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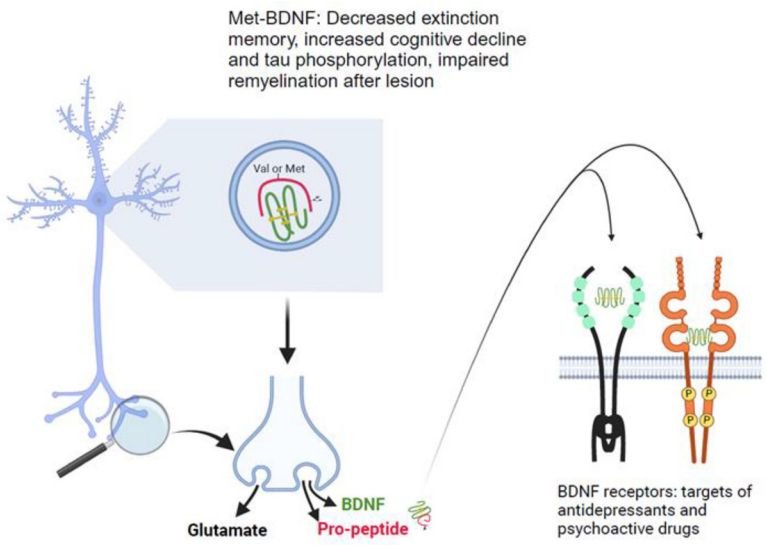
THE PHYSIOPATHOLOGY OF BRAIN-DERIVED NEUROTROPHIC FACTOR



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KEY WORDS

depression; gene polymorphisms; growth factors; memory; obesity

CLINICAL HIGHLIGHTS

Brain-derived neurotrophic factor (BDNF) is a growth factor synthesized in, and secreted by, excitatory neurons. It is a key regulator of synaptic plasticity involved in fundamental aspects of human brain function including memory. The levels of BDNF in the brain are very low and controlled by neuronal activity. Postmortem analyses of the human brain revealed that these levels inversely correlate with the rate of cognitive decline during aging, underlying the role of BDNF as an endogenous neuroprotectant. In dominant forms of inherited Alzheimer's disease, a frequent *BDNF* polymorphism is associated with Tau hyperphosphorylation and increased rates of cognitive decline. Additional human polymorphisms identified more recently underlie the multiple roles of BDNF, including the regulation of food intake and abnormal weight gain. BDNF has a complex mode of action mediated by two different cell surface receptors, recently proposed to be molecular targets of antidepressants and psychoactive drugs. Although clinical trials in neurodegenerative conditions have failed thus far to show benefits, better outcomes now appear likely as the reasons for previous failures are better understood. Meanwhile, physical exercise and activation of sensory pathways, both known to increase BDNF levels, are the most realistic options to improve memory and to retard cognitive decline during aging.

BARDE, 2025, *Physiol Rev* 105: 2073–2140
June 10, 2025;
Published by the American Physiological Society.
https://doi.org/10.1152/physrev.00038.2024

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THE PHYSIOPATHOLOGY OF BRAIN-DERIVED NEUROTROPHIC FACTOR

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Abstract

Brain-derived neurotrophic factor (BDNF) is synthesized and secreted by excitatory neurons. Decades of work with animal models prepared the ground for interpreting the results of human genome analyses associating polymorphisms with memory deficits, mood disorders, and dysregulation of food intake. These association studies include a frequent human polymorphism causing an amino acid substitution in the protein-coding sequence of BDNF. Even if its biochemical impact is still incompletely understood, this polymorphism helped understanding of the role of BDNF in humans in specific aspects of memory and neurodegeneration and in central nervous system (CNS) remyelination after lesion. BDNF is stored with its pro-peptide in the presynaptic terminals of excitatory neurons. Both are released when neurons are activated by the pattern of stimuli that also efficiently increase the transcription of the *BDNF* gene. While the restricted availability is a key aspect of BDNF's physiology, this feature greatly complicated studies of its biochemistry and cell biology. It also led to the extensive use of overexpression paradigms that generated considerable confusion. As a result, the notion that BDNF is released from neurons after intracellular processing of its precursor is not universally shared nor is its accumulation in presynaptic nerve terminals as opposed to dendrites. Beyond the use of drugs reported to activate the BDNF/TrkB pathway such as commonly used antidepressants, therapeutic successes have been limited thus far. However, as BDNF signaling is now better understood, rapid progress can now be expected. Meanwhile, physical activity remains the most realistic option to maintain BDNF levels and delay cognitive decline during aging.

depression; gene polymorphisms; growth factors; memory; obesity

CLINICAL HIGHLIGHTS

Brain-derived neurotrophic factor (BDNF) is a growth factor synthesized in, and secreted by, excitatory neurons. It is a key regulator of synaptic plasticity involved in fundamental aspects of human brain function including memory. The levels of BDNF in the brain are very low and controlled by neuronal activity. Postmortem analyses of the human brain revealed that these levels inversely correlate with the rate of cognitive decline during aging, underlying the role of BDNF as an endogenous neuroprotectant. In dominant forms of inherited Alzheimer's disease, a frequent *BDNF* polymorphism is associated with Tau hyperphosphorylation and increased rates of cognitive decline. Additional human polymorphisms identified more recently underlie the multiple roles of BDNF, including the regulation of food intake and abnormal weight gain. BDNF has a complex mode of action mediated by two different cell surface receptors, recently proposed to be molecular targets of antidepressants and psychoactive drugs. Although clinical trials in neurodegenerative conditions have failed thus far to show benefits, better outcomes now appear likely as the reasons for previous failures are better understood. Meanwhile, physical exercise and activation of sensory pathways, both known to increase BDNF levels, are the most realistic options to improve memory and to retard cognitive decline during aging.

1. INTRODUCTION

1.1. Discovery and Purification

The discovery of brain-derived neurotrophic factor (BDNF) is a direct result of much earlier work on nerve growth factor (NGF), the first growth factor ever to have been characterized and shown to be essential for the development of the vertebrate nervous system (for a review, see Ref. 1). The finding by Viktor Hamburger's student Elmer Bueker that some tumor cells can be successfully grown in chick embryos led to the unexpected

observation that "in mouse sarcoma 180 experiments the spinal ganglia which innervated this tumor were enlarged 33% (average of three cases) above those on the control side" (2). The same sarcoma cells grown onto the chorioallantois membrane of chick embryos were then used as starting material in the initial purification attempts of NGF, with a purification factor of 50- to 100-fold achieved with precipitation-based methods. This led to the conclusion that "the active material is a protein or is bound to a protein" (3). About two decades later, a biological activity supporting the survival of sensory neurons dissociated from chick dorsal root ganglia (DRGs) was detected in the conditioned medium of C6 glioma cells (4). This neuron survival-promoting activity was additive to that of NGF and may or may not have corresponded to what was later named BDNF (5). Whereas later experiments confirmed that *Bdnf* is among the genes expressed by cultured C6 glioma cells (6), cultured tumor cells secrete a number of factors supporting the survival of cultured embryonic sensory neurons, including for example ciliary neurotrophic factor (CNTF) (7). Tumor biology was an even more direct source of inspiration for the identification of neurotrophin receptors: The NGF receptor TrkA, a tyrosine kinase receptor transducing the best-known biological properties of NGF, was first identified as an oncogene generated by the fusion of the tyrosine kinase domain with a nonmuscle tropomyosin causing ligand-independent activation (8). The protooncogene version of TrkA was then recognized a few years later as the receptor transducing the most characteristic biological activities of NGF (see sect. 4). The successful purification of NGF and the generation of neutralizing antibodies administered to newborn rodents then led to what is arguably the most significant experiment ever performed in the growth factor field: the demonstration that an endogenous protein is required for the survival of specific neuronal populations (9, 10). This extraordinary conclusion could be reached long before the discovery of the mechanisms regulating programmed cell death in multicellular organisms and the realization of the generality of these mechanisms (for reviews, see Refs. 11, 12). In the absence of this knowledge, it was difficult to consider the possibility that cell suicide needed to be prevented by extracellular factors for neurons to survive during development (13). Over a few decades, NGF was discussed instead as a growth factor primarily stimulating axonal elongation and cell division (for a detailed account of the thought process, see Ref. 14). Further illustrating this point, the first in vivo demonstration that naturally occurring cell death may be a consequence of the limited availability of NGF was brought about comparatively late (15): the injection of NGF into the yolk sac of developing chick embryo decreases cell death in dorsal root ganglia (15). When sequence-grade BDNF purified from pig brain became available (**FIGURE 1**), similar results were obtained in related experiments performed with developing quail embryos selected because of their smaller sizes and compressed development compared with chick embryos (21). This was a consideration at the time, as these experiments were performed before the availability of recombinant BDNF. Pig brain-derived BDNF applied to the chorioallantois membrane reduced naturally occurring cell death of the placode-derived sensory neurons of the nodose ganglion, previously shown in vitro to respond to BDNF and not to NGF (5).

Despite its extremely low abundance, both the molecular weight and approximate isoelectric point of BDNF were determined early on, as BDNF's biological activity could be recovered after two-dimensional gel electrophoresis

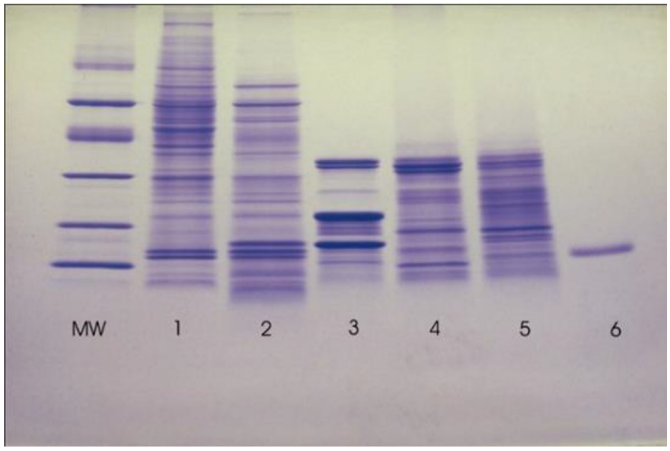


FIGURE 1. Coomassie Blue-stained 10–20% polyacrylamide gradient gel illustrating the purification steps from adult pig brain. About 20 μg of protein was applied in *lanes 1–5* and 2 μg in *lane 6*. Thirty kilograms of starting material generated ~30 μg of purified, amino acid sequence-ready brain-derived neurotrophic factor (BDNF), with an overall yield of ~10% after a purification of well over 1 million-fold. One of several technical issues with the BDNF protein is its extraction from postnatal brain tissue, especially in the absence of acidification and high salt concentration (see text for further details and implications). MW, molecular mass markers in kilodaltons. *Top*: phosphorylase b (16), bovine serum albumin (17), ovalbumin (18), carbonic anhydrase (19), soybean trypsin inhibitor (20), lysozyme (14). *Lane 1*: pig brain extract supernatant. Acidic pH and high salt concentration are needed to maximize BDNF extraction from adult brain homogenates. *Lane 2*: carboxymethyl cellulose eluate. *Lane 3*: hydroxyapatite eluate. *Lane 4*: high-ionic-strength flowthrough of octyl-phenyl-Sepharose columns used in tandem. *Lane 5*: low-ionic-strength eluate from material retained by the phenyl-Sepharose column. *Lane 6*: C8 reverse-phase column eluate (for details, see Table 1 in Ref. 21). The empirical finding that at high ionic strength BDNF does not bind to octyl- but is retained by phenyl-Sepharose greatly helped in the purification procedure. All proteolytic fragments generated from the material depicted in *lane 6* were contained in the amino acid sequence predicted from the nucleotide sequence (22). However, the amino acid composition of BDNF isolated from pig brain indicated a better fit with the omission of the 3 carboxy-terminal amino acids (RGR). A carboxy-terminal peptidase may have trimmed BDNF during the purification procedure (see sect. 3 for possible implications).

(23). However, BDNF turned out to occur as a homodimer in physiological solution, just like NGF, as isolated BDNF monomers spontaneously dimerize in physiological solutions (24).

As has been the case with several other growth factors characterized in the 1980s, the lengthy approach of protein purification and sequencing was the method of choice for the cloning of a cDNA encoding a biological activity of interest. This type of work was conducted long before the availability of whole genome sequence data that would have revealed the existence of NGF-related sequences. Intriguingly, another NGF family member, initially named NGF-2, was discovered independently at around the same time and without protein purification or the use of polymerase chain reaction (25). After the detection of an activity promoting the survival of chick sensory neurons in medium conditioned by human Hs683 glioma cells, Kaisho and colleagues (25) managed to isolate cDNA clones encoding an NGF-related sequence with a human probe, low-stringency hybridization, and a library constructed from these glioma cells. NGF-2 was then renamed Neurotrophin-3 after a series of reports by others (see below). Yet another glioma line, B49, was the source of glioma cell line-derived neurotrophic factor (GDNF), a member of the transforming growth factor (TGF)β family purified and sequenced from serum-free medium conditioned by these rat B49 cells (20). Remarkably, the purification of GDNF was monitored by using its ability to promote dopamine uptake by cultured cells dissociated from E16 rat mesencephalon. The corresponding cDNA was isolated by screening a library generated using RNA extracted from B49 cells, screened with a radiolabeled 20-nucleotide mixture corresponding to a 6-amino acid-long sequence (20). The focus from the outset on dopaminergic neurons may explain why GDNF was used comparatively early in clinical trial related to Parkinson's disease (26).

1.2. The BDNF Protein and Family Members

The publication of the nucleotide sequence of BDNF (22) not only opened the field by allowing generation of the recombinant protein and of specific nucleotide probes to localize the sites of *BDNF* expression but also greatly facilitated the rapid identification of two other neurotrophins in mammals, Neurotrophin-3 and Neurotrophin-4. As neurotrophins share ~50% amino acid sequence identities in their carboxy-terminal moieties, it was comparatively straightforward to uncover the existence of other members of the family using mixtures of oligonucleotides in polymerase chain reactions (27–33). The four neurotrophins are encoded as secreted prepro-proteins with the mature sequence generated by cleavage from the precursor (**FIGURE 2**). All neurotrophins occur as homodimers in solution and interact with the neurotrophin receptor $p75^{NTR}$ with similar nanomolar affinities. They also all bind to and activate one or more tyrosine kinase receptors of the Trk family (see sect. 4). With regard to its general characteristics, the amino acid

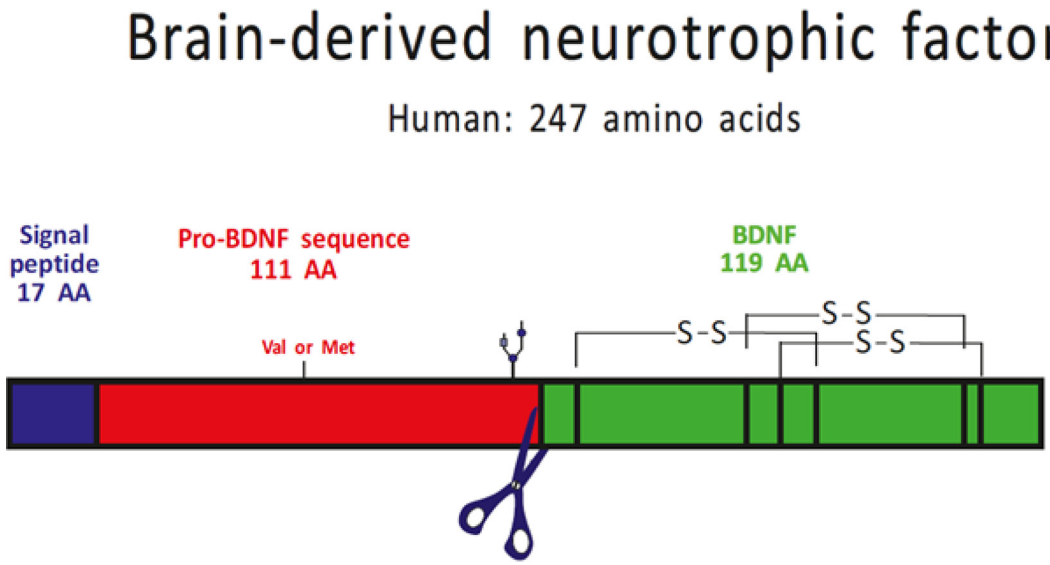


FIGURE 2. The brain-derived neurotrophic factor (BDNF) monomer. The signal peptide characterizing secreted proteins is thought to be cleaved off upon entry into the rough endoplasmic reticulum. An 8-amino acid (AA)-longer sequence generated by alternative splicing has also been described (see sects. 2 and 3). The position of the valine (Val) to methionine (Met) substitution caused by a much-discussed human polymorphism (see sect. 6) is indicated, as are the positions of the 6 cysteine residues involved in the formation of the cystine knot. The pro-BDNF sequence is 2 amino acids longer in mice, and by comparison with the human sequence there are 6 differences in the rodent amino acid sequence of pro-BDNF. The N-glycosylation site close to the cleavage site is located at an identical position in the sequence of all pro-neurotrophins and may be required for folding and secretion (see sect. 3).

sequence of mature BDNF, referred to as BDNF throughout this article, is highly conserved across species, significantly more so than the sequence of the pro-domain (see Ref. 34 for a recent compilation). Indeed, the amino acid sequence of mature BDNF is identical in a very large number of mammals.

1.3. Role of BDNF in Vivo: an Overview

Brain was used as starting material for the purification of BDNF not only because it is readily available in very large quantities but also because, at the time, it was widely assumed that the survival of central nervous system (CNS) neurons would depend on the availability of growth factors, just as had been demonstrated with NGF in the peripheral nervous system (PNS). This notion was mostly based on the widespread occurrence of naturally occurring cell death in the CNS and on its increase following axotomy (19, 35). This also led to the erroneous suggestion that sensory neurons may require a CNS-derived survival factor (36) that most likely was in fact muscle-derived NT3 in the case of the trigeminal mesencephalic nucleus (27). Although a survival-promoting activity of NGF for CNS neurons had been demonstrated early on (37), it was thought to be restricted to a small population of cholinergic neurons in the basal forebrain (37). However, the generation of *Bdnf*-null animals promptly revealed that the assumption that BDNF would be a significant survival factor for developing neurons in the CNS was incorrect (38, 39). The early postnatal death of *Bdnf*-null mutants is caused by the loss of placode-derived sensory neurons in the PNS, not by the loss of CNS neurons (see sect. 7). A number of subsequent region- or cell-selective deletions of the *Bdnf* gene in the CNS led to the overall conclusion that BDNF is a growth-promoting but not survival factor for developing CNS neurons. For example, the deletion of *Bdnf* in the developing cerebral cortex, the main source of BDNF for neurons in the striatum, only begins to cause neuronal losses after ~12 mo, at a time when even wild-type animals begin to lose neurons (40). However, striatal neurons do show much reduced dendritic arbors many months before their death (40). Likewise, the near-complete elimination of BDNF from the nervous system with Cre-mediated excision driven by the Tau (aka *Mapt*) promoter does not cause major losses of neurons or a lethal phenotype, even if the behavior of the adult mutant animals is severely impaired (41). In the absence of BDNF, no changes were observed in the number of either motoneurons (42) or retinal ganglion cells (41). These two neuronal populations were the most likely to be BDNF dependent for survival based on the results of cell culture as well as in vivo axotomy experiments (43–46). Not even the small loss of tyrosine hydroxylase-positive neurons in the pars compacta of the substantia nigra in animals carrying a Wnt-1 Cre-mediated *Bdnf* deletion can be safely taken as evidence that BDNF is required in vivo for the survival of these neurons: BDNF may be merely required for tyrosine hydroxylase expression by these neurons, as suggested by the authors (47). What has been consistently observed instead in the CNS of *Bdnf* conditional mutants is a reduction of the dendritic arbor and spine density of a large range of CNS neurons. These changes are particularly noticeable in major populations of GABAergic neurons (18, 40, 41).

The overall conclusion of this large body of work with genetically modified mice then is that BDNF is a growth-promoting factor for most CNS neurons (they all express TrkB) but not a major survival factor in the absence of lesions such as axotomy.

2. TRANSCRIPTION AND TRANSCRIPTIONAL REGULATION

2.1. Introduction

As the availability of BDNF in neurons is primarily regulated at the level of transcription, the complex topic of transcriptional regulation is of special relevance. The salient aspects are that the *BDNF* gene is expressed at very low levels and that expression in the nervous system is restricted to excitatory neurons.

2.2. Regulation of Transcription by Neuronal Activity

In humans, the *BDNF* gene spans ~70 kb on chromosome 11 and is split into 11 exons with the 3′-most exon encoding prepro-BDNF (see **FIGURE 3**). Much of the initial work centered on the genomic organization of the gene and its transcription in rodents, but work on the human gene soon revealed striking similarities, including the presence of nine functional promoters (for review, see Ref. 48). A significant difference is the presence of much longer antisense transcripts in humans, an aspect likely to receive increasing attention in the future after the identification of a human polymorphism associated with obesity and anxiety altering the levels of *BDNF* antisense transcripts (see below). By contrast, the presence of antisense transcripts in rodents was initially less clear (50–52). However, more recent work does indicate the expression of *Bdnf* antisense transcripts in the mouse, and there is now evidence for their functional relevance in vivo (53).

With regard to the regulation of expression in neurons, much of the work focused on transcripts I, IV, and VI in neurons. By contrast, less is known about the

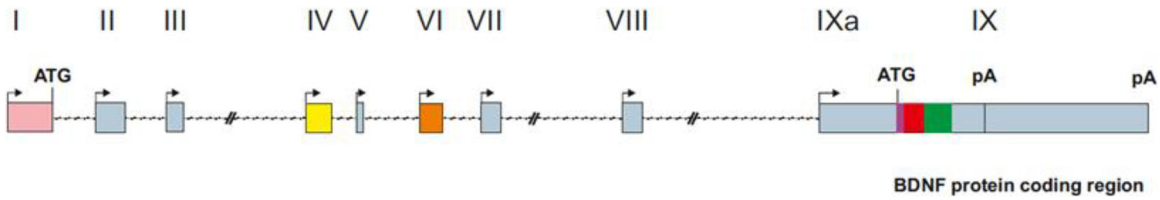


FIGURE 3. Organization of the *BDNF* gene (after Ref. 48). The *BDNF* gene is transcribed from several promoters. Some are regulated by neuronal activity, including in particular I, IV, and VI (colored). The prepro-brain-derived neurotrophic factor (BDNF) coding sequence is common to all transcripts. Exon I provides an alternative initiation site, adding 8 amino acids to prepro-BDNF (Ref. 49, see sect. 3). In publicly available databases such as UniProt, this additional amino acid sequence encoded by exon I is included in the translated sequences of *Mus spicilegus* or *Peromyscus californicus* but not of *Mus musculus*.

regulation of expression in nonneuronal tissues, even though BDNF expression in such tissues is essential for the survival of subpopulations of peripheral sensory neurons and indeed for the animal as a whole (see sect. 7).

Early studies initiated soon after specific nucleotide probes became available (22) indicated that glutamate receptor agonists increase *Bdnf* mRNA levels in rodent neurons, both in vitro and in vivo (54). As discussed in subsequent sections, these initial observations were subsequently extended, and the development of drugs activating or potentiating excitatory neurotransmission toward increasing BDNF levels in the nervous system is a topic of major interest. Massive increases of *Bdnf* mRNA were reported early on in vivo after lesion-induced seizures, especially in the cerebral cortex and the hippocampus (55). Physiological stimuli such as light were also shown early on to regulate BDNF levels in relevant brain areas (56) and the impact of activity on *Bdnf* expression noted from the earliest stages of visual system development (57). Early observations also indicated that neuronal activation in the adult rat brain involves a number of different transcripts (see **FIGURE 4**) and that these transcripts differ depending on the type of stimulus and the area investigated (63). Calcium influx in cultured neurons was then shown to trigger phosphorylation of CREB, a transcription factor modulating *Bdnf* transcription by virtue of its binding to the calcium-response element CRE within the *Bdnf* gene, later renamed CRE/Carf3 as it is also a binding site for the calcium-responsive factor transcription factor designated CaRF (58, 64–66). The observation that CREB alone is not sufficient to drive expression of promoter IV led to the subsequent identification of additional calcium-responsive elements designated CaREs, the binding sites for a transcription factor, CaRF (48, 58, 59, 66, 67). Mice lacking *Carf* show reduced expression of *Bdnf* exon IV-containing transcripts and BDNF protein in the cerebral cortex but not in the hippocampus (68). Intriguingly, the loss of *Carf* leads to enhanced *N*-methyl-D-aspartate (NMDA) receptor (NMDAR)-dependent induction of both *Bdnf* IV transcripts and the activity-regulated gene *Arc,* revealing a novel role for CaRF as an upstream regulator of NMDAR-dependent processes in the developing brain (69).

Whether transcription is differentially regulated by activity in neurons derived from the cerebral cortex or the hippocampus has been examined, with the former being more dependent on CREB than the latter (70). This study also identified a number of transcription factors by chromatin immunoprecipitation including FOXP1, SATB2, RAI1, BCL11A, and TCF4 as additional factors explaining the fine-tuning of the region-specific, activity-dependent expression of *Bdnf* (70). Although the differences observed between cultured hippocampal and

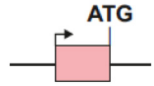
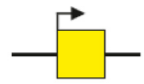
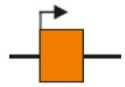
Promoters	Regulatory elements	Transcription factors
I 	PasRE	NPAS4, ARNT2
	CRE	CREB, USF1/2
	NF-κB-RE	NF-κB
IV 	PasRE	NPAS4, ARNT2
	CARE1	CaRF, MEF2
	CARE2/UBE	USF1/2
	CARE3/CRE	CEB/CaRF
	BHLHB2-RE	BHLHB2
	NF-κB-RE	NF-κB
VI 	GCbox1	Sp1
	GA	Sp1
	GCbox2	Sp1
	CCAAT	CEBP/b

FIGURE 4. Main binding sites and corresponding transcription factors regulating *BDNF* transcription. Although much of the work has focused on the 3 promoters indicated (17, 58–62), other regulatory elements have been studied, including those regulating transcription from promoter 2 that harbors a binding site for REST (see sect. 2.4).

cortical neurons were on the whole subtle, it should be noted that they were observed within a few hours after depolarization with 25 mM KCl in the presence of a NMDA receptor blocker and a 1-day pretreatment of the cultures with tetrodotoxin (70). It is thus likely that more physiological patterns of increased neuronal activity may result in larger differences in vivo with regard to the role of CREB between different brain regions. A recent study also using cultured rat hippocampal neurons and 25 mM KCl reexamined the impact of this treatment on the levels of transcripts I, II, IV, and VI and confirmed that it is mostly the levels of I and IV that are increased by this manipulation (71). Given the intrinsic limitations of in vitro studies, it is important to note that they have been usefully complemented by in vivo experiments using promoter-specific deletions. Much of the initial work focused on promoter IV given its role in activity-regulated expression of *Bdnf*, and selective mutations preventing the binding of CREB/CARF revealed that neurons from these animals form fewer inhibitory synapses in the visual cortex (58). These results are of special interest as they indicated a specific requirement for activity-dependent *Bdnf* expression for the development of the inhibitory circuitry in the cortex (58). Remarkably, this comparatively subtle disruption of just one functional element of promoter IV led to a reduction of BDNF protein levels by ~50% in lysates of the entire brain (58). This result impressively documents the role of neuronal activity in regulating BDNF levels. Subsequent studies revealed that disruption of promoter IV causes enhanced fear and resistance to fear extinction (reviewed in Ref. 72) that can be rescued by overexpressing BDNF in hippocampal neurons projecting to the prefrontal cortex (73). These studies also involved a systematic approach consisting in the deletion of the four main promoters driving the expression of transcripts I, II, IV, and VI, with the overall outcome that disruption of specific *Bdnf* transcripts leads to specific behavioral deficits. These results suggest different roles for BDNF in the assembly of neural circuits underlying social behavior. In particular, the deletion of promoter I or II promoted aggression in mutant males associated with changes in gene associated with serotonin signaling (74). The deletion of the same promoters caused reduced sexual receptivity in female mice, impaired maternal care, and decreased oxytocin expression during development (75). By contrast, deletion of promoter IV or VI did not cause enhanced aggressivity but instead impaired gene expression related to the GABAergic system (58, 74). Interestingly, the deletion of any of the four main promoters leads to the reduction by ~50% of BDNF levels in the hypothalamus and the prefrontal cortex (74). In the hippocampus, BDNF levels remained unchanged in mice carrying deletions of either promotor I or II but were reduced by about half after deletion of promoter IV or VI (74). Further highlighting the functional significance of the different *Bdnf* promoters in the mouse, deletion of promoter I or II was found to cause obesity as a result of hyperphagia, a phenotype that was not observed after deletion of promoter IV or VI (76). Among the numerous regulators of *Bdnf* transcription, the neuronal PAS domain protein 4 (NPAS4) is of special interest. It belongs to a family of transcriptional regulators and is a member of a basic helix-loop-helix (bHLH) tPer-Arnt-Sim (bHLH-PAS) protein family. It is one of the most strongly and selectively induced immediate-early genes by neuronal activity (77). This PAS domain is critical for neuronal activity-dependent transcription from promoter I. ARN2 and NPAS4 dimerize and bind to a Ca^{2+}-responsive element termed bHLH-PAS t NPAS4, and the ARNT2 and NPAS4 heterodimers formed after the first wave of immediate-early genes have been transcribed and translated in response to the activating stimulus (59). This finding may explain the later rise of exon I transcripts compared with exon IV transcripts, the latter being predominantly under the control of the CREB/CRE system (58). NPAS4 regulates the number of inhibitory synapses (77, 78) and is involved in vivo in DNA repair, thus contributing to prevention of DNA damage caused by neuronal activity by virtue of its association with NuA4 (79). As indicated in **FIGURE 4**, additional transcription factors have been implicated in mediating the neuronal activity-dependent induction. These include nuclear factor kappa B (NF-κB) through binding to NF-κB response elements in proximity of BDNF promoter I (80) and the myocyte-specific enhancer factor (MEF) via binding an upstream enhancer element (reviewed in Ref. 67). In a rat model, retrieval of contextual conditioned fear memories has been shown to activate the NF-κB pathway and to regulate histone H3 phosphorylation and acetylation at specific gene promoters in the hippocampus (80). An evolutionarily conserved sequence located ~3 kb downstream of the exon I transcription start site has also been shown to function as an enhancer (81).

