. *et al.* Stringent specificity in the construction of a GABAergic presynaptic inhibitory circuit. *Cell* **139**, 161–174 (2009).
150. Levine, E. S., Dreyfus, C. F., Black, I. B. & Plummer, M. R. Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. *Proc. Natl Acad. Sci. USA* **92**, 8074–8077 (1995).
151. Kang, H. & Schuman, E. M. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science* **267**, 1658–1662 (1995).
152. Carmignoto, G., Pizzorusso, T., Tia, S. & Vicini, S. Brain-derived neurotrophic factor and nerve growth factor potentiate excitatory synaptic transmission in the rat visual cortex. *J. Physiol.* **498**, 153–164 (1997).
153. Bolton, M. M., Pittman, A. J. & Lo, D. C. Brain-derived neurotrophic factor differentially regulates excitatory and inhibitory synaptic transmission in hippocampal cultures. *J. Neurosci.* **20**, 3221–3232 (2000).
154. Tanaka, T., Saito, H. & Matsuki, N. Inhibition of GABAA synaptic responses by brain-derived neurotrophic factor (BDNF) in rat hippocampus. *J. Neurosci.* **17**, 2959–2966 (1997).
155. Lessmann, V., Gottmann, K. & Heumann, R. BDNF and NT-4/5 enhance glutamatergic synaptic transmission in cultured hippocampal neurones. *Neuroreport* **6**, 21–25 (1994).
156. Jovanovic, J. N., Czernik, A. J., Fienberg, A. A., Greengard, P. & Sihra, T. S. Synapsins as mediators of BDNF-enhanced neurotransmitter release. *Nature Neurosci.* **3**, 323–329 (2000).
157. Thakker-Varia, S., Alder, J., Crozier, R. A., Plummer, M. R. & Black, I. B. Rab3A is required for brain-derived neurotrophic factor-induced synaptic plasticity: transcriptional analysis at the population and single-cell levels. *J. Neurosci.* **21**, 6782–6790 (2001).
158. Alder, J. *et al.* Early presynaptic and late postsynaptic components contribute independently to brain-derived neurotrophic factor-induced synaptic plasticity. *J. Neurosci.* **25**, 3080–3085 (2005).
159. Suen, P. C. *et al.* Brain-derived neurotrophic factor rapidly enhances phosphorylation of the postsynaptic *N*-methyl-D-aspartate receptor subunit 1. *Proc. Natl Acad. Sci. USA* **94**, 8191–8195 (1997).
160. Lin, S. Y. *et al.* BDNF acutely increases tyrosine phosphorylation of the NMDA receptor subunit 2B in cortical and hippocampal postsynaptic densities. *Brain Res. Mol. Brain Res.* **55**, 20–27 (1998).
161. Caldeira, M. V. *et al.* Brain-derived neurotrophic factor regulates the expression and synaptic delivery of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunits in hippocampal neurons. *J. Biol. Chem.* **282**, 12619–12628 (2007).
162. Ji, Y. *et al.* Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. *Nature Neurosci.* **13**, 302–309 (2010).
163. Wardle, R. A. & Poo, M. M. Brain-derived neurotrophic factor modulation of GABAergic synapses by postsynaptic regulation of chloride transport. *J. Neurosci.* **23**, 8722–8732 (2003).
164. Jovanovic, J. N., Thomas, P., Kittler, J. T., Smart, T. G. & Moss, S. J. Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating $GABA_A$ receptor phosphorylation, activity, and cell-surface stability. *J. Neurosci.* **24**, 522–530 (2004).
165. Lu, H., Cheng, P. L., Lim, B. K., Khoshnevisrad, N. & Poo, M. M. Elevated BDNF after cocaine withdrawal facilitates LTP in medial prefrontal cortex by suppressing GABA inhibition. *Neuron* **67**, 821–833 (2010).
166. Lemtiri-Chlieh, F. & Levine, E. S. BDNF evokes release of endogenous cannabinoids at layer 2/3 inhibitory synapses in the neocortex. *J. Neurophysiol.* **104**, 1923–1932 (2010).
167. Figurov, A., Pozzo-Miller, L. D., Olafsson, P., Wang, T. & Lu, B. Regulation of synaptic responses to high-frequency stimulation & LTP by neurotrophins in the hippocampus. *Nature* **381**, 706–709 (1996).
168. Kovalchuk, Y., Hanse, E., Kafitz, K. W. & Konnerth, A. Postsynaptic induction of BDNF-mediated long-term potentiation. *Science* **295**, 1729–1734 (2002). **This study demonstrates that the postsynaptic dendrite is the main site of BDNF's action during LTP induction, although the source of secreted BDNF is not addressed.**
169. Akaneya, Y., Tsumoto, T. & Hatanaka, H. Brain-derived neurotrophic factor blocks long-term depression in rat visual cortex. *J. Neurophysiol.* **76**, 4198–4201 (1996).