2.3. *Regulation of Transcription Downstream of TrkB Activation*

Activation of the tyrosine kinase receptor TrkB by BDNF (see sect. 4) also activates *BDNF* transcription, thus adding to the complexity of the transcriptional regulation of the *BDNF* gene. Yet given the limited availability of BDNF, this self-amplifying mechanism is of great functional relevance. An autocrine/paracrine mechanism was first suggested by the observation that in vitro the survival of isolated adult rat DRG neurons is dependent on the biosynthesis of BDNF by these neurons (82). Though the relevance of this mechanism to the early development of sensory neurons was later questioned

(83), its functional significance should not be underestimated. In particular, in the adult visual cortex of the mouse, soma size and the numbers of dendrites and of spines of single neurons deprived of BDNF are reduced compared with wild-type neighboring neurons (84). These reductions are similar in magnitude to those observed in animals deprived of BDNF in all neurons (84). It thus appears that the activation of TrkB by BDNF-secreting neurons is necessary for neurons to successfully compete with their wild-type, BDNF-producing neighbors (84). With regard to the mechanisms involved in this autoregulation, Yasuda and colleagues (85) reported a marked, biphasic increase of exon IV-containing transcripts. Activation of the ERK/MAPK pathway mostly accounts for the first wave of increased BDNF transcription and calcium/calmodulin-dependent protein kinase IV for the second (85). The mechanisms involved in increased *BDNF* transcription caused by either increased neuronal activity or TrkB activation thus significantly overlap, albeit with different time courses. In line with this notion, the transcription factors involved in both processes also partially overlap, as revealed by studies involving chromatin immunoprecipitation (70). The timing of chromatin accessibility has been compared after either BDNF addition to cultured mouse neurons or their depolarization (86). This led to the identification of BDNF-specific programs, with enhancer activation uncovered as an early event as well as the pioneering role of Fos in chromatin opening (86). Importantly, this study also includes comparisons between BDNF and depolarization using excitatory neurons generated from human induced pluripotent stem cells (86). The question of chromatin accessibility, or lack thereof, is critical in the context of transcriptional activation by external stimuli such as BDNF. A case in point is inhibitory neurons that do not express the *BDNF* gene at significant levels (see **FIGURE 7**). This is also the case after TrkB activation, yet TrkB is expressed by essentially all inhibitory neurons and at levels comparable to those expressed by excitatory neurons (see **FIGURE 7**). Using the transposon-based tagging and sequencing approach known as ATAC-seq (87), Carullo and colleagues (88) compared cultures consisting of either excitatory or inhibitory neurons to explore chromatin accessibility in neurons either depolarized with KCl or treated with tetrodotoxin. It is conceivable that the *Bdnf* locus is less accessible in striatal cultures consisting largely of inhibitory neurons. This is significant, as in the nervous system *Bdnf* expression is essentially limited to populations of excitatory, as opposed to inhibitory, neurons as revealed early on by in situ hybridization experiments (89) and *Bdnf-lacZ* reporter studies (40), see also **FIGURE 8**. This segregation of BDNF expression between excitatory and inhibitory neurons is one of the central aspects of BDNF's physiology in the CNS.

2.4. *Regulation of BDNF Transcription in Humans and in Animal Models of CNS Dysfunction*

As many as 17 different transcripts can be generated from the *BDNF* human gene (52), a complexity further increased by the use of alternative 3′ polyadenylation site. This arrangement gives rise to transcripts of substantially different length, with 3′ untranslated sequences of 0.35 and 2.85 kb, respectively. This is also the case in rodents, and the proportion of the short and long BDNF transcripts varies substantially between tissues, even between different brain regions. However, the functional significance of these 3′ untranslated regions (UTRs) is unclear and somewhat controversial with regard to the impact on the half-life, localization, and translation of the corresponding mRNAs in dendrites (90, 91). The suggestion that *Bdnf* mRNAs may be translated in dendrites rests in part on the use of overexpressed BDNF-green fluorescent protein (GFP) constructs (see sect. 3 for a critical discussion of the use of such constructs). By contrast, probes allowing direct in situ hybridization without amplification through enzymatic reaction challenge this notion, as do the results of deep sequencing (92). As can be expected given the overall picture of the regulation of BDNF's availability, BDNF transcripts are short-lived compared with most other transcripts expressed in neurons (92). Recent work involving affinity purification of actively translating ribosome and reporter constructs indicates that the prevalent 5′ UTRs exert a repressive role on translation (93). The same study also indicates that neuronal activity does not affect translation (93), suggesting that BDNF's availability is primarily regulated by mechanisms controlling transcription, as opposed to translation. The relevance of the mechanisms regulating *BDNF* transcription to human conditions is becoming increasingly evident (for review see Ref. 94) and an important aspect of current BDNF research (for a recent example, see Ref. 16). With regard to *BDNF* transcriptional repressors, methyl-CpG-binding protein 2 (MeCP2) has been much discussed in the past, given that this major repressor is expressed at high levels in the brain, comparable with histones (95, 96). There is a great deal of interest for this repressor as *MECP2* mutations are the cause of most cases of Rett syndrome, an X-linked neurodevelopmental disorder causing severe neurological dysfunction in females (97). However, the role of MeCP2 in the regulation of *BDNF* transcription is complex. Brain-specific elimination of *Mecp2* suggested early on that some of the key functional deficits resulting from *MECP2* mutations are a consequence of neuronal dysfunction (98, 99). Remarkably, restoring *Mecp2* expression in mouse mutants as late as 3–4 wk after birth, when mutant mice already begin to deteriorate because of the absence of *Mecp2,* is sufficient to

erase many of the deleterious consequences resulting from the lack of MeCP2 (100). This key result suggests a lack of widespread neurodegeneration caused by the lack of *Mecp2*, a finding that has significant therapeutic implications. Although *Mecp2* was shown to bind to *Bdnf* promoter IV and initially proposed to repress transcription (101, 102), the interpretation of a possible link between these two genes is complicated by the observation that the lack of *Mecp2* also alters neuronal excitability due to a shift in the balance between excitation and inhibition (103). Interestingly, depolarization of cultured neurons triggers a calcium-dependent phosphorylation of MeCP2 and its release from DNA, thereby facilitating transcription (101). A further contributing factor is the depolarization-induced decrease in CpG methylation within the regulatory region of the *Bdnf* gene (102). In vivo, the levels of BDNF are clearly decreased in the brain of mouse mutants lacking *Mecp2*, and increasing BDNF expression by genetic engineering significantly improves the behavior of mice lacking *Mecp2* (104). These observations prompted a number of studies aiming at counteracting the impact of the *Mecp2* mutation by attempting to restore *MECP2* expression (for recent review, see Ref. 105) or by increasing BDNF levels or TrkB signaling with drugs (106, 107). In particular, a drug widely used to treat multiple sclerosis and known to diffuse into the brain designated fingolimod, a sphingosine-1 phosphate receptor modulator, was shown to increase BDNF levels and to markedly mitigate the impact of the *Mecp2* mutation when injected intraperitoneally into mice (107).

One of the best-known factors repressing transcription in the nervous system is the repressor element 1-silencing transcription factor REST, also known as neuron-restrictive silencer factor NRSF. This is a negative regulator of transcription binding to a *cis*-acting negative regulator element designated RE1. REST binds CoREST (108) and Sin3A, and this protein complex recruits the histone deacetylases repressing transcription (109). RE-1 is located downstream of promoter II, and *Bdnf* promoter II assay with cortical neurons indicated constitutive repression by REST (110). In a later study, increased neuronal activity caused by the addition of 4-aminopyridine (4-AP) to newborn mouse cortical neurons resulted in a transient increase of *Bdnf* transcription that was more sustained after inhibition of REST with shRNA (111). The same study showed that the increase in the number of perisomatic inhibitory synapses caused by 4-AP was blocked by the BDNF scavenging reagent Fc-TrkB, suggesting increased release of perisomatic BDNF (111). A possible role for REST and its associated effectors has also been examined in the context of Huntington's disease (112). Wild-type Huntingtin has been proposed to activate *BDNF* transcription by inhibiting REST-mediating silencing at promoter II through cytoplasmic sequestering, thus preventing the binding of the REST complex (62). This important finding led to the virtual screening of large collections of molecules potentially interacting with the REST complex with the goal of disrupting the interaction of REST with mutant forms of huntingtin (112).

2.5. BDNF Antisense Transcripts

In humans and nonhuman primates, as many as 12 different antisense (AS) transcripts have been identified and shown to form RNA duplexes in the human brain by hybridizing with the sense transcripts. These results suggest an important regulatory function for these *BDNF*-AS, and AS-mediated regulation could become an important aspect of the physiopathology of BDNF. A human AS polymorphism designated rs10767664 has been recently identified on the basis of its strong association with obesity (see sect. 9). The initial notion that BDNF antisense transcripts may be primate specific has been subsequently disputed, and the deletion of rodent antisense RNAs using a siRNA strategy shown to cause increased levels of both sense RNA and protein (113). Using this knowledge, the same group went on to show that the levels of BDNF are somewhat increased in the hippocampus of mice carrying a 6-kb deletion upstream of the *Bdnf* antisense promoter and that object discrimination is improved after bouts of involuntary exercise (53). Although these studies are recent, they indicate that genomic polymorphisms found in noncoding region of the *BDNF* gene may become an important aspect of BDNF research and may help guide the diagnosis of complex behavioral traits and brain disorders.

2.6. Epigenetic Modifications

Given the multiple links between BDNF and memory-related processes (see sect. 6), there is increasing interest in the covalent modifications of histones within *BDNF* promoter sequences as a way to record and store persistent changes leading to long-lasting altered gene expression (for a recent review, see Ref. 114). Using one of the best-established behavioral paradigms related to memory persistence in mice, Bredy and colleagues (115) found that the extinction of conditioned fear is accompanied by increased histone H4 acetylation in *Bdnf* promoters I and IV in neurons of the prefrontal cortex in young males. In addition, the histone acetylase inhibitor valproic acid was found to potentiate the effects of extinction training (115). A later study using rats and a contextual fear paradigm described a regulation of *Bdnf* transcripts I, IV, VI, and IX associated with DNA methylation (116). This study also showed that infusion of a DNA

methyltransferase inhibitor altered DNA methylation and the composition of *Bdnf* transcripts in the rat hippocampus. NMDA receptor blockade prevented DNA methylation of *Bdnf* promoters in the hippocampus while also eliciting deficits in memory formation (116). In a mouse model of neurodegeneration caused by the forebrain expression of the truncated version of the kinase p25, HDAC2-mediated changes were examined and the enzyme found to be enriched at *Bdnf* promoter 4 with concomitant increased acetylation of lysine 12 of histone 4, decreased polymerase II binding, and decreased *Bdnf IV*, but not *I* and *II*, transcripts (117). Decreasing the levels of HDAC2 with shRNA restored expression of several plasticity-associated genes including *Bdnf* IV transcripts and reduced memory impairment (117). Later related studies revealed that inhibition of class II histone deacetylases (HDACs) caused a rapid increase in *Bdnf* mRNA levels in cultured rat neurons whereas inhibition of class I HDACs delayed the increase of *Bdnf* mRNA levels (118). The same study revealed that HDAC4 and HDAC5 repressed *Bdnf* promoter IV activity (118). Long-lasting epigenetic changes have been noted as a result of prenatal stress in rats, with decreased exon IV- and VI-containing transcripts in the amygdala and hippocampus as long as 3 wk after birth (119). Inhibition of histone deacetylase increases BDNF expression and promotes neuronal rewiring and functional recovery after brain injury (120). Interestingly, exercise has been proposed to increase BDNF expression in mice through the action of β-hydroxybutyrate acting as an inhibitor of histone deacetylase, particularly HDAC2 and 3, and to increase *Bdnf* transcription from promoter I (121). Interest in this all-important field of research is likely to further increase given recent results involving single-cell analyses in circuits known to be involved in specific tasks and ready to be recruited in memory tasks (122). Single neurons in the mouse lateral amygdala exhibit different degrees of intrinsic chromatin plasticity that could be correlated with increased neuronal excitability. In line with this, epigenetic alterations of neurons prevent memory formation (122). Whether these results may contribute to explain the large differences in BDNF translation from neuron to neuron (see **FIGURE 9**) remains to be investigated. In a rat model, the antidepressant effects of ketamine have been explained by the ability of the drug to inhibit histone deacetylases and restore *Bdnf* expression at promoter IV (123).

3. BIOSYNTHESIS AND DISTRIBUTION

3.1. Introduction

Given the very low abundance of BDNF, studies of its localization and biosynthesis are technically challenging. As a result, there is no consensus as to the isoforms of BDNF released from neurons or with regard to the distribution of BDNF within neurons. As discussed in this section, the problems arose in large part from the extensive use of overexpression paradigms, of insufficiently validated constructs encoding BDNF fusion proteins, and of antibodies lacking specificity.

3.2. Biosynthesis

3.2.1. Translation and folding.

The prepro-BDNF protein is encoded in exon IX included in all BDNF transcripts (see sect. 2). Translation follows the classical pathway of glycosylated precursor proteins including transit through the Golgi apparatus (**FIGURE 5**). Transcripts also containing exon I have been detected in most brain areas (51, 52). This is functionally significant, as the inclusion of exon I provides an alternative, in-frame translation initiation codon encoding a prepro-protein with 8 additional amino acids. Exon I promoter is also regulated by neuronal activity, and the transcripts are translated more efficiently than those not including exon I (49). The transcripts are expressed at significant levels in the human brain, including the hypothalamus (48).

Like many secreted neuropeptides, BDNF is synthesized as a prepro-protein, with the cleavage of the signal sequence presumably occurring during translation and translocation into the endoplasmic reticulum (ER), where an elaborate folding process takes place resulting in the formation of a cystine knot (124). As first demonstrated with NGF (125), the first cystine knot growth factor to have been structurally characterized (126), the BDNF pro-domain is required for the proper folding of mature BDNF. This process involves the formation of covalent bonds between the cysteine residues 1–4, 2–5, and 3–6, the arrangement at the core of the cystine knot motif. The chaperone function of the BDNF pro-domain can be fulfilled by other neurotrophin pro-domains, for example the pro-domain of NT3 (127). Whether or not the Val/Met polymorphism (see sect. 6) impacts the rate or other aspects of BDNF folding is unknown. The recombinant forms of BDNF used in most published experiments are produced in bacteria using constructs without the pro-domain. BDNF folding is achieved with denaturation and renaturation conditions and with methionine instead of histidine as the amino terminal of the mature protein (128), hence the designation Met-BDNF in some studies (129).

Just one set of experiments has been performed directly addressing the question of the biosynthesis of endogenous BDNF in neurons (130). Western blot analysis of immunoprecipitates of neurons pulse-labeled with

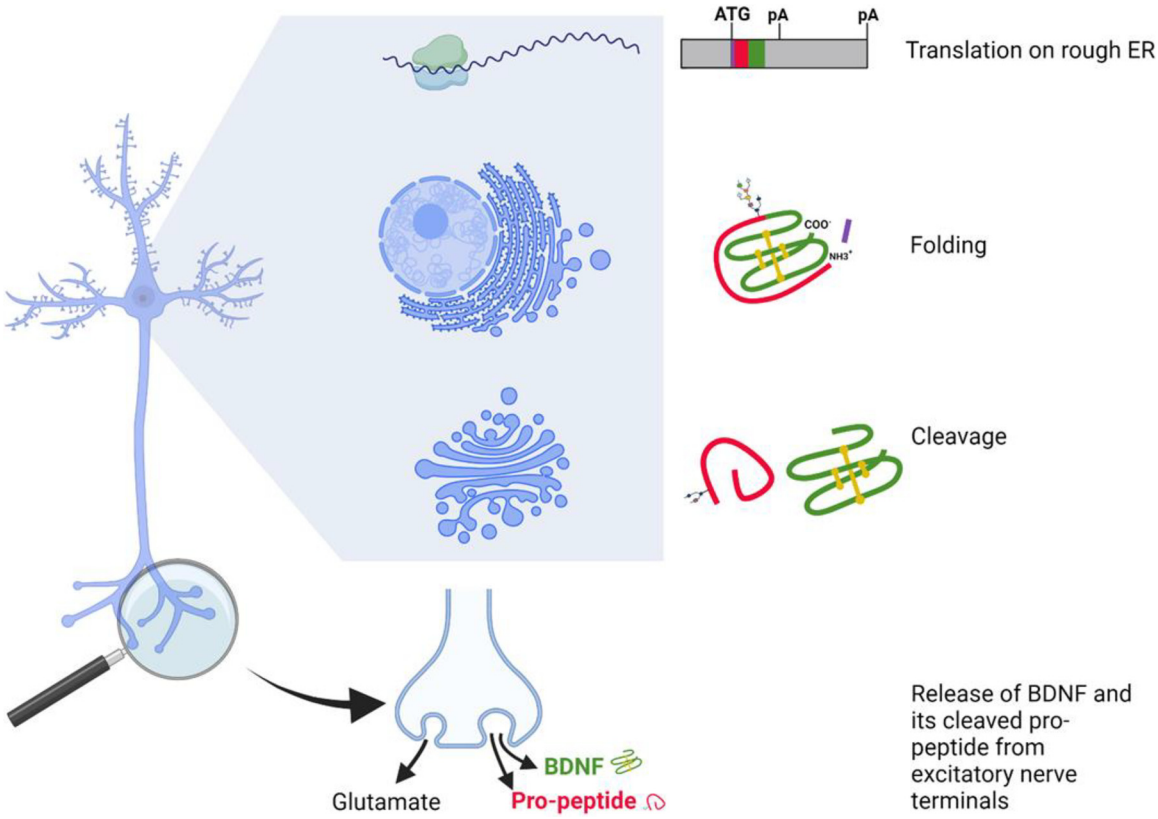


FIGURE 5. Biosynthesis of brain-derived neurotrophic factor (BDNF) in neurons. The *BDNF* mRNA is translated on the rough endoplasmic reticulum (ER). After packaging in large dense-core vesicles and anterograde transport, BDNF is released together with its cleaved pro-peptide following calcium entry triggered by high-frequency stimuli. Figure created with a licensed version of BioRender.com.

radioactive amino acids revealed that the radioactivity initially incorporated in pro-BDNF is then found in mature BDNF after a chase period with unlabeled amino acids, thus indicating that pro-BDNF is a transient biosynthetic intermediate (130). These experiments also revealed a lack of significant secretion of pro-BDNF upon analysis of the culture medium (130). After cleavage, BDNF and its pro-peptide likely remain associated (131), and a quantitative electron microscopy study revealed that the subcellular distribution of both components is identical in the mouse hippocampus (132). It would thus appear likely that both components are cosecreted. However, the notion of a complete intracellular processing of pro-BDNF within neurons is not universally shared, in large part because of the extensive use of overexpression paradigms (see below).

As first described for NGF (133), BDNF exists in solution as a stable dimer (24). Unlike the case with other members of the cystine knot family, the neurotrophin protomers are not bound by disulfide bridges, as all cysteine residues are involved in the formation of the cystine knot of the protomer forming a flat surface by virtue of the arrangements of their three antiparallel β-strands (126). BDNF monomers can be isolated under denaturing conditions, and they spontaneously reform homodimers at neutral pH, with BDNF interacting with its receptors as a homodimer (24). Neurotrophins can form heterodimers after either denaturation/renaturation of mixtures of homodimers (134, 135) or coexpression of the corresponding cDNAs in transfected cells (127). BDNF/NT3 heterodimers are biologically active and can activate both TrkB and TrkC (127), but the potency of such heterodimers is reduced when assessed in neuron survival assays compared with the respective monomers (127). There is no evidence that such heterodimers exist in vivo. The structures of BDNF/NT3 and BDNF/NT4 heterodimers have been reported (136, 137).

3.2.2. The cleavage of pro-BDNF.

The glycosylated glutamine close to the pro-BDNF cleavage site (see **FIGURE 2**) carries sulfated LacdiNAc residues (138), a posttranslational modification first recognized with NGF (139). Sulfation has been shown to be required for the secretion of pro-BDNF in overexpression paradigms (138) and N-glycosylation likely required to facilitate neurotrophin folding and prevent protein aggregation (140). In ex vivo experiments with recombinant proteins, the furin-mediated cleavage of pro-BDNF has been shown to be independent of pro-BDNF N-glycosylation (138). Furin is a member of a family of

pro-protein convertases (PCs) comprising nine members of serine proteases related to bacterial subtilases and yeast kexin, hence the abbreviation PCSKs. They cleave at single or paired basic amino acid residues (for a recent review, see Ref. 141). PC7 could be shown in vivo to be involved in the cleavage of pro-BDNF because *Pcsk7*-null mutants are viable, unlike the case with other null mutants of the PC family, with the exception of PC9 (141). Like furin, PC7 is mainly localized in the *trans*-Golgi network and is thus likely to come into contact with pro-BDNF during its biosynthesis (142). The tissue distribution of PC7 in the brain matches that of BDNF in some of the most thoroughly investigated brain areas such as the cerebral cortex and the hippocampus. The viability of *Pcsk7*-null mutants also allowed the demonstration that the levels of BDNF are reduced in the adult hippocampus and amygdala, and in line with these findings these mutant animals were found to exhibit learning deficits (142). Yet the very fact that *Pcsk7*-null mutant animals survive until adulthood indicates that other proteases can also cleave pro-BDNF. A number of different candidates have been proposed, but their in vivo relevance is questionable given that a protease with a trypsinlike specificity is sufficient to ensure cleavage. For example, tissue plasminogen activator (TPA) has been implicated because of its pattern of expression in the brain and its ability to activate plasmin, but it is unclear whether plasmin is physiologically involved in the processing of endogenous pro-BDNF [see sect. 5 for a proposed link between plasmin and long-term potentiation (LTP)].

With regard to the processing of pro-BDNF, an important question has remained unaddressed thus far: the adjustment of the pro-BDNF processing capacity with the levels of pro-BDNF to be processed. Work with cultured neurons (130) as well as in vivo with *Bassoon* mutants (**FIGURE 6**) revealed that such adjustments do take place, both in vitro and in vivo. Bassoon is a major presynaptic protein, and for reasons that are not entirely clear, its loss causes increased neuronal activity, to the extent that the mutant animals eventually die of epilepsy (for a recent review that also discusses Bassoon mutations in humans, see Ref. 143). As the brain of these animals is significantly enlarged, brain levels of BDNF were determined and found to be much higher than in age-matched control animals (144). Increased neurogenesis was also noted in the dentate gyrus of these animals (144). Subsequent Western blot experiments (**FIGURE 6**) revealed that the ratio pro-BDNF/BDNF is the same in wild-type and *Bassoon* mutants, indicating that the capacity to process pro-BDNF is also increased in mutant animals (132). How the levels and/or the activity of the protease(s) involved in the cleavage of pro-BDNF in neurons are adjusted as a function of neuronal activity remains unclear. Adaptative mechanisms have been recently reviewed in the case of pro-insulin and its processing (145), but they have yet to be investigated in the case of BDNF's biosynthesis in neurons.

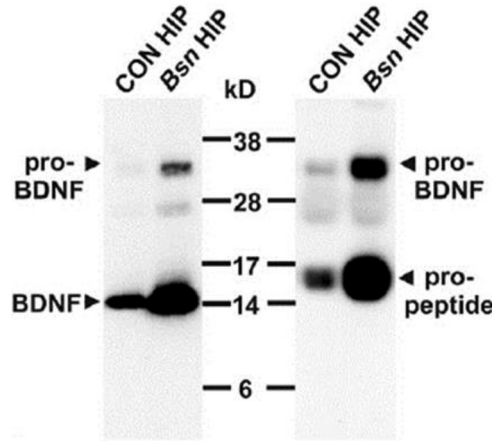


FIGURE 6. Western blot experiments performed after immunoprecipitation of hippocampal (HIP) lysates. Lysates prepared from control (CON) or *Bassoon* (*Bsn*) mutant animals were incubated with anti-brain-derived neurotrophic factor (BDNF; *left*) or anti-pro-BDNF (*right*) antibodies. After transfer, the membranes were probed with anti-BDNF antibodies (*left*) or pro-BDNF antibodies (*right*). Molecular masses are indicated in kilodaltons. The *Bassoon* mutation causes a massive increase in neuronal activity resulting in increased BDNF levels. Yet the ratio pro-BDNF to BDNF remains as in wild-type animals, indicating that the capacity to process pro-BDNF also increases when neuronal activity increases. This is not the case when neurons are acutely transfected with cDNA constructs encoding pro-BDNF. Reproduced from Ref. 132 with permission.

The much higher levels of BDNF in the hippocampus of the *Bsn* mutant compared to wild-type animals greatly facilitated the visualization of BDNF and of its pro-peptide (**FIGURE 7**). Both were found to colocalize as revealed by a quantitative immunoelectron microscopy using gold-labeled antibodies to BDNF and to its pro-peptide (132). This study also revealed a presynaptic localization of both components in CA3. Neither postsynaptic structures nor astrocytes were labeled above background. Gold particle counts well above background could also be visualized in the Golgi apparatus and in axons (132). The unresolved question of the adjustment of the processing capacity of neurons as a function of neuronal activity is also critical in view of the countless overexpression experiments using cDNAs encoding prepro-BDNF. These experiments invariably led to the secretion of pro-BDNF from transfected cells including neurons, and this has fueled the widespread notion that neurons secrete pro-BDNF (see below).

3.2.3. Challenges and controversies related to the reported secretion of pro-BDNF by neurons.

Whether or not neurons release pro-BDNF under physiological conditions is an important question given that

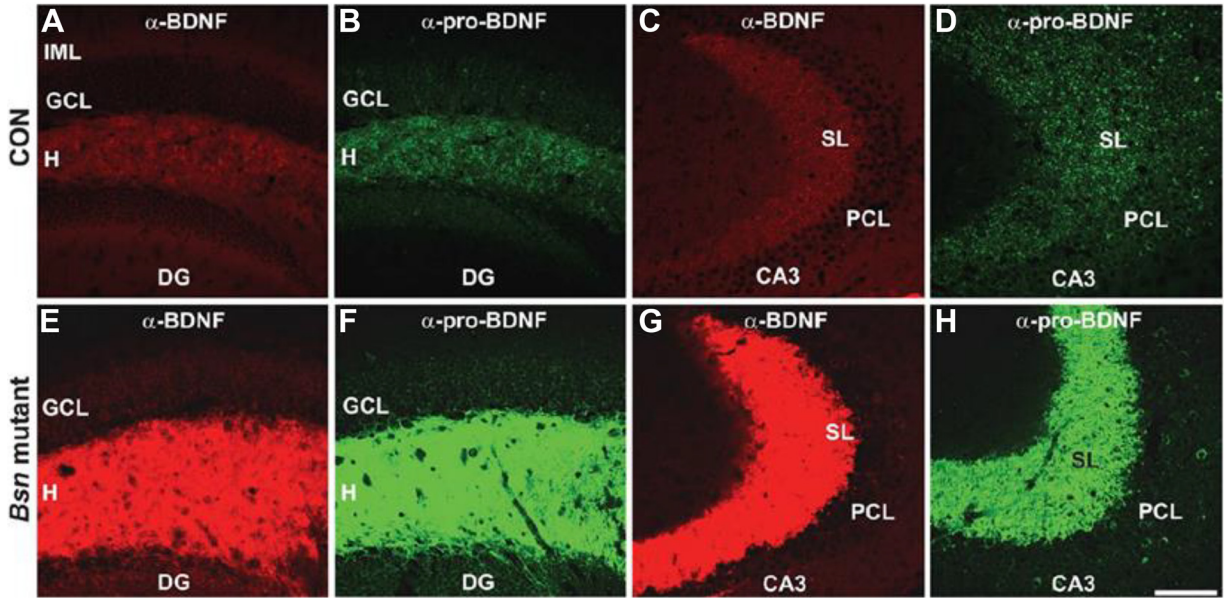


FIGURE 7. Hippocampal sections of control (CON; *A–D*) and Basson (*Bsn*) mutant (*E–H*) animals stained with antibodies to either brain-derived neurotrophic factor (BDNF) (red, α-BDNF) or the BDNF pro-peptide (green, α-pro-BDNF). Note the colocalization of BDNF and its pro-peptide and the much higher staining intensity in sections prepared from young adult *Bsn* mutant animals. This colocalization was also observed in isolated, strongly labeled principal neurons in the dentate gyrus (132). The marked increase in the staining intensity of both components is a result of the increased neuronal activity in the *Bsn* mutant, to the extent that it leads to seizure episodes. Scale bar in *H*, 100 μM. DG, dentate gyrus; GCL, granule cell layer; H, hilus; PCL, pyramidal cell layer; SL, stratum lucidum. Reproduced from Ref. 132 with permission.

BDNF has prosurvival effects through the activation of TrkB whereas pro-BDNF can cause cell death through $p75^{NTR}$ (see sect. 4). As has been the case in other fields (146), the extensive use of insufficiently validated antibodies has been quite problematic, leading to initial claims that pro-BDNF is the dominant, or even the only detectable, form of BDNF in brain lysates (90, 147). These reports were later challenged when more specific BDNF antibodies suitable for Western blotting became available, including in particular a thoroughly validated monoclonal antibody designated 3C11 developed by Icosagen (Õssu, Estonia) recognizing a defined BDNF epitope (48, 148, 149). The addition of small tags such as Myc or HA (for hemagglutinin) added to the carboxy terminal of BDNF in gene substitution experiments also helped but limited the work to the corresponding engineered animals (130, 150). Currently, the controversy about the release of pro-BDNF from neurons is mostly fueled by the extensive use of overexpression paradigms, under the implicit assumption that the processing and sorting capacity of neurons transfected with cDNA constructs, or with constructs activating specific *Bdnf* promoters (71), would not be limiting. When analyzed with suitable BDNF antibodies, overexpression paradigms invariably do lead to the secretion of pro-BDNF. By contrast, the only published pulse-chase experiment monitoring the biosynthesis of endogenous BDNF in cultured neurons indicates a simple precursor-product relationship and an intracellular conversion of pro-BDNF into BDNF (130). Increasing activity of the neurons by blocking ongoing inhibitory activity increases BDNF's biosynthesis and its release as mature BDNF, suggesting that, as discussed above in the case of the *Bassoon* mutant, increased neuronal activity scales up the pro-BDNF processing capacity of neurons (130). However, experiments also performed with cultured hippocampal neurons isolated from animals carrying an HA-tagged version of BDNF led to a different conclusion, namely that neurons do release pro-BDNF (150). Unfortunately, the reasons for these discrepant results remain unclear, but differences in the culture protocols used in these studies may be part of the explanation. In particular, the use of mitotic inhibitors and of 56 mM KCl to depolarize the neurons may have compromised neuronal survival and/or impaired neuronal maturation because of the lack of astrocytes and other supporting cells (150). Adding to the initial confusion was the notion that the cleaved BDNF pro-peptide is not detectable in brain lysates (150). However, later experiments revealed that the pro-peptide, likely costored with BDNF in presynaptic vesicles (see above), is lost from the blotting membranes unless the transferred proteins are cross-linked to the membrane (132). The notion of presynaptic storage of BDNF together with its cleaved pro-peptide was subsequently supported by the results of Western blot experiments performed with extracts of the striatum (151): The accumulation of BDNF in the striatum is widely recognized to result from the anterograde transport of

BDNF from the cerebral cortex (see for example Refs. 40, 152), and Western blot experiments with lysates of the striatum failed to detect significant levels of pro-BDNF (151). It appears then that the presynaptic pool of BDNF ready to be released consists of BDNF costored with its cleaved pro-peptide as illustrated in **FIGURE 5**. Release of the BDNF pro-peptide triggered by depolarizing neurons with 25 mM KCl has also been documented, with the levels of the pro-peptide remaining unchanged in the presence of protease inhibitors (153). In sum then, excitatory neurons are likely to secrete BDNF together with its pro-peptide, a notion that is becoming increasingly important given the finding that the secreted pro-peptide, including its Met version, may have an activity on its own (153, 154).

3.2.4. Human polymorphisms related to the pro-BDNF cleavage site.

Beyond the Val/Met polymorphism (see sect. 6), polymorphisms have been reported causing amino acid substitutions in the cleavage site of human pro-BDNF. Specifically, rs1048220 and rs104822 cause the replacement of arginine residues by methionine and leucine, respectively (see **TABLE 1**). Although both polymorphisms are often described as rare, they are nonetheless sufficiently frequent to have been included in studies attempting to correlate them with human conditions such as Alzheimer's disease (AD) and major mood disorders (see for example Refs. 158, 159). Given the outcome of related experiments in the mouse involving the BDNF cleavage site (see **TABLE 1**), it is surprising that the carriers of these polymorphisms are not more symptomatic. For example, the study by Yang and colleagues (157) indicates that mice heterozygous for a mutation in the cleavage site (**FIGURE 4**) show a strong phenotype likely caused by the secretion of pro-BDNF. This includes an abnormal weight gain postnatally that is more pronounced than in mice lacking one *Bdnf* allele (Ref. 157; see sect. 9). By contrast, the substitution of two arginine residues by methionine and leucine (**FIGURE 4**) causes a comparatively mild phenotype, to the extent that even adult animals homozygous for the mutation can be studied in detail. Although these mutant animals do show a reduction in nest building (155, 156) this is a comparatively mild outcome compared with what was observed in the 2014 study by Yang et al. (157). Early in vitro work on the pro-BDNF cleavage may offer some clue as to how to explain this surprising discrepancy. With the use of recombinant proteins expressed in a vaccinia virus-based system, it has been fortuitously observed that the substitution of the distal arginine residue by lysine at the cleavage site (**FIGURE 4**) did not prevent the cleavage of pro-BDNF but caused instead a cleavage further upstream. This leads to the generation of a glycosylated form of BDNF carrying a 19 amino-terminal extension (24). Unexpectedly, this material turned out to be fully biologically active in an in vitro neuron survival assay (24). This result potentially explains why rs1048220 and rs104822 carriers do not exhibit severe phenotypes: Sufficient amounts of biologically active BDNF may be available in the human carriers of these polymorphisms, possibly explaining why the carriers are not more symptomatic. This speculation could be experimentally tested by analyzing the isoforms of BDNF present in the blood of the corresponding human carriers (see sect. 9 for the origin of BDNF in human blood). In line with this interpretation, a close examination of the published Western blots performed with brain extracts of the mouse model generated to test the functional impact of the polymorphisms rs1048220 and rs104822 suggests the presence of a longer, possibly glycosylated form of

Table 1. ***Overview and outcome of experiments involving the cleavage site of pro-BDNF***

Context	Experiment	Cleavage Site	Outcome
Wild-type cleavage site	Cleavage of pro-BDNF at rest and under conditions of increased neuronal activity (130)	RVRR//HSDPARRGEL	Full cleavage of pro-BDNF under both conditions
Replicating human polymorphisms rs1048220 and rs104822	In vitro experiments and mouse model (155, 156)	**M**V**L**R//HSDPARRGEL	No dramatic phenotype, either in mice or in humans
Deliberate mutation preventing pro-BDNF cleavage	In vitro experiments and mouse model (157)	RV**AA**HSDPARRGEL	No pro-BDNF cleavage, strong phenotype in mice
Single deliberate mutation	In vitro experiments with recombinant proteins (24)	RVR**K**HSDPARRGEL	Glycosylated, biologically active BDNF with NH_2-terminal extension //LEEYK**N**YLDAANMSIMRVR**K**HSDPARRGEL. . .

Mutated residues are indicated by boldface characters. The small dots following the GEL sequence refer to the wild-type sequence of brain-derived neurotrophic factor (BDNF) thought to remain unaffected by the mutations introduced at the cleavage site.

BDNF, understandably interpreted as a nonspecific signal at the time (see **Fig. 1*B*** in Ref. 155).

3.3. BDNF Distribution in the Nervous System

The expression of *BDNF* in the nervous system is restricted to excitatory as opposed to inhibitory neurons. Excitatory neurons are thus well placed to play important roles in the activity-dependent development of inhibitory circuits (160, 161), given that essentially all GABAergic neurons express the BDNF receptor TrkB. This is the case not only in rodents but also in humans (see **FIGURE 8**).

A close examination of the brain of *Bdnf*-null mutant mice revealed decreased staining intensity of inhibitory neuron markers in the striatum, hippocampus, and cerebral cortex of mice lacking *Bdnf* (39). Beyond the role of BDNF in CNS myelination reported much later (for a review, see Ref. 162), this turned out to be the most prominent CNS anomaly detected in the brain of mice lacking BDNF. This key initial observation was supported by the results of parallel cell culture experiments indicating that BDNF increases the content of GABA as well as its uptake by striatal neurons (163, 164) and increased calbindin staining in cells dissociated from the rat cerebral cortex (165). Earlier in vitro observations with cholinergic neurons dissociated from the rat septum, a population previously shown to be NGF responsive (see sect. 1.3), had indicated a survival activity of BDNF (166). Activation of GABA receptors in cultured striatal neurons was further shown to increase neuropeptide Y (NPY) staining in a BDNF-dependent fashion (167) and BDNF to regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus (168). As discussed in sect. 2, the in vivo physiological relevance of activity-dependent expression of BDNF for the development of inhibitory circuitry in the visual cortex has also been suggested in experiments involving mice carrying mutations in the *Cre/Carf3* binding sites, one of the activity-dependent sites (58). Meanwhile, a wealth of information has become available on the sites of BDNF's biosynthesis thanks to major single-cell RNA sequencing efforts (see, e.g., **FIGURE 8** and http://mousebrain.org/adolescent/genesearch.html). This all-important detailed cartography

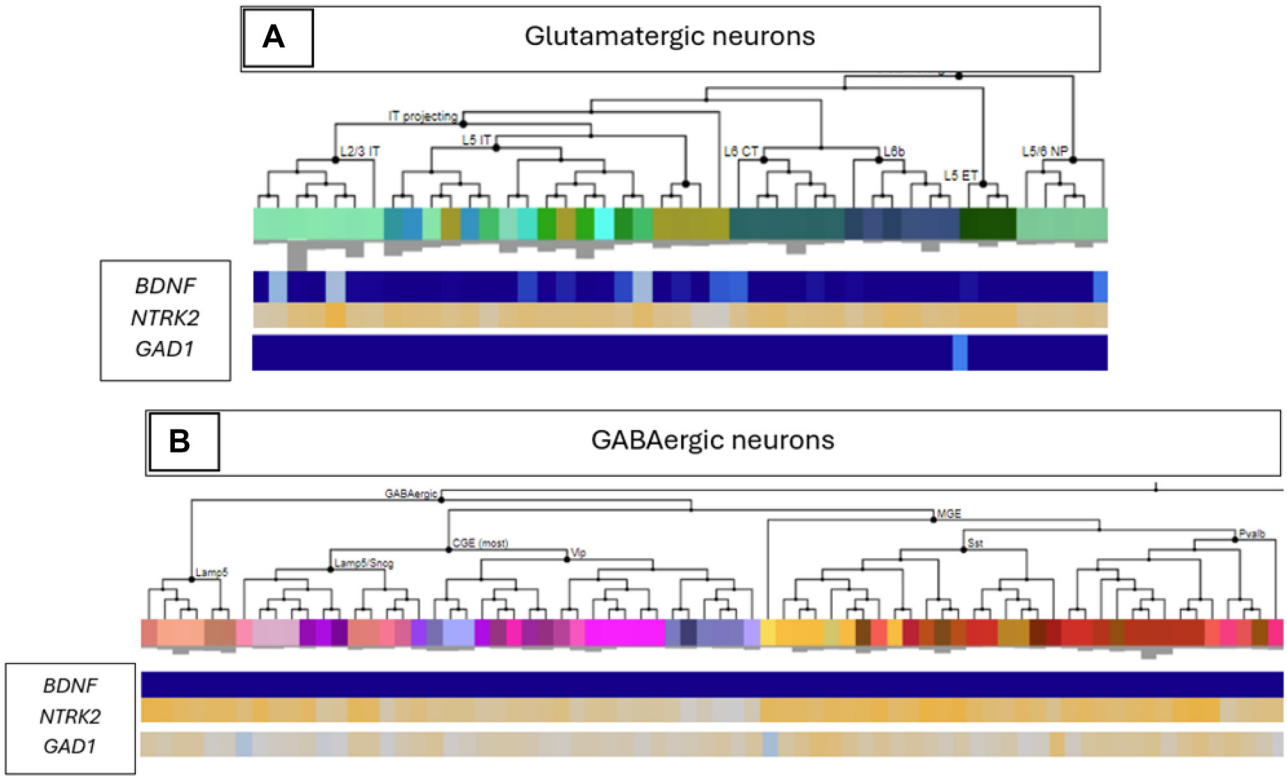


FIGURE 8. Relative levels of *BDNF*, *NTRK2* (aka TrkB), and *GAD1* expression in the adult human brain. These heat maps are based on RNAseq data and summarize key features of the brain-derived neurotrophic factor (BDNF)/TrkB signaling system Note in *A* the extremely low levels of *BDNF* expression only in some populations of excitatory, as opposed to inhibitory, neurons. By contrast (see *B*) the levels of TrkB (aka *NTRK2*) are much higher than those of *BDNF*, with all neurons analyzed expressing the gene. The levels of expression of *GAD1* (aka Glutamate decarboxylase 1), one of the major enzymes involved in the biosynthesis of the inhibitory neurotransmitter GABA, are indicated. Curiously, the reasons why inhibitory neurons transcribe the *BDNF* gene at barely detectable levels are still unclear but possibly due to different chromatin accessibility compared with excitatory neurons. This is all the more surprising given that inhibitory and excitatory neurons express TrkB at comparable levels and TrkB activation by BDNF in excitatory neurons causes an increase in *BDNF* transcription (see text). Image credit: Allen Institute (https://celltypes.brain-map.org/rnaseq/human_m1_10x?selectedVisualization=Heatmap&colorByFeature=Cell+Type&colorByFeatureValue=GAD1).

has also been greatly helped by the generation of mouse lines conditionally expressing GFP under the control of the *Bdnf* locus with strategies involving the introduction of short self-cleaving sequences preceding Cre (169). With a similar strategy involving self-cleaving sequences, the translation of BDNF could be monitored with mice engineered to express GFP under the control of the *Bdnf* promoters (**FIGURE 9**), with GFP proceeded by a short self-cleaving sequence to prevent the problems described in sect. 3.2.3 (148). This study also quantified the impact of tags of different lengths added to the carboxy terminal of BDNF with regard to the ability of these modified versions of BDNF to activate TrkB (148). Briefly, an extension up to 20 amino acids had no measurable impact on TrkB activation, but 30 and beyond did measurably decrease it (148).

The main general outcome of these studies is a confirmation of what in situ hybridization and reporter studies had long suggested, namely that the *Bdnf* gene is primarily expressed by excitatory neurons (18, 89). These include not only CNS neurons but also peripheral sensory ganglia, with some expressing *Bdnf* at levels considerably higher than those in the CNS, including the subdivisions of the hippocampus (http://mousebrain.org/adolescent/genesearch.html). The comparatively high levels of BDNF in sensory ganglia including their projections in the spinal cord greatly facilitated the subcellular localization of BDNF in neurons (170, 171). These studies indicated early on that after its biosynthesis in neuronal cell bodies, BDNF is transported anterogradely in large dense-core vesicles (172) and is stored in the presynaptic terminals of many excitatory neurons.

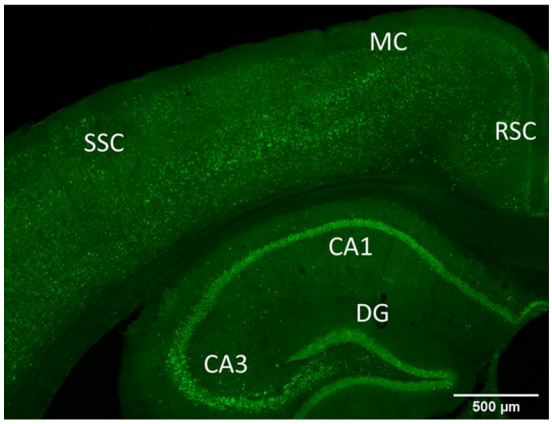


FIGURE 9. Mapping brain-derived neurotrophic factor (BDNF) translation in the mouse brain with a self-cleaving green fluorescent protein (GFP) tag. Transverse section of a young adult mouse brain expressing GFP under the control of the *Bdnf* promoters. To avoid the problems resulting from BDNF tagging (see below), a self-cleaving sequence was added between BDNF and GFP tagged with a nuclear localization signal (148). Note the relatively high levels of BDNF expression by isolated single neurons, for example in CA3 and in layers 3 and 5 of the motor cortex (MC), presumably reflecting higher levels of activity of these neurons. CA, cornus ammonis; DG, dentate gyrus, RSC, retrosplenial cortex; SSC, somatosensory cortex.

3.4. *Distribution of BDNF within Neurons and Controversies Resulting from the Use of BDNF-GFP Constructs*

Given the very low levels of BDNF, addressing the issue of its intracellular distribution within neurons is technically challenging. Yet this issue is critical to understand the role and function of BDNF in brain circuits. Although the localization of BDNF in presynaptic large dense-core vesicles is not controversial, its localization in postsynaptic structures, including dendrites and spines, is. Beyond the problems caused by the use of antibodies lacking specificity, those resulting from the extensive use of overexpression paradigms have not received sufficient attention. First, overexpression paradigms are exceptionally challenging to validate in view of the minuscule amounts of endogenous BDNF. When attempted at all, validation has been for the most part unconvincing and/or performed with cultured neurons with unclear degree of maturation. Second, experiments of this type involve the use of BDNF constructs tagged at the carboxy terminal with covalently bound GFP, under the surprising assumption that such comparatively large tags would not interfere with the folding, processing, and intracellular distribution of the protein. Unfortunately, these widely used constructs have not been sufficiently validated (see Ref. 173 and related comments in PubPeer) and their suitability is questionable. The outcome of experiments using *Bdnf* constructs directly coupled to GFP to replace the endogenous *Bdnf* gene in mice illustrates the limitations of the approach: Only half of the expected number of transgenic animals were found to be viable (174), and in the surviving animals the GFP signal was found in locations distinct from those where endogenous BDNF typically accumulates, such as the presynaptic mossy fiber terminals in CA3 (see **FIGURE 7**), where BDNF is comparatively easy to detect in wild-type animals (132, 175, 176). The mislocalization of the GFP signal in BDNF-GFP animals may have been caused by the carboxy-terminal cleavage of GFP in the surviving mice (see **FIGURE 1**). Beyond questions related to the localization of BDNF, these considerations are also important with regard to the site of release of BDNF. In particular, the widespread suggestion that BDNF may be released not just from presynaptic terminals but also from dendrites is primarily supported by the results of experiments performed with BDNF-GFP constructs (173, 174, 177, 178). By contrast, quantitative immunoelectron microscopic experiments using gold-coated, validated antibodies to BDNF and to its pro-peptide indicate that both colocalize to presynaptic large

dense-core vesicles. In CA3, no labeling above background was detected in postsynaptic structures or in astrocytes. Similar quantitative analyses performed on the hippocampus of the *Bassoon* mutants that express much higher levels of BDNF (see **FIGURE 7**) indicate a similar distribution. In sum then, it seems that mature BDNF is costored with its pro-peptide in presynaptic terminals of excitatory neurons in the brain but not in postsynaptic structure (132). This interpretation is also in line with studies related to the localization of mRNAs actively translating BDNF in microdissected areas of the hippocampus (179). The outcome of these experiments indicates that *Bdnf* mRNAs are among those most strictly localized to the neuronal soma as opposed to dendrites. Meanwhile, a small number of anti-BDNF monoclonal antibodies have been validated for their use in immunohistochemistry experiments (149). BDNF can also be reliably localized on tissue sections in mice engineered to express *Bdnf* carrying small tags such as hemagglutinin (150) or c-myc (132). Neurons actively translating BDNF can also be mapped on tissue sections in mice expressing a self-cleaving sequence linking the carboxy terminal of BDNF with GFP (Ref. 148; **FIGURE 9**). Using a similar strategy consisting of short, self-cleaving sequences preceding Cre, a reliable mapping of cells expressing the *Bdnf* gene has also been achieved by crossing these mice with reporter lines conditionally expressing GFP (169, 180). This approach turned out to be particularly useful when delineating BDNF circuitry and/or allowing the selective expression of light-activatable ion channels (181). It also revealed that beyond numerous excitatory neurons, rare astrocytes in some brain areas as well as some oligodendrocytes do express the *Bdnf* gene (182). The same strategy has also been used to map cells expressing TrkB. This led to the observation that oligodendrocyte precursors express full-length TrkB, thus explaining how BDNF can impact myelination (182), confirming previous reports that oligodendrocyte precursors express TrkB (183).

Beyond the fundamental problem resulting from the very low abundance of BDNF, its detection in fixed tissue is further complicated by the comparatively large number of surface-exposed basic residues likely to be altered by conventional fixation techniques. An early study using rapid freezing and acetone, as opposed to aldehyde-based fixation methods, reported the localization of BDNF in most rat DRG neurons cultured in the presence of NGF (184), a finding later confirmed by numerous studies (see sect. 7). With regard to the anterograde transport of BDNF, Zhou and Rush (185) convincingly reported early on the accumulation of BDNF proximal to axonal ligatures of sensory axons, an important finding confirming early suggestions based on the use of radiolabeled neurotrophins that they may act as “anterograde transport messenger” (186). The notion that in the CNS BDNF is transported anterogradely from the neuronal cell bodies to the presynaptic endings of excitatory neurons is now well established, but it was less popular initially given the attractiveness of the notion that trophic interactions in the nervous system primarily involve retrograde mechanisms. This notion was, and still is, strongly supported by much of the early work on NGF in the PNS (see Ref. 187 for an early critical discussion) and confirmed much later with work on BDNF in the PNS (sect. 7).

3.5. BDNF in Nonneuronal Cells

There are few exceptions to the notion that the levels of BDNF are so low in nonneuronal cells that they are exceedingly difficult to detect. This also applies to widely cited studies involving microglial cells and the potential role of microglia-derived BDNF in learning-dependent synapse formation (188) and neuropathic pain (189). With a genetic approach involving Cre-dependent excision of *Bdnf* in microglial cells, the effects of microglia depletion on synapse formation could be recapitulated and cultured microglial cells shown to contain and secrete BDNF (188). In the spinal cord, microglial cells have also been suggested to be a functionally relevant source of BDNF (189). Microglial cells exposed to ATP released detectable levels of BDNF measured by ELISA, and allodynia was significantly reduced when ATP-stimulated microglial cells were delivered locally to the spinal cord and were incubated with BDNF scavengers or transfected with BDNF siRNA (189). As gene expression in microglial cells is highly dependent on signals such as ATP and various cytokines released by other cells, the unequivocal histological demonstration that microglial cells in situ do release BDNF would be desirable. This has been attempted in the hypothalamus in the context of the regulation of food intake (190). Three different BDNF polyclonal antibodies are mentioned in this study, but their specificities and validation remain unclear. Beyond microglial cells, early studies with various blood cells including B and T cells as well as monocytes indicated that they secrete biologically active BDNF and that this secretion can be augmented by exposure of autoreactive T cells to myelin-derived antigen (191). Yet a convincing immunohistochemical demonstration of the presence of BDNF in nonneuronal cells in situ is still lacking. In particular, recent studies failed to confirm the presence of BDNF in microglial cells (182, 192).

One prominent cell type of the hematopoietic lineage expressing readily detectable levels of BDNF is human and rat, but not mouse, megakaryocytes (193). Toward the end of their maturation process, once these cells have endoreplicated their DNA and developed processes designated pro-platelets, megakaryocytes do

express the *BDNF* gene at high levels, allowing BDNF to be readily detected in so-called alpha granules, where BDNF colocalizes with abundant proteins such as platelet factor 4 (193). In hair follicles, the use of *Bdnf*-driven reporter constructs allowed the localization of the sites of *Bdnf* expression to the caudal, but not the rostral, side of hair follicles, in close proximity to the terminations of Aδ low-threshold, TrkB-expressing mechanoreceptors (194). Accordingly, BDNF-TrkB signaling has been proposed to dictate the polarization of Aδ-low-threshold mechanosensory neuron (LTMR) lanceolate endings underlying direction-selective responsiveness of Aδ-LTMRs to hair deflection (194), a result that further underlies the significance of the restricted availability of BDNF. In situ hybridization data also indicate the presence of comparatively high levels of *Bdnf* mRNA in several epithelia including those lining the intestinal tract and especially the duodenum and the colon (195). Other epithelia include the lung, exocrine pancreas, and those lining the kidney tubules (195). The same study also includes BDNF measurements by ELISA in various nonneuronal tissues, but the reported levels in the brain, ~5 pg per mg protein, illustrate the difficulties of extracting BDNF from adult brain tissue at close to neutral pH (195). Maximizing extraction of BDNF, especially from the adult brain, requires low pH and high salt concentrations (23), and although still very low, the levels of extractable BDNF from the postnatal brain are in the low nanogram per milligram protein range.