170. Huber, K. M., Sawtell, N. B. & Bear, M. F. Brain-derived neurotrophic factor alters the synaptic modification threshold in visual cortex. *Neuropharmacology* **37**, 571–579 (1998).
171. Kinoshita, S. *et al.* Brain-derived neurotrophic factor prevents low-frequency inputs from inducing long-term depression in the developing visual cortex. *J. Neurosci.* **19**, 2122–2130 (1999).
172. Sanes, J. R. & Lichtman, J. W. Can molecules explain long-term potentiation? *Nature Neurosci.* **2**, 597–604 (1999).
173. Minichiello, L. *et al.* Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron* **24**, 401–414 (1999).
174. Chen, G., Kolbeck, R., Barde, Y. A., Bonhoeffer, T. & Kossel, A. Relative contribution of endogenous neurotrophins in hippocampal long-term potentiation. *J. Neurosci.* **19**, 7983–7990 (1999).
175. Patterson, S. L. *et al.* Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron* **16**, 1137–1145 (1996).
176. Korte, M. *et al.* Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. *Proc. Natl Acad. Sci. USA* **93**, 12547–12552 (1996).
177. Minichiello, L. *et al.* Mechanism of TrkB-mediated hippocampal long-term potentiation. *Neuron* **36**, 121–137 (2002).
178. Lu, Y. *et al.* TrkB as a potential synaptic and behavioral tag. *J. Neurosci.* **31**, 11762–11771 (2011).
179. Aicardi, G. *et al.* Induction of long-term potentiation and depression is reflected by corresponding changes in secretion of endogenous brain-derived neurotrophic factor. *Proc. Natl Acad. Sci. USA* **101**, 15788–15792 (2004).
180. Kang, H., Welcher, A. A., Shelton, D. & Schuman, E. M. Neurotrophins and time: different roles for TrkB signaling in hippocampal long-term potentiation. *Neuron* **19**, 653–664 (1997).
181. Gartner, A. *et al.* Hippocampal long-term potentiation is supported by presynaptic and postsynaptic tyrosine receptor kinase B-mediated phospholipase $C\gamma$ signaling. *J. Neurosci.* **26**, 3496–3504 (2006).
182. Du, J. L., Wei, H. P., Wang, Z. R., Wong, S. T. & Poo, M. M. Long-range retrograde spread of LTP & LTD from optic tectum to retina. *Proc. Natl Acad. Sci. USA* **106**, 18890–18896 (2009).
183. Grover, L. M. & Teyler, T. J. Two components of long-term potentiation induced by different patterns of afferent activation. *Nature* **347**, 477–479 (1990).
184. Cavus, I. & Teyler, T. Two forms of long-term potentiation in area CA1 activate different signal transduction cascades. *J. Neurophysiol.* **76**, 3038–3047 (1996).
185. Zakharenko, S. S., Zablow, L. & Siegelbaum, S. A. Visualization of changes in presynaptic function during long-term synaptic plasticity. *Nature Neurosci.* **4**, 711–717 (2001).
186. Xu, B. *et al.* The role of brain-derived neurotrophic factor receptors in the mature hippocampus: modulation of long-term potentiation through a presynaptic mechanism involving TrkB. *J. Neurosci.* **20**, 6888–6897 (2000).
187. Pu, L., Liu, Q. S. & Poo, M. M. BDNF-dependent synaptic sensitization in midbrain dopamine neurons after cocaine withdrawal. *Nature Neurosci.* **9**, 605–607 (2006).
188. Jia, Y., Gall, C. M. & Lynch, G. Presynaptic BDNF promotes postsynaptic long-term potentiation in the dorsal striatum. *J. Neurosci.* **30**, 14440–14445 (2010).
189. Patterson, S. L., Grover, L. M., Schwartzkroin, P. A. & Bothwell, M. Neurotrophin expression in rat hippocampal slices: a stimulus paradigm inducing LTP in CA1 evokes increases in BDNF and NT-3 mRNAs. *Neuron* **9**, 1081–1088 (1992).
190. Hall, J., Thomas, K. L. & Everitt, B. J. Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. *Nature Neurosci.* **3**, 533–535 (2000).
191. Santi, S. *et al.* Hippocampal neurons recycle BDNF for activity-dependent secretion and LTP maintenance. *EMBO J.* **25**, 4372–4380 (2006).
192. Kasai, H., Fukuda, M., Watanabe, S., Hayashi-Takagi, A. & Noguchi, J. Structural dynamics of dendritic spines in memory and cognition. *Trends Neurosci.* **33**, 121–129 (2010).

193. Alonso, M., Medina, J. H. & Pozzo-Miller, L. ERK1/2 activation is necessary for BDNF to increase dendritic spine density in hippocampal CA1 pyramidal neurons. *Learn. Mem.* **11**, 172–178 (2004).
194. Amaral, M. D. & Pozzo-Miller, L. TRPC3 channels are necessary for brain-derived neurotrophic factor to activate a nonselective cationic current and to induce dendritic spine formation. *J. Neurosci.* **27**, 5179–5189 (2007).