In general, the distribution of BDNF in cells and tissues does not differ much between humans and rodents, at either the protein or mRNA level. However, a striking exception is blood. As discussed in sect. 10, human and rat platelets contain comparatively high levels of BDNF: on a total protein basis, these levels are considerably higher in human platelets compared with the brain.

4. BDNF RECEPTORS

4.1. Introduction

All neurotrophins bind to two structurally unrelated receptors with nanomolar affinities when determined in systems based on receptor expression in transfected nonneuronal cells. The association of these two very different receptors either with each other or with other membrane proteins endogenously expressed by neurons and other cells increases their binding affinities by up to 100-fold, i.e., a much higher affinity range likely required to transduce the effects of the trace amounts of neurotrophins present in tissues and body fluids.

4.2. The p75 Neurotrophin Receptor

After the early discovery of NGF, it took a comparatively long time to identify a cell surface receptor expressed by NGF-responsive cells transducing its best-known biological effects, i.e., the elongation of neuronal processes and the prevention of cell death. The first NGF receptor candidate was concomitantly identified by two groups using the same strategy involving expression cloning and cell sorting with monoclonal antibodies selected on the basis of promising staining features (196, 197). The binding characteristics of what turned out to be the first member of the tumor necrosis factor receptor (TNFR) family, now also named CD271 or TNFRF16 (**FIGURE 10**), corresponded to the low-affinity NGF binding sites characterized in suspension of NGF-responsive chick sensory neurons (213). Later binding studies with radiolabeled BDNF purified from pig brain revealed binding characteristics strikingly similar to those of NGF on the same neurons, i.e., a few hundred high-affinity sites per cell body and several thousand low-affinity sites, with dissociation constants of roughly 10^{-11} and 10^{-9} M, respectively (214). As the subsequent elucidation of the primary structure of BDNF revealed a spacing of cysteine residues similar to NGF (22), it was then of interest to explore whether the "NGF receptor" as it was then named may also bind BDNF. This turned out to be the case (214), a finding later extended to NT3 (215). Although this receptor has long been renamed neurotrophin receptor p75 ($p75^{NTR}$), the corresponding gene still carries the historical designation *NGFR* (for review, see Ref. 201). Even if the dissociation constants of $p75^{NTR}$ for NGF, BDNF, and NT3 are similar, i.e., ~1 nM, the rates of association to, and dissociation from, the receptor markedly differ for each neurotrophin (214, 215). This feature is indicative of different degrees of ligand-induced changes in the conformation of $p75^{NTR}$ and is least pronounced for NGF (216). The biological impact of these kinetic distinctions is still unknown.

The role played by $p75^{NTR}$ in the physiopathology of BDNF turned out to be particularly challenging to delineate, in large part because it is a noncatalytic transmembrane receptor, as is also the case with truncated TrkB (see below). As a result, it is comparatively difficult to determine whether $p75^{NTR}$ has been engaged after the binding of BDNF or of other ligands. In addition, any functional or biochemical outcome critically depends on which cell type expresses the receptor. The interactions of $p75^{NTR}$ with transmembrane and cytoplasmic proteins can engage several different pathways, some leading to outcomes as diverse as the promotion of cell death or of cell survival (202, 203, 205, 206). A further difficulty in assessing the role of $p75^{NTR}$ is that the phenotype of animals lacking $p75^{NTR}$ is comparatively mild; the null mutants are viable and fertile. Detailed analyses revealed modest

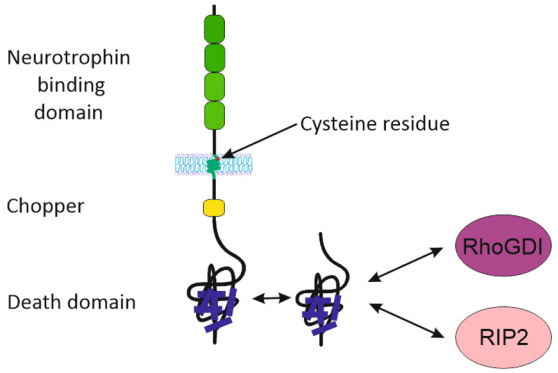


FIGURE 10. The neurotrophin receptor p75 monomer and 2 of its most discussed interactors, including the death domain itself. Four cysteine-rich repeats (green) form the neurotrophin binding site, and as is the case for the Trk receptors, $p75^{NTR}$ is heavily glycosylated. It is thought to exist at the cell surface as a covalently linked dimer, a linkage involving a conserved cysteine residue in the transmembrane domain (198). Chopper designates a 60-amino acid-long unstructured sequence generated by proteolysis of the receptor and causing cell death (199). $p75^{NTR}$ is surprisingly promiscuous, and beside the 4 neurotrophins it also binds the beta-amyloid peptide Aβ and viral capsid proteins including a glycoprotein of the rabies virus (200). The receptor also interacts with a number of other membrane proteins including the 3 Trk tyrosine kinase receptors, receptors for myelin-derived components such as the Nogo and lingo receptors, and members of the sortilin family (for review, see Refs. 201–206). Neurotrophin binding causes structural changes including a weakening of the interaction between the death domains of the $p75^{NTR}$ dimer, displacement of the Rho GDP-dissociation inhibitor RhoGDI, and interaction with RIP2 leading to NF-κB activation (207). The interaction with sortilin and related family members is also noticeable as it leads to the formation of a receptor complex transducing the death-promoting effects of pro-brain-derived neurotrophic factor (BDNF) (208). Furthermore, the BDNF Met propeptide has been reported to transduces its activity through the sortilin-related molecule Sorcs2 in a complex with $p75^{NTR}$ (154). Nuclear magnetic resonance-based experiments revealed that the 145-amino acid-long sequence referred to as death domain (DD) consists of 2 perpendicular sets of 3 alpha helices packed into a globular structure (209). DD dimerization regulates the access to cytoplasmic interactors such as the receptor-interacting-serine/threonine-protein kinase 2 (RIP2) involved in NF-κB activation. NF-κB regulates gene expression and promotes cell survival (210, 211). The binding site of RhoGDI on DD partially overlaps with RIP2 epitopes, and the binding affinity RhoGDI and RIP2 for DD differs by >100-fold (212). Neurotrophin binding causes a conformational change leading to the release of RhoGDI, thereby deactivating RhoA and allowing axon elongation (207).

deficits in the distal sensory innervation of peripheral tissues (217), contrasting with the *Bdnf*- or *Ntrk2*-null mutants (see below): both die before sexual maturity. Experiments performed with peripheral ganglia isolated from *Ngfr*-null animals indicated that higher concentrations of NGF are needed to keep sensory neurons alive compared with those of wild-type animals (218). This observation underlines the functional significance of early findings indicating that the association between $p75^{NTR}$ and TrkA increases NGF's binding affinity (219). Although it could have been expected that the association of $p75^{NTR}$ with TrkB may lower the concentrations of BDNF needed to support the survival of sensory neurons expressing both receptors, this turned out not to be the case: No changes were noted in the BDNF concentrations required to support the survival of neurons isolated from sensory ganglia of *Ngfr*-null mutants, unlike the case with NGF as indicated (218). This is somewhat surprising, as overexpression paradigms indicate that $p75^{NTR}$ associates with the three Trk receptors, an association that also significantly increases the selectivity of TrkB toward BDNF (220). In the absence of $p75^{NTR}$, TrkB can also be readily activated by NT3 and NT4 (220). More generally, predictions from biochemical experiments performed with $p75^{NTR}$ in reconstituted systems often failed to be confirmed in vivo in cells naturally expressing the receptor. However, $p75^{NTR}$ is clearly involved in both death-promoting and death-inhibiting activities. Among many other important signaling functions, it modulates the activity of Rho GTPases, which (for example) impacts the elongation of neuronal processes (see below and **FIGURE 11**). NGF, most likely in the form of pro-NGF (222), can actually cause cell death through $p75^{NTR}$ (223, 224). Related experiments later performed with $p75^{NTR}$-expressing sympathetic ganglia revealed that BDNF can also cause the death of cultured sympathetic neurons by $p75^{NTR}$-dependent mechanisms (225). In line with this, the number of sympathetic neurons is higher in early postnatal *Bdnf*- as well as *Ngfr*-null mutants (225), and there are various potential sources of BDNF during the formation of the sympathetic chain (226). The death of cultured sympathetic neurons promoted by pro-BDNF was later shown to also depend on the expression of sortilin, in addition to $p75^{NTR}$ (208), and sortilin can bind pro-BDNF as well as pro-NGF (227). Although the source of pro-BDNF in vivo remains uncertain (see sect. 3), it is possible that the nonneuronal cells expressing the *Bdnf* gene detected in the vicinity of the nascent sympathetic chain contribute to the adjustment of neuronal numbers during development by releasing pro-BDNF (226). This appears likely given that sympathetic neurons also express TrkB and that (mature) BDNF would be expected to counteract the effects of $p75^{NTR}$/sortilin signaling. $p75^{NTR}$-mediated effects of BDNF have also been noted in the development of the myelin sheath of the sciatic nerve (228) and to modestly contribute to the survival of sensory neurons. This mechanism involves the regulation of cholesterol metabolism in Schwann cells and the association of $p75^{NTR}$ with ErbB2 (229). In myelinating Schwann cells, $p75^{NTR}$ recruits and activates ErbB2 in response to BDNF, independently of ErbB3, thereby stimulating sterol regulatory element binding protein 2 (SREBP2) (229). BDNF and $p75^{NTR}$ also play a role in postnatal neurogenesis, and $p75^{NTR}$-expressing cells have been identified within the stem cell niche of the rat subventricular zone and shown to generate neurons, in both newborn and adult animals, and this

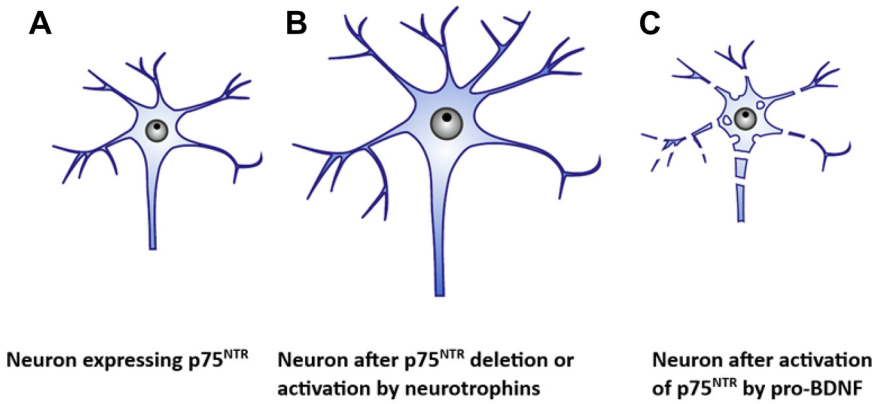


FIGURE 11. $p75^{NTR}$ and neuronal morphology. $p75^{NTR}$ expression is widespread early in development and readily detectable in many cells and tissues (*A*). It decreases postnatally in the central nervous system (CNS), correlating with increasing dendritic length and branching. Activation of $p75^{NTR}$ by neurotrophins typically increases branching, but so does its deletion (*B*) because of the inhibition of RhoA activation following the release of RhoGDI from the death domain of $p75^{NTR}$ (see text). Using slices of the visual cortex, Baho and colleagues (221) examined $p75^{NTR}$ expression in inhibitory, parvalbumin-positive neurons and observed that its downregulation in the postnatal CNS correlates with increased branching and innervation of target neurons. This study also showed that the addition of recombinant pro-brain-derived neurotrophic factor (BDNF) caused the death of $p75^{NTR}$-expressing neurons (*C*).

process is enhanced by the addition of BDNF or of NGF (230). Accordingly, precursor cells isolated from *Ngfr*-null mutants show a 70% reduction in their neurogenic potential in vitro and fail to respond to BDNF treatment (230). These observations are reminiscent of previous findings indicating that BDNF added to explant cultures of adult rat forebrain ependymal/subependymal zone allows the long terms of survival of newly generated neurons (231), an important observation that was later confirmed in vivo (232).

With regard to neurotransmission, $p75^{NTR}$ has been shown to modulate long-term depression (LTD) in the hippocampus and to regulate the expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits (233). In addition, exogeneous recombinant pro-BDNF facilitates LTD (234), but whether or not this reflects a physiological process is unclear. As discussed in sect. 3, the conditions leading to the release of pro-BDNF are unclear and subsequent work revealed that the induction of LTD is not affected in *Bdnf*−/− null mutants (130).

A neurotrophin/$p75^{NTR}$ pathway that has been investigated in considerable detail involves RhoGDI and RhoA, a well-known regulator of cytoskeleton dynamics (235). By virtue of its binding to the cytoplasmic domain of $p75^{NTR}$, RhoA was thought to be inactivated and to favor the formation/stabilization of actin filament (236). However, this association was later shown to be indirect and mediated by the binding of RhoGDI to $p75^{NTR}$ (237). In $p75^{NTR}$-expressing chick ciliary neurons that do not express Trk receptors and were reported early on to respond to NGF in vitro by a rapid outgrowth response (238), the binding of all neurotrophins enhances neurite elongation without promoting the survival of these neurons by virtue of RhoA inactivation following neurotrophin binding (236). Meanwhile, structural studies have detailed not only how RhoGDI interacts with the death domain of $p75^{NTR}$ (212) but also how this interaction competes with other effectors involved in NF-κB activation such as TRADD and RIP (207). In particular, RIP2 recruitment and RhoGDI release are linked, indicating that competition for DD binding underlies cross talk between NF-κB (239) and RhoA pathways in $p75^{NTR}$ signaling (207, 240).

It is conceivable that the physiological role of $p75^{NTR}$ in neurotrophin signaling has been underestimated thus far, especially in the adult nervous system as the expression of the receptor dramatically decreases during postnatal development. However, as more sensitive and specific methods are becoming more widely used, including fluorescence in situ hybridization and duo-link-related techniques, new roles may become apparent. For example, $p75^{NTR}$ expression has been convincingly documented in parvalbumin neurons in the postnatal mouse cerebral cortex (221). Its elimination increases perisomatic innervation (see **FIGURE 11**), in line with the notion that $p75^{NTR}$ may constitutively limit axonal branching through RhoA activation as discussed above (221). Although the addition of (cleavage resistant) pro-BDNF decreases perisomatic innervation in a $p75^{NTR}$-dependent manner, the same study also notes that endogenous pro-BDNF could not be detected by three commercially available antisera when staining specificity was assessed by Western blot (221). Still, it is conceivable that the presynaptic release of BDNF together with its associated pro-peptide may activate $p75^{NTR}$, thus mimicking the pharmacological observation that exogenous pro-BDNF decreases the perisomatic innervation of parvalbumin neurons in the postnatal cortex. As $p75^{NTR}$ has recently been suggested to be involved in the activity of some

widely used antidepressants, the topic of postnatal expression of $p75^{NTR}$ may gain further significance in the future, also because of its role in Aβ-mediated cell death (see sect. 11.3).

On the whole, it remains exceedingly difficult to fully understand and predict the role $p75^{NTR}$ may play in vivo. This is unlike with the Trk receptors, where in general the phenotype of animals lacking a Trk receptor and the related neurotrophin show noticeable similarities. With regard to the role of $p75^{NTR}$ in humans, the few genome-wide association studies (GWASs) that have been conducted have not been particularly productive thus far. Among 11 common *NGFR* single-nucleotide polymorphisms (SNPs) available at the time in the International HapMap Project, 5 were selected (rs2072445, rs2072446, rs734194, rs741072, and rs741073) and failed to reveal a significant association with AD (241). However, an association was later found between sporadic AD and polymorphism rs2072446 (see sect. 11.3).

4.3. The Tyrosine Kinase Receptor TrkB

TrkB was discovered by using a human protooncogene *trk* probe to screen a mouse brain cDNA library under low-stringency hybridization conditions (242). TrkB was then found to bind BDNF as well as NT4 with nanomolar affinities (for review, see Ref. 243). TrkB is encoded by the *Ntrk2* locus, and the corresponding null mutants show striking similarities with *Bdnf*-null mutants with regard to the loss of specific subpopulations of sensory neurons including those comprising the nodose and vestibular ganglia (244). Though not much work has been performed with *Ntrk2* hemizygotes (see example in sect. 6.2), their phenotype is typically significantly milder than *Bdnf* hemizygotes, in line with the general notion of the much higher expression levels of TrkB compared with BDNF. The reported loss of spinal cord and facial motoneurons indicated in the initial report (244), not seen with *Bdnf*-null or even in *Bdnf/Ntf4* double mutants (42), could not be confirmed in subsequent experiments (245).

The *NTRK2* gene encodes three main splice variants, with the longest including a tyrosine kinase domain showing a high degree of sequence identity (>80%) with TrkA and TrkC (243) and two variants with much shorter cytoplasmic domains designated TrkB.T1 and TrkB-Shc, the latter still containing a Y532 Shc binding site found in full-length TrkB. The ligand-mediated dimerization of TrkB causes a large number of transcriptional changes through well-delineated pathways (48, 246, 247), and some of the key intermediates are indicated in **FIGURE 12**. Detailed comparisons using cultured human neurons indicate that activations of TrkB by BDNF, NT4, and the TrkB activating antibody ZEB84 cause very similar transcriptional changes (250). Many of the transcriptional changes caused by TrkB activation involve genes known to activate *BDNF* transcription, explaining the positive feedback following TrkB activation in excitatory neurons (61). The mechanisms underlying this loop have been examined in detail with rat cortical neurons, and CREB family transcription factors were found to be required for the induction of all *Bdnf* transcripts (17). Reporter assays further showed that these mechanisms are conserved between rat and human (17).

The catalytic form of TrkB is primarily expressed by neurons, and, as is the case with BDNF, its expression markedly increases after birth in a number of species including humans (252). TrkB is transcribed from the *NTRK2* locus that spans ~400 kb in humans and is split into 24 exons potentially generating hundreds of mRNA isoforms. Estimates vary between 10 (253) and 36 (254) with regard to the number of different proteins likely to be encoded by these transcripts (for review, see Ref. 255). The main isoforms are full-length TrkB and TrkB.T1 depicted in **FIGURE 12**, with exon 16 spliced in instead of exon 17, the first exon of full-length TrkB. Exon 16 encodes an 11-amino acid peptide with identical sequences in human, rodents, and chicken (255). The generation of a mouse specifically lacking this truncated form of TrkB led to important functional insights as these mice are viable, unlike those lacking catalytic TrkB. Hippocampus-related functions seem to be unchanged, but an anxiety phenotype associated with morphological changes in the amygdala has been noted (256). In the brain, TrkB.T1 is mostly expressed by glial cells and especially by astrocytes (182), and the functional significance of this expression has been highlighted in the context of the regulation of energy and glucose homeostasis (Ref. 257; see sect. 9). TrkB.T1 expression is also widespread outside the nervous system including cardiomyocytes and pancreatic β cells (for review, see Ref. 255). In cardiomyocytes, BDNF binding to TrkB.T1 regulates the strength of contraction, an effect that is independent of the heart innervation. The selective removal of TrkB.T1 impairs calcium signaling and causes cardiomyopathy (258). The source of BDNF is the cardiomyocytes themselves, indicating an autocrine or paracrine function of BDNF, and the possibility that sensory endings may release BDNF remains to be investigated. Given recent evidence that there are also measurable levels of BDNF in mouse blood (see sect. 10), it is conceivable that TrkB.T1 may also be activated by BDNF originating from other sources including skeletal muscle as has been shown for the activation of TrkB.T1 expressed by pancreatic β-cells leading to the release of insulin (259). Beyond these few examples, the function of BDNF/TrkB signaling outside the nervous system has not received much attention. However, this may become a more

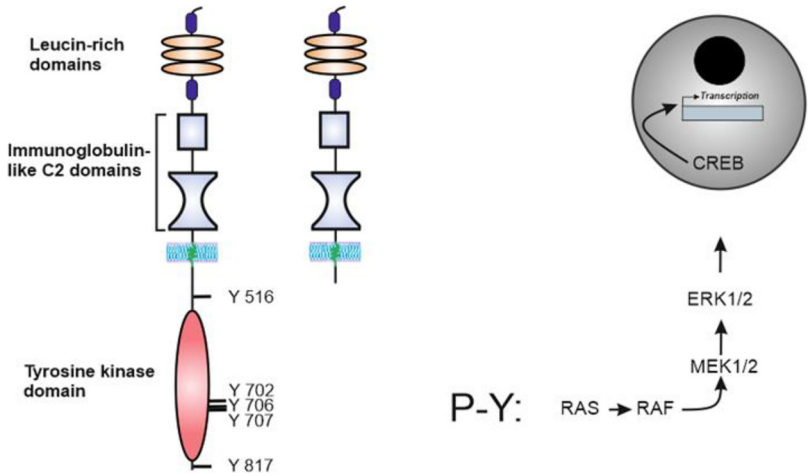


FIGURE 12. The tyrosine kinase receptor TrkB and its main splice variant T1. The full-length human TrkB receptor (*left*) is 822 amino acids long (https://www.uniprot.org/uniprotkb/Q16620/entry#sequences), and the positions of the tyrosine residues refer to the sequence of the human *NTRK2* gene. This gene is expressed by most central nervous system (CNS) neurons, both inhibitory and excitatory. Its splice variant T1 (*right*) lacking the kinase domain is mostly expressed by nonneuronal cells throughout the CNS, particularly on astrocytes, as well as on a number of cells outside the CNS (see text). Interestingly, TrkB.T1 also associates with RhoGDI like $p75^{NTR}$, an association impacting the morphology of cultured astrocytes in a brain-derived neurotrophic factor (BDNF)-dependent fashion (248). The extracellular domain of TrkB receptors consists of 2 cysteine-rich domains (purple) flanking 3 leucin-rich motifs (orange) and 2 immunoglobulin-like domains (blue), with the domain closer to the membrane thought to account for the binding of BDNF and NT4. High-resolution structural data are still lacking. As is the case with $p75^{NTR}$, the TrkB extracellular domain is heavily glycosylated and its apparent molecular mass on SDS gel is ~145 kDa compared with ~90 kDa predicted from the amino acid sequence. TrkB exposed at the surface of neuronal processes is part of a large group of proteins that are core glycosylated, i.e., carrying a pattern of immature sugar residues typically found in the Golgi apparatus (249). Activation of the kinase domain of Trk receptors is caused by ligand-induced dimerization. The figure also indicates critical tyrosine residues involved in the kinase activity, with some becoming docking sites for intracellular interactors after their phosphorylation. A cascade of protein interactions initiated by phosphorylation of some of the tyrosine residues ultimately leads to increased gene transcription (for review, see Refs. 48, 246, 247). As dimerization is the key feature triggering TrkB activation, antibody-mediated activation of TrkB is an attractive option also because of its selectivity, given that BDNF also activates the $p75^{NTR}$ pathway. A number of such reagents have been developed, with some replicating in human neurons the complex pattern of transcriptional changes caused by both BDNF and NT4 (250). Activation of TrkC also expressed by human neurons leads to similar changes when activated by NT3 (251).

prominent topic in the future given current interests in neuropeptides controlling insulin secretion, the regulation of food intake (260), and, more generally, the role of the nervous system in tissue repair and its interaction with the immune system (261).

As can be expected given their sequence relatedness, Trk receptors share many features. In particular, binding of the four neurotrophin dimers causes dimerization of TrkA, B, and C, thus facilitating transphosphorylation following activation of the kinase domains after ligand binding (for review, see Ref. 262). With immunoelectron microscopy and specific TrkB antibodies raised against its cytoplasmic domain coupled to horseradish peroxidase or decorated with gold particles, TrkB could be localized in the adult rat hippocampus to the sites of synaptic contacts of both excitatory and inhibitory neurons (263). Strikingly dense labeling in axon initial segments was also noted (263), and a number of observations indicate that the bulk of TrkB may not be exposed to the cell surface and requires activation of other receptors for the transport to occur (for review, see Ref. 264). A recent publication also indicates that calnexin, a lectin with a chaperone function, targets TrkB either to the cell surface or to autophagosomes for degradation (265). This does not seem to be the case to the same extent for TrkA and TrkC. Also, TrkB-mediated signaling is rapidly downregulated, compared with TrkA (210, 266), and TrkB and TrkA are differentially regulated by ubiquitination (267). This downregulation involves the cytoplasmic domain of TrkB and is not observed with TrkB.T1 (266). Downregulation was reported early on in cultured rat hippocampal neurons and found to be accompanied by a reduction of *Ntrk2* mRNA (268). These observations were complemented by similar in vivo observations (268). As discussed in sect. 10, the levels of TrkB, but not TrkB.T1 levels, have been reported to be decreased after exposure to Aβ (269).

Despite the functional and structural similarities between the Trk receptors, there are also differences beyond the mechanisms involved in their downregulation mentioned above. Perhaps the most surprising difference is that TrkB does not cause the death of neurons in the absence of ligands, whereas both TrkA and TrkC do (270). This difference has been observed in neurons derived from embryonic stem cells allowing expression of each of the three receptors under the exact same conditions from a neuron-specific promoter (270). The death induced by TrkA and TrkC expression

could be prevented by the addition of their respective ligands, just as has been observed with a number of previously identified dependence receptors (271). The use of the tetraploid complementation system allowed the generation of mouse embryos expressing each of the three Trk receptors from the same neuron-specific locus. Examination of these embryos revealed that at midgestation the entire nervous system of embryos expressing either TrkA or TrkC had disappeared, whereas the nervous system of TrkB-expressing embryos remained indistinguishable from controls (270). The mechanisms involving TrkA- and TrkC-induced neuronal death involve the proteolysis of $p75^{NTR}$ and can be prevented with a selective gamma secretase inhibitor (270). This TrkA- and TrkC-mediated death has also been observed in vivo, and neuronal death is reduced in the DRG of animals lacking both TrkC and NT3, compared with those lacking just NT3 (272).

5. SYNAPTIC TRANSMISSION

5.1. Introduction

BDNF accumulates in the presynaptic vesicles of excitatory neurons, and its neurotransmitter-like properties have attracted a great deal of attention. BDNF is regarded as a major effector of synaptic plasticity in the CNS, and, like many low-molecular-weight neurotransmitters, it also acts on presynaptic receptors, explaining its autocrine/paracrine trophic effects that have been reported both in vitro and in vivo.

5.2. Electrophysiology of Synaptic Transmission

The first report of a fast action of BDNF on synaptic transmission was at the developing neuromuscular junction (273). These initial observations were made in vitro with neural tubes and myocytes dissociated from *Xenopus laevis* embryos. They generated a great deal of interest as they indicated that neurotrophins may be involved in rapid, physiologically relevant phenomena, beyond traditional trophic activities such as the promotion of neuronal survival and of axon elongation. These observations were later extended to the mammalian CNS, and perfusion of slices of young adult rat hippocampus with BDNF or NT3 was found to enhance synaptic transmission within minutes at the Schaffer collateral-CA1 synapses (274). These effects were later found to critically depend on parameters such as the speed of perfusion of the slices (275), a complication possibly related to the rapid desensitization of TrkB and/or its disappearance from the cell surface following BDNF exposure (210). This may explain why others initially found the effects of BDNF on excitatory transmission in the hippocampus difficult to reproduce (276). With the use of slices of the hippocampus and double-barreled pipettes filled with either BDNF or glutamate, BDNF was later reported to cause depolarization of the neurons within the same milliseconds time frame as glutamate (277). Heterologous systems were then used to demonstrate that these very rapid effects could be recapitulated by the coexpression in HEK293 cells of TrkB and the sodium channel $Na_V1.9$ (278). Perhaps even more surprising at the time, binding of BDNF to truncated TrkB expressed by astrocytes was shown to trigger within a few seconds the release of calcium from intracellular stores (279). Unfortunately, the significance of these observations, i.e., the neurotrophin-induced depolarization of TrkB-expressing cells within milliseconds, is still difficult to evaluate. As pointed out by some of the initial contributors, these observations all originated from one group of investigators (see corresponding comment in Ref. 280) and there have been no convincing explanations yet as to why they could not be replicated and extended by others. Still, rapid or very rapid actions of BDNF remain a possibility of great potential significance, given the well-documented presence of full-length TrkB not only on postsynaptic sites but also on presynaptic nerve terminals in the CNS (281). More recently, the activation of truncated TrkB by BDNF has also been reported to cause within seconds rapid calcium transients both in cardiomyocytes (258) and in pancreatic β-cells (259). In this latter case, BDNF derived from the skeletal musculature was also shown to trigger insulin release, pointing to a novel and potentially significant role of BDNF in glucose homeostasis (259).

With regard to the role of BDNF in the physiology of synaptic transmission, a large number of experiments have been performed, initially mostly with neurons dissociated from the hippocampus. Although there is little doubt that the addition of BDNF or its elimination with scavenging agents impacts neurotransmission, it does so in so many different ways that it is difficult to distill a consistent picture (see for example Ref. 282). Part of the complexity stems from the fact that BDNF impacts both excitatory as well as inhibitory neurotransmission (282) and that the degree of maturation of the culture also plays a role. Perhaps the most widely recognized role of BDNF is its requirement for the long-term potentiation (LTP) of synaptic transmission, first recorded in postnatal hippocampal slices at CA1 synapses following high-frequency stimulation of hippocampal Schaffer collateral (283). Crucially in the context of the central notion of BDNF's limited availability, this requirement was also noted in slices prepared from mice lacking just one allele of *Bdnf* (283). These results were replicated and extended in a number of subsequent studies (284, 285),

including in the intact adult hippocampus (286) and in brain areas other than the hippocampus including the visual cortex (287). An immediate requirement for BDNF after stimulation of the Schaffer collateral was suggested by the uncaging of a BDNF blocking antibody during the process of LTP induction (288). A correlative study later suggested that mice lacking tissue plasminogen activator (TPA) show defects in LTP and that the addition of the protease plasmin restores LTP (289). However, the interpretation that LTP is restored because of the cleavage of pro-BDNF released from neurons triggered by repeated bursts of presynaptic stimulation critically depends on the assumption that pro-BDNF is released in the first place (289). As discussed in sect. 3, the notion of a physiological release of pro-BDNF from neurons is controversial, and the four publications cited by Pang et al. (289) in support of this view are all based on overexpression paradigms. With regard to the important question of the site of release of BDNF, the results of selective genetic deletion studies suggest a presynaptic, as opposed to postsynaptic, requirement for BDNF (290). By contrast, subsequent studies suggested a postsynaptic requirement of newly synthesized (291) or released BDNF (177). However, the study by Tanaka and colleagues (291) does not explicitly show that it is BDNF that is translated in postsynaptic dendrites, and the study by Harward and colleagues (177) suffers from the limitations of overexpression paradigms and the use of a BDNF probe directly coupled to a fluorescent reporter (see sect. 3). In a similar vein, the notion that *Bdnf* mRNA may be localized in, and even translated in, dendrites is based on studies using overexpressed reporter constructs and/or on staining methods based on enzymatic reactions, with the problem inherent to such methods of the diffusion of the reaction product from the neuronal cell bodies into dendrites (292). High-resolution, fluorescence-based in situ hybridization revealed instead that both the short and the long *Bdnf* transcripts are primarily localized to the cell bodies, not in distal dendrites, unlike the case for the CAMKII mRNA, long established to localize to dendrites (92). Furthermore, detailed studies based on the differential localization of actively translating mRNA in CA1, the most-studied brain region with regard to the physiology of BDNF, localized the corresponding ribosomes to the cell bodies, not to the dendrites (179). This key publication also contains an informative map indicating that *Bdnf* is among the transcripts translated at comparatively low levels and in the cell bodies of CA1 neurons, not in dendrites (see https://public.brain.mpg.de/dashapps/localseq/).

With regard to long-term depression (LTD), a role has been proposed for pro-BDNF mediated by $p75^{NTR}$ (234). However, this notion has been challenged, with subsequent studies failing to detect the release of pro-BDNF by cultured hippocampal neurons (see sect. 3), also showing that LTD is unimpaired in neurons lacking BDNF (130). In addition, the induction of LTD has been shown to be counteracted by BDNF through the activation of TrkB and the PLCγ pathway (293). The notion that BDNF is involved in NMDA receptor-dependent hippocampal LTD was reactivated a decade later after the unexpected observation that the addition of the BDNF pro-peptide to hippocampal slices prepared from wild type or from *Bdnf*-null mutants facilitates LTD in a $p75^{NTR}$-dependent manner (294). The same study also indicated that the BDNF pro-peptide can be detected in the culture medium of hippocampal neurons, confirming previous results (153). Remarkably, the Met version of the BDNF pro-peptide was shown instead to prevent the induction of LTD (294). This is surprising in view of the stronger and less pH-sensitive association between BDNF and the Met pro-peptide compared with the Val pro-peptide (131). Meanwhile, other activities have been proposed for the Met pro-peptide (see sect. 6), including a disassembly of dendritic spines (153).

5.3. BDNF Release from Neurons

Given the technical challenges involved, the important question of the release of endogenous BDNF from neurons has only been addressed by a small number of studies. Using rat neurons dissociated from the nodose ganglia, Balkowiec and Katz (295) managed to quantify the release of endogenous BDNF by ELISA by plating dissociated neurons onto microwells precoated with BDNF antibodies. They found that high- as opposed to low-frequency bursts of stimulation were effective in triggering BDNF release. Specifically, short pulses of depolarizing stimuli delivered at 50 Hz every 18 s over a period of 60 min were effective, whereas the same number of stimuli delivered at 20 Hz over the same period of time did not trigger any BDNF release (295). Similar studies were later conducted with neurons dissociated from the newborn rat hippocampus. The results confirmed that high-frequency patterns of stimulation known to induce hippocampal LTP were significantly more effective at releasing BDNF from hippocampal neurons than low-frequency stimulation (296). BDNF release was observed in the presence of the glutamate receptor antagonists CNQX and APV but not of tetrodotoxin or conotoxin or in the absence of external calcium (296). The conditions leading to the release of BDNF from presynaptic terminals have also been examined in sections of the rat spinal cord with dorsal roots attached (297). This landmark study revealed that activating C fibers with short bursts of high-frequency stimulation, as opposed to continuous or even tetanic high-frequency stimulation, led to the release of BDNF as well as of substance P (297). Remarkably, the NMDA

antagonist D-AP-5 inhibited electrically evoked BDNF release that was also increased after NGF administration (298). Similarly, presynaptic NMDA receptors were later shown to be involved in the release of endogenous BDNF from cortical afferents to the striatum (299). Using slices of rat perirhinal cortex under conditions also allowing LTP induction to be monitored, Aicardi and colleagues (300) managed to demonstrate that measurable levels of BDNF were released for short periods of time (5–12 min), after afferent stimulation by theta burst inducing long-lasting LTP, in a BDNF-dependent manner. Although the processing of pro-BDNF has not been specifically studied in DRG, it appears likely that BDNF is processed and cleaved in the neuronal cell bodies as is the case in CNS neurons and that both BDNF and its pro-peptide are coreleased in the spinal cord, presumably by peripheral sensory endings as well. How long BDNF remains associated with its pro-peptide after their corelease from presynaptic vesicles is unclear yet likely to be functionally relevant. The affinity of this association has been determined by plasmon surface resonance using solid-phase bound pro-peptide and soluble BDNF, with the dissociation constant determined to be ~40 nM and barely measurable when NGF, NT3, or NT4 were used in the mobile phase instead of BDNF (131). This affinity was found to be sufficient to allow the precipitation of the soluble moiety with the solid-phase bound partner (131). Remarkably, the solid-phase bound Met pro-BDNF peptide was determined to have higher affinity for BDNF, a result of a slower rate of dissociation, and its association with BDNF to be less pH sensitive than the Val pro-peptide. It is conceivable then that the association of BDNF with its pro-peptide impacts BDNF's ability to activate its receptors, potentially more so in the case of the Met pro-peptide (131). Although it will be technically challenging to further explore the duration of the postrelease association of BDNF with its pro-peptide, a better understanding of this question may shed new light on the physiopathology of BDNF. Specifically, it is conceivable that BDNF associated with its cleaved Met pro-peptide may have a reduced ability to activate TrkB.

With regard to the physiological impact of BDNF release in the spinal cord, a study using a preparation allowing measurements of monosynaptic and polysynaptic reflexes evoked by primary afferents revealed that *Bdnf*- unlike *Ntf4*-null mutants showed selective deficits in ventral root potentials evoked by stimulating nociceptive primary afferents (301). These results indicate an important role for the presynaptic release of BDNF in the spinal cord in modulating synaptic transmission and the modulation of pain-related mechanisms (see sect. 7).

5.4. *Postsynaptic Changes Involving Translational Regulation*

BDNF-mediated enhancement of synaptic transmission was recognized early on to require local protein synthesis (302). Synaptic transmission was recorded in CA1 with the cell bodies separated from the dendritic arbor where postsynaptic responses were recorded after stimulation of the Schaffer collaterals (302). CA3 afferents were also severed from their cell bodies, and after a 30-min bath application of BDNF synaptic transmission reached twice the initial level within ~10 min and lasted for the whole 200-min recording period (302). Similar results were obtained after bath application of NT3. This potentiation of synaptic transmission was blocked by the prior addition of anisomycin or cycloheximide, indicating a requirement for protein translation for the potentiation of synaptic transmission by BDNF and NT3 (302). BDNF was later shown to stimulate mRNA translation in dendrites by mechanisms involving the mammalian target of rapamycin (mTOR) pathway downstream of TrkB activation (303). This pathway included the initiation factor 4E binding protein 1 (4EBP1) and p70S6 kinase (p70S6K) (303).

In cultured hippocampal neurons, the addition of BDNF increases the levels for GluR1 and GluR2 subunits and cell surface expression of GluR1 within 30 min by mechanisms involving both increased transcription and translation (304). Conversely, the neutralization of extracellular BDNF with TrkB-IgG selectively decreased GluR1 (304). Importantly, positive allosteric modulators of AMPA receptors designated ampakines (for a recent review, see Ref. 305) can pharmacologically activate this pathway by mechanisms involving the activation of TrkB as a result of increased BDNF secretion caused by the drug (306).

BDNF-induced translation in dendrites necessitates mRNA transport into dendrites driven by kinesin motors and microtubules (for reviews, see Refs. 307–310). Obviously, transport of mRNAs also occurs in axons (for review, see Ref. 311), in RNA granules with the transcripts interacting with specific proteins that help stabilizing mRNAs encoding proteins typically present in dendrites such as CAMKII and Arc (312) as well as NMDA receptor subunits and the scaffolding protein Homer2 (313). The RNA-binding protein hnRNP K (for heterogeneous nuclear ribonucleoprotein K) has been identified as one of several components of RNA transport granules in neurons (314), and although the speed of the granules remained unchanged by activity or by the application of BDNF, the latter, but not activity, was found to increase the proportion of moving granules (314). Interestingly, hnRNP K interacts with Abelson-interacting protein 1 (Abi1) at postsynaptic densities of hippocampal

neurons. Abi1 has been proposed to play a role in cytoskeletal organization and synaptic maturation and in the regulation of cytoskeleton reorganization and synaptic maturation (315). Given this context, it is interesting to note that hnRNP K has been identified as a mediator of the effects of BDNF in RNA metabolism in the dendritic compartment of hippocampal neurons (316). Specifically, the punctate accumulation of hnRNP K in dendrites caused by neuronal activity was found to involve a BDNF-dependent mechanism, including a reduction of the interaction of hnRNP K with transcripts encoding synaptic proteins, including glutamate receptor subunits (316).

Given the later realization that BDNF also activates the translation of mRNAs not only in postsynaptic structures but also, to a lesser extent, in excitatory and inhibitory presynaptic terminals (317), the BDNF-mediated potentiation of synaptic transmission is likely to be caused by increased translation in both pre- as well as postsynaptic mRNAs. In particular, high-resolution microscopy has revealed that the addition of BDNF to cultured motoneurons triggers the translocation of ribosomes to ER-like structures (318). The potentiation of synaptic transmission has been typically recorded after the application of BDNF to the rat dentate gyrus (286, 319), and although these results make a compelling case for a postsynaptic activation of TrkB by exogenous BDNF, there is also a strong possibility that presynaptic TrkB may also be required to trigger functionally relevant presynaptic changes leading to an enhancement of synaptic transmission. In line with this, the use of mice expressing reduced levels of presynaptic TrkB revealed a decreased ability of tetanic stimulation to induce LTP (320).

The consolidation of long-term potentiation (LTP) in the dentate gyrus of live rodents requires sustained BDNF/TrkB signaling, a result achieved by maintaining an initially labile signaling pathway from TrkB to the MAP kinase-interacting kinase (MNK) promoting the formation of an eukaryotic initiation factor 4F (eIF4F) translation initiation complex, a highly regulated step in protein synthesis in eukaryotes (321). Early in the process of mRNA translation, MNK triggers the release of the cytoplasmic fragile-X mental retardation protein (FMRP)-interacting protein CYFIP1/FMRP repressor complex from the 5′-mRNA cap (321), whereas later in the cascade MNK regulates the canonical translational repressor 4E-BP2 in a synapse compartment-specific manner. This later stage is coupled to MNK-dependent enhanced dendritic mRNA translation (321). A remarkable aspect of these biochemical studies is that they have been conducted with the tissue used for electrophysiological experiments, with synaptoneurosomes isolated from the dentate gyrus (321). With regard to the nature of the protein translated in dendrites after BDNF treatment, much attention has focused on the activity-regulated cytoskeleton-associated protein Arc that is translated from a short-lived mRNA (for review, see Ref. 322). Briefly, this intriguing gene product (also designated Arg3.1) is thought to be critical for both long-term potentiation and long-term depression of synaptic transmission as well as for homeostatic synaptic scaling and for complex adaptive functions including long-term memory formation (322). It is not only rapidly induced in structures such as the hippocampus during learning paradigms but also is rapidly transported to dendrites (323, 324). The selective localization of *Arc* mRNA at active synapses in dendrites was later found to be in part due to localized mechanisms of RNA degradation, also found to be activity dependent (325). *ARC* is among the genes most rapidly induced after TrkB activation by BDNF and other TrkB agonists in cultured human neurons (250) and of TrkC by NT3 (251). Among the reasons why Arc is a protein of such interest in the context of synaptic plasticity is that it interacts with components of the clathrin-mediated endocytosis machinery, thus promoting postsynaptic internalization of AMPA-type glutamate receptors and recruitment to recycling endosomes (reviewed in Ref. 322) as well as with actin-binding proteins, underlying its role in morphological aspects of synaptic plasticity known to involve changes in the structure of spines (see sect. 5.6). Arc has been described as a repurposed retrotransposon gag protein (326), and it can readily form polymers. It is also the target of various posttranslational modifications, explaining its short half-life (322). With regard to the pathways of translational control involving BDNF, increased translation of a protein designated GADD34 has been shown to facilitate eukaryotic initiation factor 2α (eIF2α) dephosphorylation and subsequent de novo protein synthesis (327). GADD34 is also of special interest with regard to cytoskeletal changes as it requires G-actin generated by cofilin to dephosphorylate eIF2α to enhance protein synthesis (327).

5.5. *The Potassium/Chloride Exchanger KCC2 as a Major Target of TrkB Activation*

During the development of the nervous system, the upregulation of KCC2 explains the switch of the GABA receptor from a depolarizing to the familiar hyperpolarizing response seen in the mature nervous system (328). KCC2 is also a remarkable gene product interacting with a number of different proteins and is a potential drug target (329). Its developmental upregulation is mediated by early growth factor response 4 (Egr4), a DNA binding protein that is strongly and rapidly induced downstream of TrkB activation in immature neurons (330). A massive but transient increase in the levels of *EGR4* has also

been observed in subsequent studies with cultured human neurons downstream of TrkB and TrkC activation (250, 251). The mechanisms underlying the transcriptional upregulation of the major transcript *Kcc2b* in cultured cortical neurons involve two distinct RE-1 sequences, the target of REST, identified as the critical elements regulating *Kcc2b* transcription (331). BDNF potentiates the developmental upregulation of *Kcc2b* in this culture system (331). By contrast, TrkB activation in mature neurons leads to the opposite result, namely a rapid downregulation of KCC2 (332). Membrane-inserted KCC2 intrinsically turns over rapidly, and its downregulation decreases, suppresses, or even inverts the inhibitory responses normally mediated by GABA receptors (332) because of the decreased ability of neurons to extrude chloride ions (333). This property of KCC2 is of special relevance to pathological conditions linked with hyperexcitability of neuronal circuits in neuropathic pain (see below) and in epilepsy (334). A brain-specific serine/threonine kinase designated LMTK3 targets KCC2 and decreases chloride extrusion (335). KCC2 also interacts with cytoskeletal proteins to promote spine development. This latter function seems to be independent of its function as ion transporter (336), and in the absence of KCC2 spine maturation is prevented in immature neurons (336).

5.6. Cell Biology of Synaptic Plasticity

Spines are the main sites of contact between excitatory nerve terminals and postsynaptic structures and have long been recognized as "the locus of structural and functional plasticity" (for review, see Ref. 337). Spine growth during LTP has been well documented (338, 339), and multiple biochemical mechanisms have been described underlying the local effects of BDNF on spine and dendritic growth. BDNF has been shown to enhance the number of densely phalloidin-labeled spines caused by theta burst stimulation (340). Conversely, scavenging BDNF with TrkB-Fc constructs blocked the increase in F-actin typically seen after theta burst stimulation (340). The actin regulatory proteins p21 activated kinase and cofilin are involved in the BDNF-mediated effects on the state of actin polymerization (340). With a method allowing the local uncaging of glutamate, the gradual spine enlargement of CA1 pyramidal neurons has been shown to be dependent both on protein synthesis and on BDNF (291). Some of the mechanisms involved in the regulation of spine morphology are mediated by microRNAs such as miR-134. It is localized in dendrites and has been shown to negatively regulate the size of dendritic spines by inhibiting the translation of Limk1, a kinase involved in regulating actin filament dynamics (341). MicroRNAs are generated by the sequential processing of precursors, with the last processing step involving the endonuclease Dicer as part of a protein complex redistributed after exposure to BDNF (342). The release of Dicer from this complex prevents the generation of microRNAs, thus inhibiting dendritic growth. It is conceivable that the redistribution of Dicer after TrkB activation following the presynaptic release of BDNF may increase translation of Limk1 and contribute to the dendritic growth and the generation of new spines (342). However, the role of BDNF/TrkB signaling in the biochemistry and cell biology of microRNA distribution and generation is complex and somewhat controversial (343). Refined imaging techniques allowing localization of the protein complex involved in the maturation of microRNAs will greatly help progress in the critical area of local control of spine growth (344). This area is likely to develop further given intriguing recent findings suggesting that microRNAs might be contained in extracellular vesicles transferred from postsynaptic neurons to their presynaptic partners (345), with BDNF reported to mediate the sorting of three different microRNAs that increase the formation of excitatory synapses (345). To study a possible morphogenetic role of BDNF in the brain of early adult mice once its expression has reached its peak, viable mice were generated with a Cre driver, *Mapt*, allowing the deletion of *Bdnf* in all neurons, as opposed to the nonneuronal cells (41). When examined postnatally at 2 mo, a dramatic reduction of spine numbers was found on the dendrites of medium spiny neurons in the striatum as well as shorter and less branched dendrites, replicating previous results obtained with a Cre driver line, *Emx2*, deleting *Bdnf* just in the cerebral cortex (40), further emphasizing the significance of anterogradely transported BDNF. An important related study also revealed that the transport of TrkB at the cell surface of striatal neurons is facilitated by dopamine through the activation of DRD1 receptors (346). This result is relevant to the physiopathology of Parkinson's disease (see sect. 11), as increased numbers of intracellular aggregates of TrkB were found in postmortem samples (346).

With regard to spine growth induced by BDNF, intriguing differences have been noted between excitatory and inhibitory neurons. In general, it seems that spine shape and/or numbers and dendritic length and branching are more affected in GABAergic neurons compared with excitatory neurons (see in particular Ref. 41). This is line with previous observations using mixed cultures of *Bdnf*+/+ and *Bdnf*−/− neurons distinguished on the basis of GFP expression or lack thereof in neurons from *Bdnf* mutant animals (347). A possible contributing factor is a chronic desensitization of TrkB in BDNF-expressing excitatory as opposed to inhibitory neurons that do not express BDNF at significant levels. In general, the morphological effects of BDNF added to cultures of excitatory neurons are quite modest and variable (348). But however modest, these effects are clearly measurable,

and the notion of autocrine or paracrine effects of BDNF on neuronal morphology is supported by the results of elegant in vivo experiments using a sparse *Bdnf* deletion approach (84). This finding also has important implications for the interpretation of results involving Cre-mediated deletion of *Bdnf* in slices for example, as this maneuver is likely to alter the postsynaptic structure of the contacts being investigated (177). BDNF has also been uncovered as a regulator of autophagy, a process involving the degradation of synaptic proteins (349). Specifically, the activation of TrkB suppresses autophagy in the hippocampus, and this suppression is essential for BDNF-induced synaptic plasticity in the hippocampus of adult mice (349). Increased autophagy is thus part of the explanation as to why LTP is compromised in the absence of BDNF and, accordingly, it can be rescued by pharmacologically blocking autophagy (349). In a follow-up study, hippocampal LTD was also shown to involve autophagy and the resulting degradation of postsynaptic components (350).

6. LEARNING AND MEMORY

6.1. Introduction

A one-page contribution published as "Scientific correspondence" first reported increased levels of *Bdnf* mRNA in the brain of adult rats voluntarily using running wheels (351). The same year, a publication came out indicating that hippocampal LTP is compromised in mice lacking one *Bdnf* allele (Ref. 283; see sect. 5). Subsequently, the role of BDNF in memory-related processes became a major area of investigation, further enhanced 8 years later with the discovery of a human polymorphism associated with a somewhat compromised memory performance (352).

6.2. Learning: Rodent Models

Given the notion that BDNF levels are regulated by the activity of excitatory neurons, increased transcription of the *BDNF* gene can be expected in the circuits that are active during learning processes. This notion has been experimentally verified in rodents in the well-established paradigm of the hidden platform that the animal learns to localize using visual cues (353). With this paradigm, *Bdnf* mRNA levels were shown to increase in the hippocampus after 3 and 6 days of spatial memory training but not after 1 day (354). Interestingly, the control group of rats swimming in the absence of platform showed a smaller increase in the hippocampus, with *Bdnf* levels remaining essentially unchanged in other brain areas (354). With regard to the important notion that exercise may help decreasing the impact of depressive episodes (see sect. 8), a study using rats and a combination of physical activity and antidepressant treatment reported additive effects on the levels of *Bdnf* mRNA in the hippocampal formation (355). Related results were reported in an important follow-up study centered on contextual learning, a well-established paradigm in which rats are exposed to an environment where they have previously received a foot shock (356). This study revealed a marked increase in the levels of *Bdnf* mRNA in the hippocampus, above and beyond the increase normally seen by just exposing rats to a novel environment without foot shock (356). This latter type of maneuver is sufficient to increase the expression of genes just reflecting increased neuronal activity such as for example *Zif268* (356). With regard to the use of mouse mutants in classical learning paradigms, *Bdnf* hemizygotes were used early on to explore their ability to locate a platform in the Morris water maze test (357). Although the observed deficits compared with control mice were not overwhelming and not replicated in a related study (358), the study by Linnarsson and colleagues (357) is also interesting as it reported a marked decrease of *Bdnf* expression with age throughout the cortex including the hippocampal formation, to the extent that even wild-type animals were unable to learn the location of the platform. Specifically, brain levels of *Bdnf* mRNA at 10 wk of age were reduced compared with those determined in 7-wk-old animals, to the levels typically measured in mice lacking one *Bdnf* allele (357). Subsequent studies using a conditional deletion of TrkB in the forebrain revealed massive deficits in the same Morris water maze paradigm, while the behavior of these animals was otherwise normal (359). Whether or not the discrepancies observed in related behavioral tests between mice lacking one *Bdnf* allele and those carrying a forebrain-specific deletion of TrkB may be due to unchanged levels of NT4 potentially activating TrkB is unclear. A further interesting aspect of the study by Minichiello and colleagues (359) is that mice lacking just one allele of *Ntrk2* did not show any deficits in their ability to locate the platform, suggesting that the levels of TrkB are not limiting, unlike those of *Bdnf*. In a similar vein, LTP induced by either theta burst or tetanic stimulation of the Schaffer collateral was less compromised in hemizygote *Ntrk2* mice compared with conditional *Ntrk2* animals, in which TrkB levels are reduced to almost background levels in the forebrain (359). The lack of significant abnormalities in hemizygote *Ntrk2* animals is thus in marked contrast with those obtained with *Bdnf* hemizygotes, despite the continued expression of NT4 in the latter animals. These results fit with the general notion that TrkB levels are not limiting. Subsequent studies showed that exercise prevents the decrease of BDNF levels observed following a 2-h period of immobilization stress, with the

level of stress monitored by measuring the levels of circulating corticosteroids (360). Measurements of BDNF levels by ELISA in hippocampal extracts indicated a marked decrease after 5 and 10 h followed by a recovery after 24 h. Interestingly, voluntary access to a running wheel before immobilization stress not only increased the levels of BDNF but also prevented the decrease normally seen 10 h after stress (360). The timing of the benefits of exercise on memory has been further explored in mice after periods of voluntary exercise (361). After a 3-wk period of exercise, mice were either assessed immediately or after a 1- or 2-wk delay for their performance in a radial water maze. The fewest errors and shortest latency occurred in animals trained after a 1-wk delay, whereas the best memory performance was observed in those trained immediately after exercise (361). Injections of BDNF into the rat hippocampus were later shown to improve performance in spatial memory tests (362), thus confirming previous results indicating that the delivery of anti-BDNF antibodies into the lateral ventricles impairs spatial learning and memory in adult rats (363).

Although the main focus of memory-related investigations has been on the hippocampus, the role of BDNF and TrkB has also been studied in the amygdala (364). *Bdnf* levels were examined by in situ hybridization in the basolateral amygdala and found to increase during the course of the Pavlovian fear conditioning paradigm used, whereas the levels of *Ngf*, *Ntf3*, *Ntf4*, *Fgf1*, and *Fgf2* remained unchanged. The activation of TrkB monitored by Western blot was shown to be required for the acquisition of conditioned fear, as this acquisition was blocked by the lentivirus-mediated expression of TrkB.T1 (364).

6.3. Memory and the BDNF Val/Met Polymorphism

The accidental but critical discovery of the association between a frequent polymorphism in the protein-coding region of BDNF and memory performance in humans (352) generated an extraordinary volume of follow-up research. This missense mutation, designated rs6265, causes a replacement of valine (Val) by methionine (Met) in position 66 in the BDNF precursor human pro-BDNF (see **FIGURE 2**). Although it was found not to be associated with schizophrenia as initially speculated, mild memory impairments were detected in the assembled cohort (352). Using functional (f)MRI, this key initial study also reported abnormal hippocampal activation in Met carriers (352). The association with modest impairments of episodic memory was later independently confirmed (365) and further extended in a number of studies, including one reporting a 11% reduction in the volume of the hippocampus in Met carriers in a study involving 36 male and female volunteers and 15 Val/Met heterozygotes (366). A crucial next step was to establish causality with animal models. This was important not least because the frequency of the Met66 allele in humans varies considerably across populations, from 0 to 72% (367). The Met allele is virtually absent in sub-Saharan African and some American indigenous populations but dominant in various Asian ethnic groups (367). In addition, there are three additional protein-encoding genes, designated *LIN7C*, *LGR4*, and *CCDC34*, within the 500-kb region on chromosome 11 that include the *BDNF* gene (367), itself spanning ~70 kb (see sect. 2). By and large the work with animal models supports the notion that the polymorphism may be symptomatic and associated with functional changes in the human brain.