195. Kumar, V., Zhang, M. X., Swank, M. W., Kunz, J. & Wu, G. Y. Regulation of dendritic morphogenesis by Ras–PI3K–Akt–mTOR and Ras–MAPK signaling pathways. *J. Neurosci.* **25**, 11288–11299 (2005).
196. Rex, C. S. *et al.* Brain-derived neurotrophic factor promotes long-term potentiation-related cytoskeletal changes in adult hippocampus. *J. Neurosci.* **27**, 3017–3029 (2007).
197. Fukazawa, Y. *et al.* Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance *in vivo*. *Neuron* **38**, 447–460 (2003).
198. Jaworski, J. *et al.* Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. *Neuron* **61**, 85–100 (2009).
199. Gu, J., Firestein, B. L. & Zheng, J. Q. Microtubules in dendritic spine development. *J. Neurosci.* **28**, 12120–12124 (2008).
200. Hu, X. *et al.* BDNF-induced increase of PSD-95 in dendritic spines requires dynamic microtubule invasions. *J. Neurosci.* **31**, 15597–15603 (2011).
201. Verpelli, C. *et al.* Synaptic activity controls dendritic spine morphology by modulating eEF2-dependent BDNF synthesis. *J. Neurosci.* **30**, 5830–5842 (2010).
202. Du, J. L. & Poo, M. M. Rapid BDNF-induced retrograde synaptic modification in a developing retinotectal system. *Nature* **429**, 878–883 (2004).
203. Mu, Y. & Poo, M. M. Spike timing-dependent LTP/LTD mediates visual experience-dependent plasticity in a developing retinotectal system. *Neuron* **50**, 115–125 (2006).
204. Liao, G. Y. *et al.* Dendritically targeted *Bdnf* mRNA is essential for energy balance and response to leptin. *Nature Med.* **18**, 564–571 (2012).
205. Ribases, M. *et al.* Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Mol. Psychiatry* **8**, 745–751 (2003).
206. Yano, H. *et al.* BDNF-mediated neurotransmission relies upon a myosin VI motor complex. *Nature Neurosci.* **9**, 1009–1018 (2006).
207. Narisawa-Saito, M. *et al.* Brain-derived neurotrophic factor regulates surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors by enhancing the *N*-ethylmaleimide-sensitive factor/GluR2 interaction in developing neocortical neurons. *J. Biol. Chem.* **277**, 40901–40910 (2002).
208. Li, H. S., Xu, X. Z. & Montell, C. Activation of a TRPC3-dependent cation current through the neurotrophin BDNF. *Neuron* **24**, 261–273 (1999).
209. Lesser, S. S. & Lo, D. C. Regulation of voltage-gated ion channels by NGF and ciliary neurotrophic factor in SK-N-SH neuroblastoma cells. *J. Neurosci.* **15**, 253–261 (1995).
210. Lesser, S. S., Sherwood, N. T. & Lo, D. C. Neurotrophins differentially regulate voltage-gated ion channels. *Mol. Cell. Neurosci.* **10**, 173–183 (1997).
211. Ahn, M., Beacham, D., Westenbroek, R. E., Scheuer, T. & Catterall, W. A. Regulation of $Na_V1.2$ channels by brain-derived neurotrophic factor, TrkB, and associated Fyn kinase. *J. Neurosci.* **27**, 11533–11542 (2007).
212. Tucker, K. & Fadool, D. A. Neurotrophin modulation of voltage-gated potassium channels in rat through TrkB receptors is time and sensory experience dependent. *J. Physiol.* **542**, 413–429 (2002).
213. Woodall, A. J., Richards, M. A., Turner, D. J. & Fitzgerald, E. M. Growth factors differentially regulate neuronal Ca_V channels via ERK-dependent signalling. *Cell Calcium* **43**, 562–575 (2008).
214. Gaiddon, C., Loeffler, J. P. & Larmet, Y. Brain-derived neurotrophic factor stimulates AP-1 and cyclic AMP-responsive element dependent transcriptional activity in central nervous system neurons. *J. Neurochem.* **66**, 2279–2286 (1996).
215. Ginty, D. D., Bonni, A. & Greenberg, M. E. Nerve growth factor activates a Ras-dependent protein kinase that stimulates c-*fos* transcription via phosphorylation of CREB. *Cell* **77**, 713–725 (1994).
216. Je, H. S., Yang, F., Zhou, J. & Lu, B. Neurotrophin 3 induces structural and functional modification of synapses through distinct molecular mechanisms. *J. Cell Biol.* **175**, 1029–1042 (2006).
217. Takei, N. *et al.* Brain-derived neurotrophic factor induces mammalian target of rapamycin-dependent local activation of translation machinery and protein synthesis in neuronal dendrites. *J. Neurosci.* **24**, 9760–9769 (2004).

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