The first mouse model that was generated was reported to display an anxiety type of phenotype rather than overt memory deficits, a phenotype that could not be ameliorated by the administration of the antidepressant fluoxetine (368). As the extinction of conditioned fear is known to depend on the formation of new memory in the prefrontal cortex (369), it was then of interest to test whether BDNF may be involved in this specific form of memory. This turned out to be the case, as revealed in a gain-of-function approach: the infusion of BDNF in the medial prefrontal cortex of rats subjected to fear conditioning markedly reduces the duration of conditioned fear and substitutes for behavioral extinction (370). In line with this, the subpopulation of rats unable to learn extinction turned out to have reduced levels of BDNF in the hippocampus, but not in the prefrontal cortex or the amygdala (370). Strikingly, the mouse model of the rs6265 polymorphism reveals similarities with what was later observed in human Met carriers (371). Although the Met allele does not affect the process of fear conditioning in either mice or humans, both show an impairment in extinguishing the conditioned fear response and an atypical fronto-amygdala activity (371). These results suggest then that BDNF also plays a role in anxiety disorders, a notion supported by subsequent findings indicating that in individuals suffering from posttraumatic stress disorder (PTSD) the frequency of the Met/Met genotype is three times higher than in a control group with a similar history of stress exposure (372). A study focused on PTSD also revealed that the presence of a Met allele (25 patients) led to a poorer response to therapy compared with patients also suffering from PTSD carrying the Val alleles (30 patients) (373).

A number of different explanations have been put forward to account for the biochemical impact of the Val/Met substitution, including different subcellular distribution of BDNF and/or of its mRNA within neurons, reduced secretion, or decreased steady-state levels of BDNF in the hippocampus with no overall changes in

the brain (352, 368, 374, 375). None of these explanations is fully convincing, and most suffer from the familiar shortcomings resulting from the low abundance of BDNF and especially from the extensive use of overexpression and transfection paradigms discussed in sect. 3. Still, reduced availability of BDNF, however it comes about, is a common thread as also suggested by findings indicating that Met-BDNF mice gain weight abnormally when placed on a high-fat diet (376). Subsequent to the initial mouse model still used in most experiments (368), additional rodent models have been generated in both mice (377) and rats (378), the latter showing a selective impairment of fear memory. Measurements of BDNF levels in hippocampal lysates of these rats indicate no differences between Val/Val and Met/Met homozygote animals, in males or in females (378).

In sum then, the biochemical and cell biological consequences of the rs6265 polymorphism remain largely unclear and likely to be subtle and/or brain area specific. Part of the explanation may include a signaling function of the Met pro-peptide coreleased with BDNF as discussed in sect. 3. The Met pro-peptide causes growth cone collapse (153) and retraction of dendritic spines by mechanisms involving Sorcs2 and $p75^{NTR}$ (154). The tighter binding of the Met pro-peptide to BDNF may also play a role (131), and it is conceivable that this tighter association may impair TrkB activation.

Beyond the association between the rs6265 polymorphism and specific aspects of memory, another stunning finding related to this human polymorphism helps to highlight less well-known aspects of BDNF's biology, namely its role in CNS myelination (see sect. 6.4). A longitudinal study involving >300 amateur soccer players who self-reported how frequently they had hit the ball with their heads during the preceding 12-mo period showed signs of delayed remyelination by diffusion tensor imaging compared with Val/Val carriers (379). The association between "habitual" physical activity and brain volume has also been compared in 114 cognitively healthy women and men aged 60 yr and older and compared in Val/Val versus Val/Met polymorphism carriers (380). In Val/Val homozygotes, but not in Met carriers, higher levels of physical activity were associated with larger hippocampal and temporal lobes.

6.4. BDNF and CNS Myelination

Early studies aimed at understanding role of BDNF in the development of retinal ganglion cells in vivo, known by then to be supported by BDNF in cell culture (46), unexpectedly revealed a strong deficit in the postnatal myelination of the optic nerve (381). Whereas the number of axons was unchanged compared to wild-type animals, their diameter was significantly reduced when examined at 3 wk (381). As recently reviewed (162), it took a comparatively long time to understand the role of BDNF in myelination, especially in the CNS. This is in part because of the technical challenges linked with in vitro studies of CNS myelination but also because of a later publication indicating that myelination of the optic nerve was unaffected at 2 mo in mice engineered to lack *Bdnf* in neurons (41). However, myelination is a dynamic process, and parallel studies published at the same time (382) revealed that mice lacking one *Bdnf* allele had reduced levels of myelin basic protein, myelin-associated glycoprotein, and proteolipid protein (383) and that BDNF promotes myelination by a direct action on oligodendrocytes involving TrkB and not p75NTR as is the case in the PNS (see sect. 4). Hemizygous *Bdnf* animals do show transient defects in the development of the myelin sheath surrounding CNS axons that are no longer apparent in the adult (382). Oligodendrocyte precursors express catalytically active TrkB, and its activation by axon-derived BDNF is then likely to play a significant role in activity-dependent myelination (183, 384).

7. PAIN

7.1. Introduction

NGF-responsive DRG neurons have long been known to be involved in pain-related mechanisms (for review, see Ref. 385). NGF neutralizing antibodies are being trialed to treat chronic conditions such as back pain where prolonged treatment with opioids is problematic. As discussed in this section, BDNF is also involved in multiple ways in pain-related mechanisms and has been recently shown to play a key role in the analgesic action of morphine (386).

7.2. Physiopathology of BDNF-Dependent Sensory Afferents

Bdnf-null mutants die because of the loss of cranial sensory neurons derived from epidermal placodes, not because of the loss of CNS neurons (see sect. 1). *Bdnf* expression has been documented in the target structures of BDNF-dependent sensory neurons, including in particular in tissues innervated by the nodose and the vestibular ganglia, such as the baroreceptors in the aortic arch and the inner ear, respectively (387, 388). The loss of these neurons causes severe balance deficits and feeding and breathing abnormalities incompatible with an extended postnatal life (38, 39, 195, 387, 388). More generally, the sensory neurons comprising the nodose ganglion play an important role in the physiology of BDNF, including in the regulation of food intake

as discussed in sect. 9. The delineation of this role is likely to become an important topic given increasing interest in the nodose ganglion as the key relay station between a number of internal organs (see **FIGURE 13** in sect. 9) and the brain stem (recently reviewed in Refs. 390–392). As noted in sect. 3, a large number of cells in epithelia lining the gut and the airways express BDNF, and so do essentially all nodose neurons (393). Beyond neuronal survival, an in vivo role for BDNF in sensory physiology was established early on with the demonstration in *Bdnf* mouse mutants that slowly adapting mechanoreceptors (SAMs), but not other types of cutaneous afferents, require BDNF for the development of normal mechanotransduction (394). These SAM neurons showed a profound and specific reduction of mechanical sensitivity (394), quantitatively similar in young hetero- and homozygote mutants, as is the case when long-term potentiation (LTP) is recorded in the hippocampus (see sect. 5). Postnatal treatment of *Bdnf* heterozygote animals with recombinant BDNF rescued the mechanical sensitivity deficits, suggesting a role for BDNF independent of its survival-promoting activity (394). The comparatively high levels of *Bdnf* expression in sensory ganglia (see sect. 3) allowed an early and reliable demonstration of its presence in sensory neurons and of its anterograde transport as discussed in sect. 3. Indeed, the specificity of the polyclonal anti-BDNF antibodies used in these early studies could be convincingly validated with antibodies purified over a BDNF affinity column and some of the results verified using brain sections prepared from *Bdnf*-null mutants (395, 185). Specifically, endogenous BDNF was shown to localize to small-diameter DRG neurons and to be transported anterogradely to dorsal laminae of the spinal cord (185). The same study also showed that BDNF is transported down the peripheral branch of DRG neurons and that it accumulates proximally to the ligated sciatic nerve (185). Surprisingly, the functional implications of BDNF released from peripheral sensory endings in tissues such as the skin, heart, lung, pancreas, and gut do not seem to have been investigated thus far. Yet a role would appear likely given the expression of TrkB.T1 by various nonneuronal cells including those of the cardiovascular system (396) and pancreatic β-cells (259). As there is increasing interest in the possible function of neurotransmitters and peptides released by the peripheral branch of sensory nerves, it can be expected that BDNF and its pro-peptide could join the list in the future. For example, it has been shown recently that the calcitonin gene-related peptide (CGRP) released by nociceptors plays an important role in tissue healing (397). Similarly, there is accumulating evidence for the role of other peptides including VIP and substance P also released by nerve terminals on cells of the immune system (for review, see Ref. 261). Recent data also document the role of peptides released by nerve terminals in tumor growth (398).

In mammals, most DRG neurons express TrkA and are NGF dependent, while the proportion of neurons expressing TrkB is comparatively small (399). In adult rat DRG, whereas BDNF protein and mRNA are both mainly found in a subpopulation of TrkA-positive neurons also containing calcitonin gene-related peptide (CGRP), intrathecal administration of NGF increases BDNF expression in most TrkA-positive neurons (171), presumably by mechanisms similar to those involved in TrkB-mediated upregulation of *BDNF* transcription (see sect. 3). The study by Michael and colleagues (171) also includes an immunoelectron microscopic analysis revealing the presence of BDNF in finely myelinated and unmyelinated axon terminals in the spinal cord with immunoreactivity concentrated over large dense-core vesicles. A remarkable follow-up ultrastructural study by Salio and colleagues (170) using gold particles of different sizes to decorate antibodies revealed that BDNF colocalizes with CGRP and substance P, in both rats and mice, in the terminals of sensory afferents in the spinal cord as well as in the parabrachial nucleus of the amygdala.

7.3. BDNF and Pain-Related Mechanisms in the Spinal Cord

The presence of BDNF in the C-fiber terminals in the spinal cord has long intrigued scientists working in the pain field. As such it is not surprising to find BDNF stored in glutamatergic nerve terminals (see sect. 3), but this feature also suggests that BDNF may be involved in pain-related mechanisms (reviewed in Refs. 298, 400). As summarized below, this role is quite complex and not fully understood. Yet a better understanding of this role is important if only because it may complicate the outcome of clinical trials based on the activation of the BDNF/TrkB pathway, should sensory discomfort become apparent in volunteers. Early results indicated that injections of BDNF directly into the rat midbrain exerted clear, rapid, and prolonged analgesic effects that can be reversed by the administration of naloxone (401, 402). This early observation is possibly related to the recent finding (see below) that the analgesic effects of morphine depend on BDNF and TrkB expression by a group of supraspinal neurons (386). These morphinelike effects of midbrain BDNF injections were also observed with NT3, either because it activated TrkB at the concentrations used or else because of the extensive overlap between TrkB and TrkC expression throughout the CNS (see Ref. 251 for discussion and illustration). By contrast, these effects were not observed with NGF in the same experimental setting (401). After these initial experiments, attention progressively shifted to the spinal cord following reports that BDNF is transported

from DRG cell bodies to the presynaptic terminals of nociceptive afferents (185) and that its levels massively increase after NGF administration (171). Also, peripheral inflammation caused by intraplantar injection of complete Freund's adjuvant upregulates the levels of BDNF mRNA and protein in the spinal cord and in DRG, an increase that is prevented by the administration of NGF antibodies (403). This type of inflammation was also shown to increase the expression of TrkB in the dorsal horn (403). As sequestration of endogenous BDNF by TrkB-Fc fusion constructs injected in the spinal cord blocked the progressive hypersensitivity elicited by low-intensity tactile stimulation of inflamed tissue, it is difficult to escape the conclusion that at least this form of hyperalgesia depends on the release of BDNF in the spinal cord (403). The role of BDNF in synaptic transmission in the spinal cord has been firmly established with *Bdnf*-null mutants in experiments involving repetitive stimulation of C fibers (301). These investigations revealed selective deficits in the ventral root potential, a polysynaptic response evoked by stimulating nociceptive afferents. By contrast, the nonnociceptive portion of the ventral root potential remained unaltered (301). In addition, activity-dependent plasticity evoked by repetitive, low-frequency stimulation of nociceptive primary afferents, termed windup, was substantially reduced in *Bdnf*-mutant mice, indicating a role for presynaptic BDNF release from sensory neurons in the modulation of pain-related neurotransmission (301). Still, the role played by BDNF in neuropathic pain remains one of the most complex topics of the physiopathology of BDNF (400), not least because of the suggestion that there are additional sources of BDNF in the spinal cord, including in particular microglial cells (189). Spinal microglial cells stimulated by exposure to ATP and brought into contact with the lumbar spinal cord of naive rats caused a progressive decrease in the threshold of paw withdrawal (189). Recording from L1 dorsal root neurons revealed a progressive shift in the anion reversal potential caused by activated microglial cells, an effect that was mimicked by the application of BDNF and blocked by the addition of a BDNF scavenger (189). The shift in the anion reversal potential is presumably caused by the downregulation of KCC2 in L1 neurons following TrkB activation by BDNF. Subsequent work using a model of neuropathic pain induced by chronic nerve construction indicated a role for decreased presynaptic inhibition also caused by a shift in the E_{GABA} reverse potential that could also be prevented by the same TrkB-Fc scavenger reagent (404). This study also used a genetic model involving the selective deletion of a GABA receptor subunit in DRG neurons and showed that the mice display hypersensitivity to noxious heat and mechanical stimuli (404). As these experiments point to a role for DRG-derived BDNF in neuropathic pain, it is interesting to note that in the corresponding experimental models of unilateral constriction of the sciatic nerve or spinal nerve ligation, increased ipsilateral BDNF immunoreactivity was observed in axons projecting to the dorsal horn and to deeper dorsal layers than on the contralateral side (405). Importantly, a massive increase in BDNF immunoreactive profiles was also observed in the ipsilateral gracile nucleus, a major proprioceptive relay in the medulla oblongata (405). In sum then, it seems that multiple sources of BDNF and targets may contribute to the development of neuropathic pain, with NGF being just one among several mediators explaining BDNF increases in DRG neurons, as not all DRG neurons express the NGF receptor TrkA. The notion that there are other sources of BDNF in the PNS than NGF-dependent neurons is also supported by the unexpected observation that in embryonic DRG the BDNF content of mouse *Ngf*−/− mutants lacking detectable expression of TrkA is not significantly different from that of control animals (see **Fig. 6** in Ref. 406).

A further complication in evaluating the role of BDNF in pain is that this role may vary over time. As such, this notion is not particularly surprising given that synaptic plasticity and its modulation are at the core of BDNF's physiology in the nervous system. With the use of Avil-CreERT2 mice to delete *Bdnf* from all adult peripheral sensory neurons, spinal excitability was found to be normal in control animals but BDNF to play a critical role in mediating the transition from acute to chronic pain (407).

Currently, NGF remains the most directly relevant neurotrophin with regard to the development of pain treatment in humans (for a recent review, see Ref. 408), a notion strongly supported by human genetics. Mutations in the *NGF* gene have long been linked with congenital insensitivity to pain (409) and loss of pain sensation by mutations in *NTK1*, the gene encoding TrkA (410, 411). These crucial findings spurred the development of anti-NGF antibodies to treat chronic pain in conditions such as back pain (408, 412). Whether the neutralization of endogenous NGF may decrease the expression of BDNF in nociceptive afferents and contribute to the success of the anti-NGF treatment is a possibility worth considering (R. M. Lindsay, personal communication). Beyond NGF and ATP-activated microglial cells there is little doubt that other mediators play a role in pain transduction mechanisms including for example GDNF and substance P (for review, see Ref. 413) and neurotensin (414).

Given this complexity, novel approaches to chronic pain treatment may involve the targeting of common downstream effectors including chloride transporters

such as KCC2, the short-lived ion exchanger now at the center of some of the current efforts to treat pain (415).

7.4. Pain and Supraspinal Mechanisms

Beyond peripheral sensory inputs, pain perception has long been known to be regulated by supraspinal mechanisms that are also involved in morphine-mediated analgesia (416–418). In the hindbrain, one of the relevant areas involved in these supraspinal mechanisms is the rostral ventromedial medulla (RVM). This is the last relay nucleus collecting information from various brain areas and also the origin of a descending pathway terminating in the dorsal spinal cord on galanin-expressing inhibitory neurons (386). It turned out that a subpopulation of glutamatergic neurons in the RVM express *Bdnf* and that decreasing *Bdnf* levels in these neurons by shRNA prevents the analgesic action that morphine normally exerts after noxious mechanical stimulation (386). Remarkably, similar results were obtained after the downregulation of *Ntrk2* (TrkB) in these same neurons. Beyond the stunning demonstration that BDNF mediates the analgesic effects of morphine in this experimental paradigm, these results also further emphasize the role of TrkB expressed by the very neurons secreting BDNF, i.e., the functional significance of autocrine/paracrine mechanisms.

8. MOOD DISORDERS

8.1. Introduction

A major development in the field is the recent suggestion that antidepressants and psychoactive drugs facilitate BDNF signaling by virtue of their binding to TrkB. Although structural work is still lacking to explain these extraordinary findings, they were preceded by over two decades of work indicating that altered synaptic plasticity and BDNF/TrkB signaling are implicated in major depressive disorders (MDDs).

8.2. Work with Rodents

At an observational level, it has long been appreciated that stressful life events are associated with major depressive episodes (for review, see for example Ref. 419). Also, the dysregulation of the hypothalamus-pituitary-adrenocortical system has been thoroughly investigated for its involvement in depression (420). With regard to the role of neurotrophin signaling in MDDs, an early contribution revealed that in a rat model, stress caused by a 2-h period of forced immobilization in the morning was accompanied by a dramatic reduction of *Bdnf* mRNA levels throughout the rat hippocampus (421). This reduction was particularly severe in the dentate gyrus, an area of adult neurogenesis in rodents much discussed in the context of the mode of action of antidepressants (422). Remarkably, parallel in situ hybridization experiments with *Ntf3* probes revealed if anything an increase in mRNA levels in the dentate gyrus after repeated episodes of immobilization (421). These observations on the levels of neurotrophin mRNAs could be replicated by the administration of corticosterone. These important findings were supported by the observation that the effects of immobilization stress on *Bdnf* mRNA levels were largely prevented when performed on rats after bilateral removal of the adrenal medulla (421). However, this only applied to the levels of *Bdnf* mRNA in CA3 and CA1, not in the dentate gyrus, where a marked decrease was still observed in the absence of both adrenal glands (421). This seminal study also mentions that no changes were observed in the levels of *Ntf4* expression. With regard to the levels of *Ntf3*, adrenalectomy decreased them in the dentate gyrus as well as in CA1 and CA2. These results not only revealed a correlation between a recognized aggravating factor of MDD, namely stress, but also they indicated that BDNF and NT3 behave differently. This is all the more remarkable given that NT3 and BDNF share important features with regard to the pattern of expression in their respective receptors TrkB and TrkC throughout the brain. However, the organization of the respective genes differs substantially (59), and so do the mechanisms regulating their expression. In particular, the expression of *Ntf3* is not induced by the traditional stimuli increasing neural activity in the CNS (59). The role of corticosteroids in the regulation of *Bdnf* mRNA levels is also important to consider as steroids have long been known to alter dendritic morphology of pyramidal neurons in the hippocampus (423). Contrasting with stress-related effects, electroconvulsive therapies and chronic administration of antidepressants have been shown to increase BDNF levels in the rat prefrontal cortex and hippocampus (424).

As detailed in sect. 10, there is ample evidence that physical exercise increases BDNF levels in the cerebral cortex and in the hippocampus, and this is relevant given the recognized beneficial effects of physical exercise in MDD (for review, see Ref. 425). Additional early key findings relevant to rodent models of depression include the discovery of a link between BDNF and the serotoninergic system (426) and the observation that BDNF injections into the rat midbrain exert antidepressant-like effects in the learned helplessness and swim tests (427). The case for the involvement of BDNF signaling in rodent models of depression was subsequently further strengthened by the demonstration that TrkB

activation is necessary for imipramine and fluoxetine to deploy their beneficial effects in the forced swim test (428). The same publication also indicated that the efficacy of imipramine was blunted in mice lacking one *Bdnf* allele and that both fluoxetine and imipramine cause the phosphorylation of TrkB in the cerebral cortex and the hippocampus (428). These observations were later extended to inhibitors of monoamine transporters and metabolism that were also shown to rapidly activate TrkB in the rodent anterior cingulate cortex and the hippocampus (429). In a further extraordinary development, the suggestion was then made that many, if not all, antidepressants directly bind to TrkB and potentiate BDNF signaling through its receptor (430), a finding later extended to the psychoactive drugs lysergic acid diethylamide (LSD) and psilocin (431). These experiments involve binding assays using TrkB expressed in HEK293 cells and a TrkB mutant lacking a tyrosine residue located in the transmembrane domain thought to be a point of contact with membrane cholesterol. This TrkB mutant showed a much reduced binding affinity for antidepressants. Crucially, mice expressing this TrkB receptor mutant show a decreased response to antidepressants (430). The overall idea emerging from this set of data is that TrkB embedded in cholesterol-rich patches of membrane potentiates signal transduction initiated by BDNF by favoring the formation of TrkB dimers (430). A striking aspect of these observations is that none of the antidepressants and psychoactive drugs has been developed on the basis of its ability to bind to TrkB and yet they apparently all do, despite their structural diversity (430, 431). Yet not all previously published data support this notion, and, for example, the antidepressant imipramine has been found by others not to activate TrkB (432). The same also applies to a group of substances named flavonoids (432) previously reported to activate TrkB in the case of 7,8-dihydroxyflavone (433). This latter observation is potentially very significant given that 7,8-dihydroxyflavone diffuses into the brain. Although there is ongoing skepticism for the notion that TrkB may be a docking site for so many different ligands, it is important to note that, as recapitulated above, the involvement of BDNF and TrkB in the action of antidepressants has been independently documented in a large number of observations by several groups over a long period of time using different experimental approaches. What is lacking at this juncture is structural data, and it is conceivable that several TrkB molecules may come together to form a multibundle of alpha helices able to accommodate different ligands at different positions as recently shown for the dopamine transporter (434–436). Also, some of these substances may bind to BDNF and increase its binding affinity for TrkB, as recently suggested for one such flavonoid derivative designated apigenin, a common flavone found in vegetables (437). Indeed, the affinity of BDNF for aromatic residues has been noted and used in the initial purification scheme of BDNF from brain extracts (see **FIGURE 1**). Remarkably, the other component of the neurotrophin signaling pathway, namely $p75^{NTR}$, has also been shown to bind ketamine and fluoxetine with an affinity comparable to what had been previously reported for TrkB and to trigger the proteolysis of $p75^{NTR}$ expressed in HEK293 cells (438). Furthermore, related in vivo experiments indicated that the ability of both antidepressants to shift ocular dominance, previously used as evidence of enhanced CNS plasticity caused by antidepressants (for review, see Ref. 439), is lost in *Ngfr* hemizygote animals (438). The ameliorating effects of ketamine and fluoxetine in a paradigm of fear extinction were also prevented after treatment with a JNK inhibitor, one of many downstream effectors of $p75^{NTR}$, and with LM11-31A, a small molecule preventing the binding of pro-neurotrophins to $p75^{NTR}$ (440), further discussed in sect. 11. It is thus conceivable that the effects of ketamine and fluoxetine reported to trigger $p75^{NTR}$ proteolysis may impair a ligand-independent function of $p75^{NTR}$ known to activate RhoA, thereby limiting axonal elongation and branching by virtue of its interaction with RhoGDI as discussed in sect. 4.

8.3. Depression and Human Genetics

In a postmortem study involving 27 suicide subjects and 21 nonpsychiatric control subjects, mRNA as well as protein levels of BDNF and of TrkB were found to be significantly reduced, both in the prefrontal cortex and in the hippocampus (441). By contrast, the levels of the truncated form of TrkB remained unchanged. These results were then independently confirmed in a follow-up study conducted with brain tissue dissected from patients who also committed suicide. Compared with nonsuicide subjects, a significant decrease in BDNF levels was found in all 15 cases examined, both in the hippocampus and in the ventral prefrontal cortex, but not in entorhinal cortex (442). This decrease was only found in untreated patients, not in seven individuals with symptoms of major depression who were drug treated (442). NT3 levels were also decreased in the hippocampus of drug-free patients. In a study comprising a cohort involving 87 *BDNF* and *NTRK2* single-nucleotide variants established on the basis of DNA extracted from blood samples and hundreds of patients suffering from major depressive disorders (MDDs), polymorphisms associated with *NTRK2*, but not *BDNF*, were found in the cohort that included patients who had committed suicide attempts (443). With regard to plausible mechanisms explaining reduced levels of BDNF and TrkB, several promoter methylation studies have been conducted on various patient samples (for review, see Ref.

444). For example, postmortem brain samples from suicide subjects showed a significant increase of DNA methylation at specific CpG sites in the *BDNF* exon IV promoter, compared with control subjects (445).

With regard to therapies not involving pharmacological approaches and antidepressants, the benefits of physical exercise have long been recognized (446). Based on extensive work in rodents, it would seem very likely that physical exercise also increases BDNF levels in the human brain (see sect. 10 for additional details). As physical exercise is often not a realistic option for patients suffering from MDD, there is a great deal of interest in the use of noninvasive brain stimulation techniques (for a recent review, see Ref. 447). These techniques include transcranial magnetic stimulation (TMS) and especially transcranial direct current stimulation (tDCS), a comparatively simple technique that can be self-administered at home. Also, there is no shortage of positive reports suggesting efficacy (for a recent example, see Ref. 448). Although such noninvasive approaches are a priori attractive and a theoretical framework has been proposed detailing how tDCS may affect motor learning (449), it is exceedingly difficult to firmly establish in humans whether tDCS is truly useful to treat depression and other conditions likely to benefit from increased BDNF levels in relevant brain areas. There are problems at essentially all levels including anatomical variability of the skull between different subjects, the near impossibility of verifying the impact of tDCS by fMRI when different subjects are compared, and the known placebo effects that are particularly difficult to rule out in the context of depression and its treatment (for a recent critical of the use of tDCS in humans, see Ref. 450). Other noninvasive approaches are being developed including transcranial alternating current stimulation (tACS), transcranial ultrasound stimulation (TUS), and more recently temporal interference (TI) brain stimulation (451). TI is of special interest as it aims at targeting deep brain structures including the hippocampus, obviously a major issue in humans with the noninvasive techniques used thus far. In a mouse model TI allows the stimulation of the hippocampus without recruiting neurons in more superficial brain areas (451). Yet whether or not it can be applied safely and with efficacy to humans remains to be determined, and a set of recommendations has just been published (452).

9. BODY WEIGHT REGULATION

9.1. Introduction

Experiments performed with adult rats soon after recombinant BDNF became available fortuitously revealed that repetitive intraventricular injections of BDNF markedly reduced food intake (453). Meanwhile, the regulation of food intake and of energy expenditure by BDNF/TrkB signaling has become an important focus of research increasingly driven by the association of human polymorphisms with obesity.

9.2. Regulation of Food Intake

A physiological role for BDNF as a body weight regulator became apparent upon examination of adult mice lacking one *Bdnf* allele (454). These mutant animals were found to progressively become hyperphagic and to abnormally gain weight starting at ~2 mo. Increased intermale aggressivity was also noted, a phenotype ameliorated by the administration of the serotonin reuptake inhibitor fluoxetine (454). The finding of abnormal weight gain in *Bdnf* hemizygotes was confirmed in a related study that also reported *Bdnf* expression in hypothalamic nuclei known to be involved in the regulation of food intake (**FIGURE 13**), including the ventromedial hypothalamic (VMH) nuclei (455). Subsequently, *Bdnf* expression in the VMH was shown to be regulated by melanocortin-4 receptor (MC4R) signaling and to be markedly and selectively decreased after food deprivation (456). Glucose administration rapidly increases exon 1 transcripts in the VMH, whereas virus-mediated excision of *Bdnf* in the VMH in adult animals causes hyperphagic behavior and obesity, without altering energy expenditure (457). Notably, the nucleus-specific deletion of *Bdnf* did not affect other behavioral parameters including locomotor, aggressive, or depressive-like behaviors (457). In a recent, remarkable development, BDNF-expressing neurons in the VMH were shown to control food consumption and jaw movements by a surprisingly simple, subcortical circuitry (181). VMH neurons are connected to leptin-sensing neurons in the arcuate nucleus (ARH) and project to the premotor area of the trigeminal nucleus (pMe5) onto neurons likely to include muscle spindle afferents (see **FIGURE 13**). The activity of these neurons decreases during food consumption and increases when food is in proximity (181). Unlike VMH neurons, ARH neurons do not express the *Bdnf* gene, with some secreting the neuropeptide α-melanocyte stimulating hormone (α-MSH), a ligand of the melanocortin receptor 4 (MC4R) generated by cleavage from proopiomelanocortin (POMC). The ARH nucleus is located outside the blood-brain barrier and is a key structure in the response to circulating levels of leptin (458). As very little work has been done thus far in this area at the BDNF protein level, it is worth noting that activation of MC4R by the agonist MK1 does trigger the release of BDNF from hypothalamic explants (459) and that the injection of a BDNF monoclonal antibody blocking its

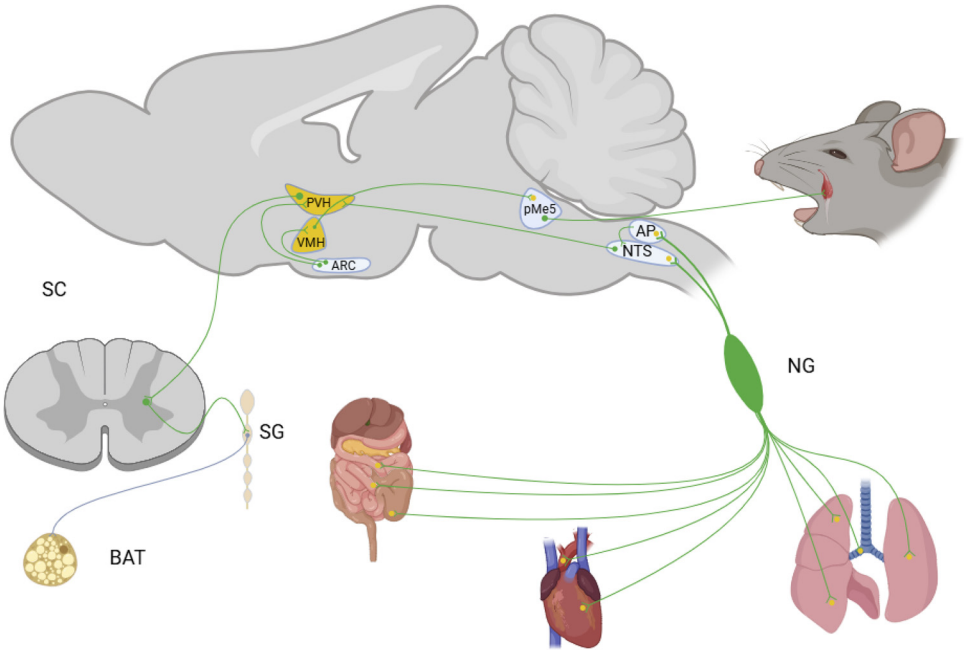


FIGURE 13. Overview of some of the circuitry regulating food intake and energy expenditure. The nodose ganglion (NG) is a key relay station, and sensory afferents innervate most internal organs as well as specialized structures such as the baroreceptors of the aortic arch. Brain-derived neurotrophic factor (BDNF; yellow dots) is expressed in epithelial cells lining the gut and other organs as well as by the baroreceptors. The hypothalamic nuclei most discussed in the context of BDNF and the regulation of food intake are indicated, and those expressing the *Bdnf* gene are indicated in yellow (for review, see Ref. 389). Major outputs include axons innervating the trigeminal mesencephalic nucleus (pMe5) and the preganglionic column of Terni in the spinal cord (SC) innervating brown adipose tissue (BAT) through a relay in the stellate ganglion (SG). AP, area postrema; ARC, arcuate nucleus; NTS, nucleus tractus solitarii; PVH, paraventricular nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus. Figure created with a licensed version of BioRender.com.

biological activity (406) into the third ventricle before the administration of the MK1 agonist completely blocks its antiorexic effect (459). These observations support a previous conclusion that TrkB activation is downstream of MCR activation (456). In addition to the role of BDNF in the VMH, its role in the paraventricular hypothalamic nucleus (PVH) has also received a great deal of attention because of its involvement in leptin signaling (see below). The connectivity of PVH neurons has been explored in considerable detail with *Bdnf-Ires-Cre* animals, rabies virus-based retrograde transsynaptic labeling, and adeno-associated virus anterograde tracing techniques (460). The results indicate that several brain regions provide a dense input to, and receive a dense input from, PVN BDNF neurons (460). Many neurons in the PVH express MC4R, and restoring MC4R expression in the PVH in transgenic mice reduces obesity by suppressing the increased food intake typically seen in animals lacking MC4R, whereas reduced energy expenditure was unaffected (461).

9.3. Energy Expenditure

The connection between BDNF and energy expenditure was recognized early on, during the course of experiments performed with leptin mutant *db/db* mice aimed at understanding the role of BDNF on glucose metabolism (462–464). Remarkably, treatment of the mutant mice with intraventricular injections of BDNF enhanced norepinephrine turnover and increased the levels of uncoupling protein-1 (UNC1) in the interscapular brown adipose tissue (462). Fat tissue including in particular the brown fat adipose tissue (BAT) is innervated by the sympathetic system. Its activation massively increases energy dissipation through the oxidative degradation of the lipids accumulated in this tissue, an important component of nonshivering thermogenesis (465). The depolarization of sympathetic terminals contained in explants of rat BAT triggers within minutes a large increase in oxygen consumption caused by the release of norepinephrine (466). In BAT, mitochondria are uncoupled from ATP generation by virtue of the expression of UNC1 (for review, see Ref. 467). In an extraordinary further development, the delineation of the circuitry explaining the role of BDNF in the PVN and energy dissipation uncovered a link with the mode of action of leptin (468). Leptin, initially discovered using an unbiased genetic approach (for review, see Ref. 469), is the best-known hormonal regulator of body weight, and its null mutation causes obesity as a result of defective thermogenesis and lipolysis. These defects can be reversed by the chronic, but not acute, administration of recombinant leptin

that restores the innervation of the adipose tissue by the sympathetic system through the activation of neurons located in the arcuate nucleus (468). These neurons express the leptin receptor and are connected to *Bdnf*-expressing neurons in the PVH (see **FIGURE 13**) via agouti-related peptide and proopiomelanocortin neurons (468). Deletion of the leptin receptor gene in ARC, but not in other hypothalamic nuclei, causes a marked decrease in the density of innervation by the sympathetic nervous system of both white and brown adipose tissue. Behaviorally, this is accompanied by a reduced thermogenic response upon exposure of the animals to cold. The selective knockdown of *Bdnf* in the PVH decreases innervation of thermogenic inguinal white and brown adipose tissues, and in mice lacking leptin this selective *Bdnf* reduction blunts the effects of exogenous leptin treatment toward increasing the sympathetic innervation of inguinal fat and brown adipose tissue (468). Animal lacking leptin and BDNF in the PVH show a significant decrease in the ability of recombinant leptin to induce weight loss, reduce food intake, and restore BAT temperature compared with animals only lacking leptin (468). Given subsequent work on the innervation of the bone marrow and the demonstration that cells expressing the leptin receptor express the *Ngf* gene, thereby regulating sympathetic innervation (470), similar mechanisms may operate in *ob/ob* mice chronically treated with leptin. BDNF is transported anterogradely from the PVH and increases the size of preganglionic sympathetic neurons located in the intermediolateral column (IMLC) of the spinal cord (471). This in turn may increase the ability of noradrenergic nerve terminals to capture the presumed limiting amounts of NGF produced by fat tissue. The use of a mouse line expressing lacZ under the control of the endogenous *Bdnf* promoters allowed detailed mapping of BDNF neurons in the PVH and revealed that BDNF-expressing neurons in the medial and posterior PVH participate in energy dissipation through a circuit regulating sympathetic outflow and thermogenesis in fat tissue (471). By contrast, BDNF-expressing neurons in the PVH were found to be involved in suppressing feeding (471). The suggestion was then put forward that BDNF transported anterogradely from the PVH toward cholinergic, TrkB-expressing neurons located in the intermediolateral column (IMLC) regulates the presynaptic to sympathetic ganglia innervating brown fat and white adipose tissue (471). This feedforward mechanism positively regulates the size of the cholinergic neurons presynaptic to the stellate ganglion innervation of brown fat tissue (471). Related mechanisms have also been proposed to explain how chronic leptin treatment regulates fat tissue innervation by the sympathetic system (468). Using an elegant approach allowing the unbiased identification of warm-sensitive neurons, later investigations uncovered an increased translation of *Bdnf* mRNA in the preoptic area of the hypothalamus in response to heat (169). Conversely, optogenetic activation of these neurons triggered hypothermia and a cold-seeking behavior (169). In the VMH, BDNF secretion has been shown to activate TrkB.T1 expressed by some of the surrounding astrocytes (257). Interestingly, the depletion of TrkB.T1 in VMH astrocytes enhances glutamate reuptake caused by a change in the morphology of astrocytes, thus decreasing neuronal activity that would normally accompany the antiorexic effects of BDNF (257).

9.4. Peripheral Sensory Input

Whereas the role of BDNF as an antiorexic and energy-dissipating agent in the hypothalamus is now well understood, a possible role of BDNF in sensory afferents in mediating the regulation of food intake and energy expenditure is still not entirely clear. As mentioned in sect. 3, BDNF is expressed in the epithelium lining as well as in other layers of the digestive tract innervated by nodose ganglion neuron afferents (195). These neurons represent an important relay between the gut and the brain (see sect. 7 and **FIGURE 13**). It has been recently shown that meal-related signals modulate neuronal activity in the caudal nucleus of the tractus solitarius (cNTS), where these signals are integrated (472). The cNTS represents the area of projection of the nodose ganglion, and neurons expressing the glucagon gene *Gcg* in cNTS are activated by mechanical feedback from the gut (472). Previous single-cell RNAseq experiments have revealed that most neurons in the mouse nodose ganglion express the *Bdnf* gene, including those expressing mechanoreceptors (393). This is significant as hunger-promoting signals are potently suppressed by the activation of intestinal mechanoreceptors that have their cell bodies in the nodose ganglion (473). Nodose ganglion neurons are also likely to upregulate BDNF levels as a function of gut signals, given the results of c-fos monitoring in this ganglion in response to gut luminal stimulation (474). As BDNF levels increase in nodose ganglion neurons when bursts of high-frequency stimuli are applied (475), it seems likely that gut-derived signal may increase BDNF levels in nodose neurons and trigger its release in the cNTS. This interpretation is suggested by previous observations on the role of nodose neuron in the regulation of respiratory input (476) and the activity-dependent BDNF release from these neurons (295). In a recent, highly significant study, BDNF-expressing neurons have been identified in the medial NTS (mNTS) (477). These neurons are required for the weight-reducing actions of both growth differentiation factor 15 (GDF15) and the glucagon-like peptide-1 (GLP-1) receptor agonist exendin-4 (477).

Activation of BDNF-expressing mNTS neurons reduces food intake and drives fatty acid oxidation (477).

With regard to patterns of food intake, intermittent fasting has been hypothesized to be potentially beneficial and to retard cognitive decline during aging (478). Kumar and colleagues (479) attempted to replicate aspects of intermittent fasting by feeding cultured mouse neurons with the glucose analog 2-deoxyglucose. The resulting endoplasmic reticulum stress caused levels of the transcription factor ATF4 to increase *Bdnf* transcription as a result of ATF4 binding to a *Bdnf* enhancer region (479). In an AD model consisting of the overexpression of amyloid precursor protein (APP) and presenilin mutant constructs, the administration of 2-deoxyglucose has been shown to improve LTP deficits observed in hippocampal slices prepared from these animals. Modest improvements were also observed in a behavioral task testing spatial memory and in functional recovery in an ischemic stroke model (479).

9.5. Human Genetics

Mutations in the *BDNF* as well as *NTRK2* genes have been associated with severe obesity in humans (480). A *NTRK2* mutation discovered in an 8-yr-old male has been associated with a syndrome including hyperphagic obesity as well as learning and memory impairment (481). The cause of this dramatic phenotype was attributed to a heterozygous de novo missense mutation resulting in a Y722C substitution in TrkB. This mutation was found to markedly impair receptor autophosphorylation and MAP kinase signaling, suggesting that this mutation acts like a dominant-negative impairing signaling through TrkB encoded by the intact allele. The loss of one functional BDNF allele discovered in an 8-yr-old girl has been associated with hyperphagia and severe obesity (482) as well as with impaired cognitive function (482). Together with the wealth of data accumulated with mouse models, these human genetic data further add to the notion that BDNF/TrkB signaling is essential for normal development, including the regulation of food intake and memory (482). The WAGR syndrome is also important to consider in this context. WAGR stands for Wilms tumor, aniridia, genitourinary anomalies, and mental retardation and is caused by 11p13 deletions of variable size. These deletions may in some cases include the *BDNF* locus. Two other genes are in its vicinity, namely *WT1* encoding the Wilms tumor protein and *PAX6,* a homeobox gene of critical significance in neurodevelopment. The *BDNF* locus is located ~4 Mb from the *PAX6* gene. In an initial study with 33 WAGR patients, 19 had a *BDNF* deletion and all had a body mass index higher than the patients without *BDNF* deletion (483). Interestingly, this is also an ultrarare case where BDNF measurements in the serum of the corresponding patients are informative, with the measurements indicating a reduction by ~50% in the 19 patients versus 14 control subjects without *BDNF* deletion (483). In a related study, 15 WAGR patients with a *BDNF* deletion had lower adaptive behavior, reduced cognitive functioning, and higher levels of social impairment, with some meeting the criteria of autism (484).

In Prader–Willi syndrome (PWS), a condition characterized by severe hyperphagia, a transcriptome analysis performed on the hypothalamus of affected individuals revealed an overlap with the transcriptome of mouse hypothalamic neurons signaling hunger, whereas downregulated genes overlapped with those activated by feeding (485). The levels of *BDNF* and *NTRK2* transcripts were found to be markedly decreased in the VMH of PWS patients (485), suggesting that decreased BDNF/TrkB signaling may underly the hyperphagic behavior of PWS patients.

As obesity is a readily quantifiable phenotype, it can be predicted that the number of associations between *BDNF* polymorphisms and human conditions will continue to increase (486, 487). For example, a study that examined the expression levels of *BDNF* transcripts in the human hypothalamus and possible associations with 44 single-nucleotide polymorphisms reported that the minor C allele rs12291063 is associated with lower expression of *BDNF* in the human VMH and greater adiposity, in both adult and pediatric cohorts (488). The study also revealed that the major T allele, unlike the C allele, binds a transcriptional regulator designated D0B (488). In addition, a recent study that focused on a regulatory region selected on the basis of its high degree of conservation between species led to the discovery of a sequence (designated BE5.1) that functions as an enhancer, with obesity associated with the A, as opposed to T, allele of this polymorphism (16). A GWAS cohort analysis in humans also demonstrated a significant association between rs10767664 and anxiousness. Interrogation of the human GTEx expression quantitative trait locus (eQTL) database revealed a highly significant effect on the levels of *BDNF*-antisense (*BDNF-AS*) transcripts, with the deletion of BE5.1 significantly reducing BDNF-AS expression in mice. This remarkable set of results underscores the power of GWAS studies in humans in helping the discovery of new regulatory mechanisms that had thus far escaped detection in rodents (16). However, the impact of the rs10767664 polymorphism on the BDNF protein level in human cells is unknown at this point and the relevance of the results obtained in mouse models unclear given differences in the organization of the gene encoding *BDNF* antisense transcripts between mice and humans (see sect. 2).

Contrasting with overwhelming evidence for the antiorexic effects of BDNF in humans and rodents, it turned out that intravenous administration of the agonistic TrkB antibody or of NT4 caused increased appetite, body weight, and fat deposits when administered peripherally to rhesus and cynomolgus monkeys (489). By contrast, the intraventricular injections of this agonistic antibody exerted the expected antiorexic effects (489). A follow-up study indicated that peripheral injections of the TrkB activating antibody decreased food intake and/or body weight in mice, rats, hamsters, and dogs but increased food intake and body weight in monkeys (490). Based on these results with nonhuman primates, a clinical trial was initiated with indications related to the dysregulation of food intake as observed in cachexia and anorexia. However, the trial was halted early for reasons that are unclear.

10. PHYSICAL EXERCISE AND BDNF MEASUREMENTS

10.1. Introduction

As discussed in sect. 6, physical activity has long been known to increase BDNF levels in the brain of rodents. This is unlikely not to be the case in humans but cannot be readily documented at present. Although BDNF levels are readily measurable in human blood, they do not reflect the levels of the nervous system, as explained in this section. There are major differences between primates and mice with regard to the presence and the levels of BDNF in blood, an important limitation when using mouse models to understand the pathophysiology of BDNF in humans.

10.2. Physical Exercise

It is widely appreciated that physical exercise helps in retarding cognitive decline during aging even if the definition of exercise in humans often lacks specificity with regard to frequency, duration, and intensity (for review, see Ref. 491). Accordingly, there is a great deal of interest in better understanding the mechanisms underpinning the multiple benefits of physical exercise and in using biomarkers (492). Although the impact of physical exercise on BDNF levels in the human brain cannot be quantified in living individuals, there is no reason to hypothesize that exercise would not increase BDNF levels in areas such the cerebral cortex and the hippocampus. Briefly, what has been learned with cultured neurons in particular suggests that the regulation of BDNF expression shows striking similarities between rodents and humans (see for example Refs. 52, 250, 493). In the rat brain, BDNF protein levels are elevated in the hippocampus immediately after exercise and only return to preexercise levels after 3–4 wk (361). The potential benefits of physical exercise have also been examined in the context of aging. Rats of ~2 yr of age show a marked deficit in their ability to locate a submerged platform compared with younger rats (494). This deficit is largely compensated in older rats by voluntary running, but not the decrease in object recognition also observed during aging (494). Voluntary exercise also increases presynaptic density in the hippocampus of aged rats compared with sedentary rats (494). With regard to possible molecular mechanisms, the cognitive benefits of exercise have also been examined in mice and compared with the effects of sodium butyrate, a histone deacetylase (HDAC) inhibitor previously shown to increase the effects on subthreshold learning (495). The effects of both exercise and sodium butyrate are thought to involve BDNF as both are blocked after the infusion of BDNF short interfering RNA into the hippocampus (495). Both exercise and sodium butyrate increase the levels of *Bdnf* transcripts I and IV that are associated with promoter acetylation on H4K8 but not H4K12 (495). With regard to BDNF levels during aging in nonhuman primate models, a pronounced decrease in BDNF staining intensity has been noted in the hippocampus and entorhinal cortex of macaque monkeys between 10 and 30 yr (496).

Increasing brain levels of BDNF levels in the human brain by physical exercise is most likely to be beneficial not only with regard to memory-related processes but also in retarding cognitive decline during aging (see also the 2024 report of the Lancet standing commission on dementia prevention, Ref. 497). Among a range of specific recommendations, the report underlines the benefits of sensory inputs, including the correction of hearing and visual deficits (497). This notion is also directly supported by postmortem measurements of *BDNF* mRNA levels in the prefrontal cortex (see sect. 11) that have established an inverted correlation between these levels and the rate of cognitive decline (498). The benefits of various forms of activity, including straightforward descriptions of how to increase the strength of various sensory inputs, referred to as “neurobic exercises,” have been usefully advertised in a booklet destined for the general public entitled *Keep Your Brain Alive*, written by Larry Katz and Manning Rubin (499). As discussed in sect. 8.3, noninvasive techniques involving extracranial stimulation are being developed, and it appears likely that some may eventually be used in humans to help increasing neuronal activity in functionally relevant pathways when physical exercise is not an option.

In rodent models, the crucial but technically challenging question of quantifying BDNF at the protein and

single-cell levels has been addressed (149). This key study quantifies and compares BDNF levels in relevant brain areas during the course of learning tasks and aging (149). It also deals with the all-important question of causality between the levels of BDNF in the cerebral cortex and the ability of mice to learn specific motor tasks. Its focus is on the connectivity between the motor cortex and the striatum, the brain areas long known to be at the center of motor learning (500). Presynaptic cortical neurons were identified by retrograde labeling and subsequently stained with validated anti-BDNF monoclonal antibodies that identified BDNF-positive neurons in layers II, III, and V (149). The number of such neurons was found to be highest in 3-wk-old mice and to be significantly reduced already by 12 wk. Free access to running wheels for 3 days not only significantly increased the number and, to a lesser extent, the staining intensity of BDNF-positive neurons in layers II and III of the motor cortex in juvenile mice but also restored the number of BDNF-positive neurons to juvenile levels in 12-wk-old mice with access to running wheels. Similarly, somewhat less pronounced changes were observed in layer V. The study also identifies BDNF-positive neurons in layer IV of the somatosensory cortex, and in contrast to the motor cortex their numbers and staining intensity did not change much between 3 and 12 wk, nor did it change after use of the running wheel. Most important in the context of the role of BDNF in exercise and motor learning, the study also shows that even a modest reduction of BDNF levels in the cerebral cortex in genetically engineered animals causes a selective impairment of motor leaning skills (149).

10.3. BDNF Measurements in Blood

A very large number of studies have reported BDNF levels in human serum or plasma after physical exercise as well as in a number of conditions such as depression. BDNF levels in human blood are readily measurable with standard BDNF ELISAs, even if the reported levels vary significantly depending on which ELISA kit is used (501). Besides technical issues, there is overwhelming evidence that BDNF levels in human blood do not reflect levels in the brain, despite an intriguing study involving eight volunteers that reported increased BDNF levels after exercise in venous compared with arterial blood (502). Not only is there is no clear evidence for the diffusion of BDNF across the blood-brain barrier (503) but also brain levels of BDNF are quite similar in a wide range of mammals including humans and mice. Yet the difference in blood levels of BDNF between these two species differs by orders of magnitude and can be accounted for by a straightforward explanation (see **FIGURE 14**): human blood contains significant levels of BDNF derived from platelets (504), and this turned out not to be the case in the mouse (505). Blood platelets are short-lived cellular fragments derived from megakaryocytes residing in the bone marrow and are best known for their central role in blood coagulation. However, platelets also contain a number of cytokines and growth factors such as platelet factor 4 (PF4) packed in secretory vesicles designated α-granules that release their content during the process of blood coagulation. Although the functional significance of most of these growth factors in platelets is uncertain, it is widely hypothesized that some may play a role in blood vessel and tissue repair. The

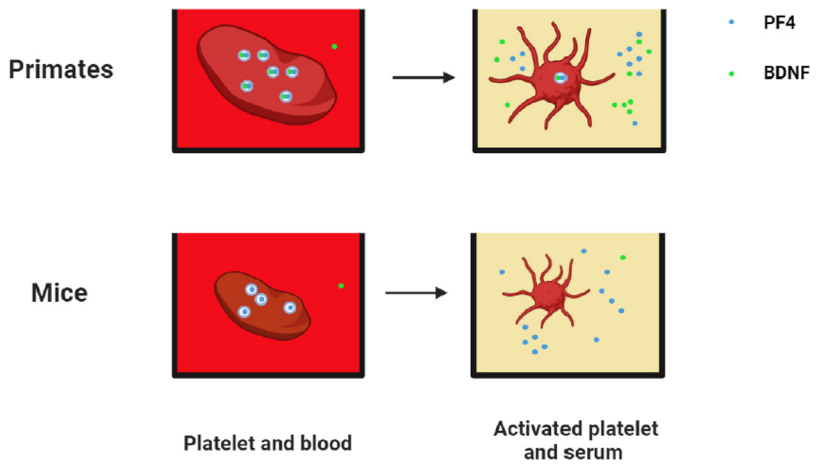


FIGURE 14. Brain-derived neurotrophic factor (BDNF) in primate and mouse blood. Primate but not mouse platelets store large amounts of BDNF (green) in secretory granules together with other secretory proteins such as platelet factor 4 (PF4, blue). BDNF in human serum derives from platelets that release their content during the process of blood coagulation. BDNF levels are ~3 orders of magnitude higher than in mouse blood, serum, or plasma. BDNF stored in human platelets originates from megakaryocytes, the progenitors of platelets. During the course of their maturation in the bone marrow, megakaryocytes express the *BDNF* gene at high levels in humans. This is not the case in mice (193). Figure created with a licensed version of BioRender.com.

platelet proteomes of mice and humans largely overlap, but they are not identical, and BDNF is absent in mouse platelets (506). As a result, BDNF levels are readily measurable in human serum by conventional BDNF ELISAs but not in mouse serum (505). The differences in BDNF levels in serum between human, rat, and mouse can be readily accounted for by comparing mRNAs extracted from the corresponding megakaryocytes (193). Whereas human and rat megakaryocytes actively transcribe the *BDNF* gene by the time these cells stop dividing and generate platelets, this is not the case with mouse megakaryocytes (193).

BDNF measurements in human blood are quite variable, even when samples are collected under rigorously controlled conditions. Specifically, a study involving >200 volunteers retested within a period of 12 mo under the exact same conditions to minimize variability indicated that group sizes of at least 60 individuals are required to detect a 20% change with confidence (507).

A similar point had been made previously cautioning the use of BDNF levels in blood as a potential marker in Huntington's disease (508). One of the reasons for this variability is that the release of BDNF from platelets during the process of blood clotting is quite incomplete and variable (509). However, when the differences are expected to be large as in the case of the loss of one functional BDNF allele, measuring BDNF levels in serum and especially in plasma could be useful and meaningful. One such rare case has been documented with blood from WAGR patients (Ref. 483; see sect. 9.5).

Whether or not the ratio of pro-BDNF to mature BDNF in human blood can be used to help the diagnosis of autism spectrum disorder (ASD) as recently suggested (156) also needs to be cautioned. The human polymorphism rs1048220 and rs104822 involving amino acids substitutions in the pro-BDNF cleavage site (see sect. 3, **TABLE 1**) has been used as a basis to generate a mouse model and the behavioral phenotype of these animals recently interpreted as being evocative of ASD in humans (156). As expected, this mutation altered the ratio pro-BDNF/mature BDNF in mouse tissues (but see comment in sect. 3), a finding that prompted the authors to compare BDNF levels in the plasma of a control group with a group of individuals diagnosed with ASD (156). Although the difference in BDNF levels between these two groups is among the largest ever reported between two human cohorts, the levels of mature BDNF reported in the plasma of the control group are also among the highest ever reported, at the high end of what is typically measured in human serum (156). It is the difference between the levels of mature BDNF in the plasma of the control group and the ASD cohort that contributes to the altered ratio pro-BDNF/BDNF in plasma. The origin of pro-BDNF in human blood (plasma or serum) is also unclear, and its presence would need to be independently validated given that pro-BDNF is virtually undetectable in human platelets (193). In sum, it is doubtful that the ratio pro-BDNF/BDNF can be used to help the diagnosis of human conditions. Unfortunately, no information is available yet on the molecular characteristics of BDNF in the blood of rs1048220 and/or rs104822 carriers, and it would be interesting to investigate its molecular weight and glycosylation status (see sect. 3.2.4).

Recently, a much more sensitive BDNF ELISA became available that does allow BDNF measurements in mouse blood (510). It is also sensitive enough to measure BDNF levels in human samples that can be collected noninvasively such as human saliva (511). The levels of BDNF in mouse serum or plasma turned out to be ~1,000-fold lower than in human serum (510). In line with the notion that the bulk of BDNF measured by conventional ELISA in human and rat serum originates from platelets, no difference was found between mouse serum and plasma (510). One source of BDNF in mouse blood is the skeletal musculature (259). The results of this study also indicate that other sources are likely, as the skeletal muscle-specific deletion of *Bdnf* gene reduced, but did not completely eliminate, the levels of BDNF from mouse blood (259). There are multiple possible alternatives as a number of tissues express the *Bdnf* gene (195), including peripheral sensory endings given the comparatively robust expression of the *Bdnf* gene by many sensory neurons in the PNS (see sect. 3). Given that it is now possible to measure BDNF levels in mouse blood in the absence of any contributions from platelets, it would be interesting to explore whether BDNF levels in mouse blood correlate with any of the many conditions affecting the function of the nervous system using relevant mouse models. Also, whether motor activity in the mouse measurably changes the levels of BDNF in blood can now be tested. The skeletal musculature does contribute to blood levels of BDNF in the mouse, and the *Bdnf* gene is expressed in some types of skeletal muscle (259), where it has been reported to play a role in the composition of muscle fibers (512). Such measurements could be informative, for example in the context of the regulation of insulin secretion or heart contraction given the presence of truncated BDNF receptors in these tissues (255).

Regarding the role of platelet BDNF in humans and other primates, it is conceivable that BDNF may contribute to peripheral nerve regeneration following traumatic injuries (513), a possible reason as to why this system evolved in long-lived species. As platelets are a major source of blood microvesicles and as these have been shown to contain BDNF in humans

(514), it is also theoretically possible that BDNF may find its way into the nervous tissue when carried by microvesicles. The possible physiopathological relevance of this hypothetical traffic has been recently suggested with a mouse model in which the expression of BDNF has been engineered to take place specifically in platelets (515). After optic nerve lesion in these animals, the retraction of dendrites of retinal ganglion cells was found to be significantly reduced compared with control animals (516). Also, there is experimental support for the notion that platelet-derived proteins may reach the CNS areas following the observation that PF4 enhances neurogenesis in the mouse hippocampus (517). This is also interesting in the context of physical exercise that been shown to "activate," i.e., cause the release of some of the platelet content (518).

11. NEURODEGENERATION

11.1. Introduction

Animal models and human genetic data both suggest that increasing BDNF signaling is a desirable objective in a number of conditions associated with dysfunction of the human nervous system. Although the few early clinical trials with recombinant BDNF have not been successful, it appears likely that the knowledge accumulated in the meantime will lead to more favorable outcomes in the future.

11.2. Amyotrophic Lateral Sclerosis

Following the demonstration that the administration of BDNF prevents the death of axotomized motoneurons (43, 44) and ameliorates motor functions of *Wobbler* mice (519), a large multicenter trial was initiated over two decades ago (520). It involved >1,000 randomized patients suffering from amyotrophic lateral sclerosis (ALS) who received subcutaneous placebo injections or injections of 25 or 100 μg/kg BDNF over a period of 9 mo. Survival in patients treated with 25 μg/kg BDNF was identical to placebo, and there was a trend toward increased survival in the 100 μg/kg group (520). As the analysis of the primary end point failed to demonstrate a statistically significant survival effect of BDNF, the study was discontinued (520). Post hoc analyses showed that ALS patients with early respiratory impairment and those developing altered bowel function showed statistically significant benefit. A clinical trial was also initiated in parallel with 25 patients with probable or definite ALS treated with BDNF delivered intrathecally (521). Doses of up to 150 μg per day were found to be well tolerated, but within days of treatment the majority of treated patients reported mild sensory symptoms, including paresthesia or a sense of warmth, usually confined to the lower limbs (521). Sleep disturbance, dry mouth, agitation, and other behavioral effects were encountered at higher doses (521). It thus seems that neither subcutaneous nor intrathecal administration of BDNF caused significant sensory symptoms of the type that may have been later observed in volunteers after peripheral administration of TrkB activating antibodies and that may have contributed to the early discontinuation of this trial (see sect. 7). Whereas these early ALS trials failed to show significant clinical benefits during the time frame of the studies, effects were noted on gastrointestinal motility (520). Among the reasons discussed for the failures of these early ALS trials was a long-lasting downregulation of TrkB caused by chronic exposure to BDNF (522). Recent findings with cultured human neurons support this view: the exposure of neurons to saturating concentrations of BDNF does trigger a profound and long-lasting downregulation of TrkB (251), in line with previous observations with rodent neurons (266, 268, 523, 524). Interestingly, the use of much lower concentrations of BDNF than those typically used in preclinical studies, i.e., in the picomolar rather than nanomolar range, is sufficient not only to massively activate TrkB in human neurons but also to allow its reactivation 24 h later (**FIGURE 15**), unlike the case when higher BDNF concentrations are used (251).

11.3. Alzheimer's Disease

Efforts to explore a possible link between BDNF and AD were initiated soon after the publication of the nucleotide sequence encoding BDNF (22). In situ hybridization studies with *BDNF* probes performed on postmortem sections of the hippocampus of nine AD patients and six control subjects revealed a marked decrease of *BDNF* mRNA levels in AD patients, whereas those of *NGF* and *NTF3* were not different from control subjects (525). The negative result with the NGF probe is in line with the outcome of a previous study indicating unchanged *NGF* levels in the cerebral cortex of AD patients compared with control subjects (526). RNase protection assays confirmed a roughly 50% decrease in *BDNF* mRNA levels in the hippocampus of AD patients compared with control subjects, though it was also noted that these control subjects were somewhat younger, with an average age of ~67 yr compared with 75 yr for AD donors (525). The initial focus on NGF and a possible link with AD was driven by the early realization that the dysfunction of CNS cholinergic neurons may be a major cause of memory deficits in the disease (527). Basal

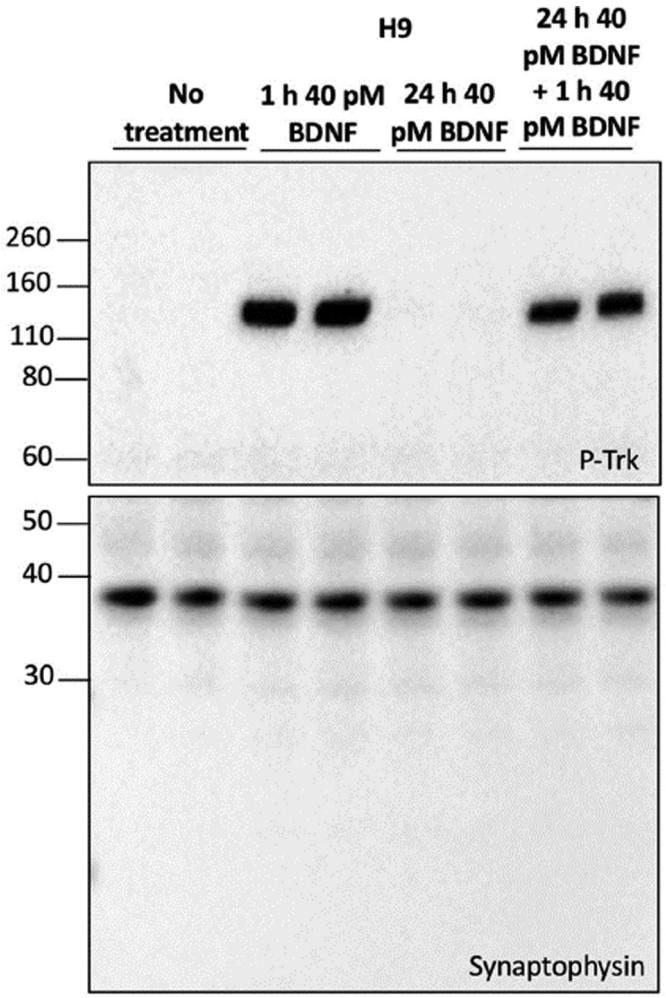


FIGURE 15. Activation and reactivation of TrkB by low concentrations of brain-derived neurotrophic factor (BDNF) in cultured human neurons. Lysates of GABAergic neurons differentiated from human embryonic stem cells and either left untreated (first 2 lanes) or treated with 40 pM BDNF for either 60 min (*lanes 3* and *4*) or 24 h before lysis (*lanes 5* and *6*). Activation of TrkB was assessed with a monoclonal antibody recognizing adjacent phosphorylated tyrosine residues 706 and 707 (**FIGURE 12**). Note the absence of any TrkB activation in the first 2 lanes. These GABAergic neurons are cultured in the absence of BDNF and do not secrete BDNF as expected (see **FIGURE 8**). Note also the complete loss of phospho-TrkB signal after a 24-h exposure to BDNF and its reappearance when the neurons are reexposed to 40 pM BDNF after initial exposure of the neurons to 40 pM (*lanes 7* and *8*). By contrast, when TrkB is initially activated with 1 nM BDNF, a saturating concentration in the range typically used in preclinical experiments, TrkB cannot be reactivated for prolonged periods of time (see Ref. 251 for additional details). The lower part of the transfer membrane was incubated with synaptophysin antibodies. MW, molecular mass markers in kilodaltons. Figure from Ateaque et al. (251), used with permission under CCBY-NC-ND license.

forebrain cholinergic neurons were already known at the time to be among the comparatively small number of CNS neurons responding to NGF (528). The restricted response of CNS neurons to NGF was later explained by the finding that TrkA expression is limited to small populations of cholinergic neurons in the CNS (529), later found to also respond to BDNF (166).

The progressive cognitive decline and the shrinking or loss of CNS neurons characterizing AD both suggest that this condition would benefit from enhanced BDNF/TrkB signaling. This view is supported by findings reported in an impressive postmortem study indicating that the levels of *BDNF* mRNA inversely correlate with the rate of cognitive decline (498). In this study, RNA was extracted from the prefrontal cortex from >500 elderly patients and their rate of cognitive decline had been monitored over an average period of >6 yr preceding death (498). Higher levels of brain *BDNF* expression were associated with slower cognitive decline ($P < 0.001$). Cognitive decline was ~50% slower in the 90th percentile BDNF expression compared with the 10th percentile (498). The study also included histological examination of the tissue, and *BDNF* expression was found to be lower in individuals with typical AD features, with no association with macroscopic infarcts, Lewy body disease, or hippocampal sclerosis (498). An association between AD and BDNF was also uncovered in a bioinformatic analysis correlating differentially expressed genes with Braak stages, Aβ levels, and beta secretase activity (530). Also, the levels of TrkB have been repeatedly reported to be decreased in postmortem analyses of human brain and correlated with AD. Specifically, Aβ has been proposed to cause the cleavage of TrkB through a mechanism dependent on *N*-methyl-D-aspartate (NMDA) receptors and calcium influx, leading to calpain overactivation and TrkB cleavage (531). A number of human genetic analyses have also been conducted attempting to correlate neurotrophin signaling with either sporadic or inherited forms of AD. In one such study conducted in Italy including 151 sporadic AD patients, 100 familial AD patients and 97 healthy subjects, an association was found with the polymorphism rs1048218. This polymorphism causes a glutamine replacement by histidine at position 77 in the BDNF prodomain and the study revealed an allele-wise association with late-onset sporadic AD (158). So did the rs1047856 *NTRK2* polymorphism (leading to an asparagine replacement by tyrosine at position 338 in the second Ig-like domain) with sporadic AD in the same study. *NGFR* was also investigated and rs2072446 (causing a serine replacement by leucin in the extracellular domain) and an association found with familial AD (158). An association of the same rs2072446 polymorphism with sporadic AD and Abeta deposit was later found in a Chinese Han population (532).

The situation with the Val/Met polymorphism (rs6265) remained unclear for a long time, with most initial studies

failing to document a clear association (533). However, in healthy individuals followed over a period of 36 mo, Met carriers did show a significant decline in episodic memory, executive function, and language and greater hippocampal atrophy compared with Val/Val homozygotes, but only in the subpopulation with high Aβ accumulation (534). Irrespective of whether Val/Val BDNF carriers are able to prevent the accumulation of Aβ or are better protected against detrimental effects of Aβ accumulation, these results suggest a link between BDNF signaling and AD. In subsequent studies focused on dominantly inherited forms of AD consisting of individuals carrying mutations either in presenilin genes (*PSN1* or *PSN2*) or in *APP*, Met-BDNF carriers showed a faster memory decline, greater loss of hippocampal volume, and higher levels of tau and P181 phosphorylated tau in the cerebrospinal fluid (CSF) (535). These changes were not observed in the group without mutations in *PSN1, PSN2*, and *APP* (535). A follow-up study with 374 individuals further refined these findings and revealed that presymptomatic *PSN1, PSN2*, *APP* mutation carriers who also carry the *BDNF Met66* allele showed significantly poorer episodic memory, smaller hippocampal volume, and higher p-tau217, p-tau181, and total tau, compared with *BDNF Val66* homozygotes (536). In symptomatic mutation carriers, *BDNF Met 66* carriers showed poorer cognition and higher levels of p-tau217, total tau, and p-tau205, compared with *BDNF Val66* homozygotes (536). These results suggest then that increasing BDNF signaling during aging is likely to be beneficial. However, the question remains of how this could be achieved over prolonged periods of time. As discussed in previous sections, physical exercise would appear to be the best way to prevent neurodegeneration during aging. Obviously this is not a realistic option for many, given frequent associations with other pathologies restricting mobility. Promising alternatives would consist in the development of drugs improving BDNF/TrkB signaling over prolonged periods of time with minimal side effects as well as further development of techniques allowing noninvasive extracranial stimulation. The notion that increased levels of BDNF may help in improving brain function during aging is also supported by observations made with aging rhesus monkeys (age range: 21–27 yr) injected with lentivirus delivering BDNF in the entorhinal cortex and tested with visual discrimination tasks (537). BDNF-injected monkeys showed a clear improvement over GFP-lentivirus treatment when reassessed after 1 mo (537). This is all the more remarkable given that, as discussed in sect. 3, viral delivery of BDNF includes the complication of the codelivery of pro-BDNF. This is likely to be problematic given the notion that the $p75^{NTR}$ receptor binds and seems to be activated by Aβ causing the death of neurons (538–541).

In a recent phase 2a trial with a drug designated LM11A-31A known to prevent the binding of pro-NGF and of Aβ to $p75^{NTR}$ (542), twice daily oral administration to a cohort of patients presenting signs of early stages of AD revealed significant drug-to-placebo differences in structural magnetic resonance imaging, positron emission tomography, and CSF markers (543). However, no effects were observed in cognitive tests in this 26-mo-long trial (543). TrkB levels have been reported to be decreased in AD autopsy material, with Western blot experiments revealing a selective decrease of the catalytic form of TrkB compared with TrkB.T1 (269). This decrease was also observed when compared with the levels of TrkB in brain lysates of age-matched control subjects (269). Subsequently, TrkB was reported to be cleaved by Aβ by a calpain-dependent mechanism (531), a finding that led to the development of a cell-permeant compound preventing the cleavage of TrkB by calpain (544).

11.4. Huntington's Disease

Huntington's disease is a condition of special relevance with regard to BDNF/TrkB signaling. The pathology resulting from CAG triplet repeats is primarily caused by dysfunctional medium spiny neurons thought to result from an impaired or decreased supply of anterogradely transported BDNF from the cerebral cortex (40, 152, 545, 546). Early Western blot and immunohistochemical data generated with a polyclonal anti-BDNF serum and autopsy material suggested reduced BDNF levels in the caudate and putamen but normal levels in the parietal and temporal cortex and the hippocampus (547). Follow-up studies indicated that the wild-type product of the gene encoding Huntingtin is a positive regulator of the mouse *Bdnf* gene but not in its mutated form (548). Huntingtin has been suggested to interact with the transcription inhibitor REST and to keep it in the cytoplasm (62, 549). An additional physiopathological mechanism has been proposed for the mutant form of Huntingtin, namely interference with the anterograde transport of BDNF vesicles from the cortex with the wild-type protein thought to be part of the motor machinery driving vesicle transport (545). With regard to the relevance of animal models, the interesting observation has been made that cerebral cortex-specific excision of *Bdnf* causes transcriptional changes in the striatum more closely related to the changes observed in the human striatum of Huntington disease patients than those observed in the widely used R6/2 model of overexpression of the huntingtin gene (550). The R6/2 model refers to the insertion in the mouse genome of the promoter and exon 1 fragment of human gene with 150 CAG repeats. Molecular analyses of the changes caused by the CAG repeats in Huntington's disease revealed an overactivity

of a repressor complex consisting of REST-mSIN3a-mSIN3b-CoREST-HDAC. This complex silences transcription through REST binding to the RE1/NRSE silencer within the *BDNF* locus, normally prevented by wild-type Huntingtin (62, 551). In a small-molecule screen the REST complex formation was used to target the PAH1 domain of mSIN3b (112), but activating the BDNF/TrkB pathway in humans with drugs does not seem to have been successful thus far despite promising results in mouse models. Specifically, the upregulation of the levels of BDNF levels with the ampakine designated CX929, a positive allosteric regulator of AMPA-type receptors injected twice daily, rescued CA1 LTP in slices prepared from young mutant animals carrying 140 CAG repeats in the *Huntingtin* gene but did not improve locomotor deficits (552). Further emphasizing the role of physical exercise in increasing BDNF levels in the CNS (see sect. 10), detailed guidelines and recommendations have been published to help patients suffering from Huntington's disease underlining the benefits of specific types of physical exercise (553).

11.5. Parkinson's Disease

Dopaminergic neurons were recognized early on to respond to BDNF: its addition to neurons dissociated from the embryonic rat mesencephalon increased the survival of these neurons (554). Since then, numerous studies in rodent models have confirmed that BDNF can prevent the death of dopaminergic neurons. However, with regard to the use of growth factors in Parkinson's disease patients it is GDNF that has dominated the field. As indicated in sect. 2, GDNF was discovered with an approach focused on the monitoring of dopamine uptake by mesencephalic neurons (20). This may explain why a first clinical trial could be initiated only 3 years after the first publication of the GDNF sequence (26). The trials involved both injections of the protein into the striatum and viral delivery using adenoviruses (26). As the success of growth factor treatment depends on the presence of the receptor at the cell surface, it is important to consider the impact of existing therapies on the TrkB pathway. In the case of Parkinson's disease, patients will have typically been treated with L-DOPA, as this therapy offers considerable, albeit temporary benefits. A well-known side effect of long-term L-DOPA treatment is dyskinesia, and the role played by TrkB in this context has been recently investigated in a hemiparkinsonism mouse model consisting of a unilateral 6-hydroxydopamine injection into the right medial forebrain bundle (555). This study involved >350 animals, and chronic L-DOPA treatment was found to increase protein and mRNA levels of TrkB compared to the contralateral side or to sham-operated animals. These increases were shown to be mediated by the dopamine receptor D1 (555). It turned out that the selective deletion of TrkB in neurons expressing D1 as opposed to D2 receptor aggravated L-DOPA-induced dyskinesia, suggesting a protective role of BDNF/TrkB signaling in L-DOPA-induced dyskinesia (555). Interestingly, a related study emphasized the role of dopamine in facilitating the cell surface exposure of TrkB through activation of dopamine receptor D1 (346). Activation of this receptor subtype was found to enhance the translocation of TrkB to the cell surface, whereas, conversely, intracellular clusters of TrkB were observed in the striatum not only of rats treated with 6-hydroxydopamine but also in postmortem samples of Parkinson's patients (346).

11.6. Glaucoma

Glaucoma is a widespread eye condition affecting tens of millions worldwide (556). It is characterized by a gradual increase of intraocular pressure and the progressive degeneration of retinal ganglion cells accompanied by a decrease of the visual field. Beyond attempts to decrease intraocular pressure in affected individuals, therapeutic options are currently limited. Retinal ganglion cells have long been recognized to respond to BDNF, with initial studies indicating that BDNF increased the survival of these cells dissociated from perinatal rat (46) and embryonic chick (557) retinas. A growth-promoting effect of BDNF was also noted in explants of adult rat retinas (558). Later studies centered on the dendrites of retinal ganglion cells indicated that BDNF can delay the progressive retraction of retinal ganglion cell dendrites, even when BDNF was added 3 days after explantation of adult mouse retina (559). Beyond in vitro effects, BDNF has also long been shown to promote the survival and axonal elongation of retinal ganglion cells in vivo after lesions of the optic nerve (45, 560) or after ischemic injury (561). TrkB is known to be expressed by neonatal and adult rat retinal ganglion cells (562), and in *Bdnf*-null mutants the diameter of retinal ganglion cell axons is decreased (381). Given this background and the critical notion that that the dendrites of retinal ganglion cells undergo a prolonged period of atrophy preceding cell death (563), a desirable objective is to activate TrkB early in the disease to delay the progressive deterioration of vision. Given that VEGF signaling antagonists are now routinely injected into patients' eyes to combat age-related macular degeneration caused by the proliferation of blood vessels (564), injection of TrkB agonists into the eye of glaucoma patients seems to be a realistic prospect. TrkB agonists including antibodies would seem preferable to BDNF itself given their selectivity toward TrkB activation as opposed to $p75^{NTR}$, whose

activation may be undesirable as noted in previous sections. A number of TrkB-activating antibodies have been developed (250, 565–568), and the effects of one of them, designated 1D7, examined after injection into the eye of rats subjected to unilateral section of the optic nerve or cauterization of three scleral veins causing sustained increased intraocular pressure (568). When the survival of retinal ganglion cells was assessed 2 wk after axotomy and 42 days in the glaucoma model, significant differences were observed in the 1D7-injected animals compared not only with a control antibody but also with BDNF-injected animals (568). Detailed comparative analyses later performed with human neurons incubated with a TrkB activating antibody designated ZEB85 raised against human TrkB revealed that it mimics the transcriptional changes caused by BDNF or NT4 activation of TrkB, with regard to both the multiple targets that are up- and downregulated after TrkB activation as well as the time course of the changes (250).

12. CANCER

12.1. Introduction

As recapitulated in sect. 1, tumor biology played an important role in the history of the neurotrophin field. As tyrosine kinase receptors transduce some of the key biological activities of neurotrophins, it is not surprising that TrkB has been implicated in malignancies given the involvement of tyrosine kinases in multiple forms of cancer (for review, see Ref. 569).

12.2. Trk Receptors in Tumors outside the Nervous System

The three Trk receptors have been systematically examined for their oncogenic potential as fusion proteins and found to account for ~1% of solid tumors (570, 571). These include secretory carcinoma of the salivary gland, infantile fibrosarcoma, thyroid, colon, lung, and gastrointestinal tumors, as well as melanoma (571). A significant number of patients benefited from the Breakthrough Therapy FDA approval for the Trk inhibitor larotrectinib for adult and pediatric patients with solid tumors (572). A study including >7,300 patients with hematologic malignancies uncovered *TRK* fusions in 0.1% of patients with either acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), histiocytosis, multiple myeloma, or dendritic cell neoplasms (572). Responsiveness to Trk inhibition by larotrectinib could be detected both in vitro and in vivo (570). A potential difficulty in the field may result from the extensive use of mouse models to try and analyze the significance and relevance of the BDNF/TrkB pathway in tumors. As discussed in sect. 9, the blood levels of BDNF levels in mice versus humans differ by ~1,000-fold (510). Although in humans plasma levels of BDNF are sufficient to activate TrkB, this is unlikely to be the case in the mouse.

12.3. Neuroblastoma and Medulloblastoma

There has been considerable interest in the role of the Trk receptors in neuroblastoma, one of the most common pediatric tumors (573, 574). Neuroblastoma cells derive from the neural crest, and the spontaneous regression of neuroblastoma resembles the developmentally regulated programmed cell death of PNS neurons (575). In these tumor cells the Netrin-1 receptor UNC5D was found to be highly expressed in neuroblastoma with a favorable outcome. UNC5D is cleaved by caspases 2/3 in the absence of Netrin-1 and the released intracellular fragment translocated into the nucleus to selectively transactivate proapoptotic target genes (575). Remarkably, mice lacking *Unc5d* have increased numbers of DRG neurons, and their sympathetic neurons are less dependent on NGF for their survival (575). The majority of favorable neuroblastomas express high levels of TrkA, an observation in line with the serendipitous discovery that TrkA causes the death of neurons expressing this receptor in the absence of NGF (see sect. 4). The same observation was also made with developing neurons expressing TrkC in the PNS, in line with the previous discovery that TrkC is a dependence receptor (576), i.e., a receptor that causes the death of the cells expressing it in the absence of ligands as described for UNC5D (577). As noted in sect. 4, subsequent experiments revealed that in mice lacking both NT3 and TrkC cell death is decreased in the DRG compared with mice lacking just NT3 (272). Importantly, tumor cells expressing TrkB have also been noted to express the *BDNF* gene at a significant level (573, 574) and TrkB-expressing neuroblastomas are associated with a worse prognosis compared with those expressing TrkA or TrkC (574). This is in line with the finding that TrkB does not cause cell death in the absence of ligand (Ref. 270; see sect. 4).

12.4. Glioma

Even if this specific type of brain tumor was recognized very early on (578), glioma remains a condition that is exceedingly difficult to treat. Recently, some of the reasons why this is so have begun to emerge and may relate to the deep integration of glioma cells within brain tissue. Patient-derived tumor cells grown in culture for extended periods of time and subsequently transplanted into the mouse brain were found to extend

lengthy membrane protrusions designated microtubes interconnecting cells over long distances through gap junctions (579). These microtubes were also observed in glioma resected from patients (579). Neurons were then found to even form chemical synapses along these microtubes and to generate postsynaptic currents mediated by AMPA receptors that contribute to tumor growth and invasiveness (580). Optogenetic control of cortical neuronal activity in mice transplanted with patient-derived pediatric glioblastoma cells had previously revealed that neuronal activity promotes cell proliferation (581). Remarkably, culture medium from active cortical slices was found to promote the growth of cultured tumor cells, with a soluble form of Neuroligin-3 identified as one of the mitogenic components (581). Subsequent work by the same group implicated BDNF as a promoter of plasticity in these malignant synapses, with TrkB activation enhancing AMPA receptor trafficking (582). Crucially, TrkB inactivation with entrectinib, an alternative to larotrectinib as Trk inhibitor, prolonged the survival of mouse recipients in the xenograft model (582).

13. CONCLUSION AND PERSPECTIVE

This review emphasizes throughout the limited availability of BDNF in the nervous system as the central feature of its physiopathology. As BDNF is a key mediator of activity-dependent synaptic plasticity, any decrease of its levels can be predicted to be detrimental, as exemplified by decreased memory performance and cognitive decline during aging in humans. The tyrosine kinase receptor TrkB transduces the bulk of the trophic effects of BDNF following its temporary activation by BDNF released from presynaptic nerve terminals. The failures of the small number of BDNF clinical trials may have been caused by the continuous delivery of large quantities of BDNF, resulting in the prolonged downregulation of TrkB. Local applications of minimal amounts of selective TrkB agonists avoiding activation of the $p75^{NTR}$ pathway are thus worth considering in future trials. These may include indications such as glaucoma since repeated, local delivery is an option as illustrated by work with anti-VEGF reagents in neovascular glaucoma (583). The role of physical activity toward increasing BDNF levels in functionally relevant brain areas has been thoroughly documented in animal experiments, and there is no reason to hypothesize that the situation may be different in humans. Unfortunately, physical activity is often not an option in some of the conditions where maintaining BDNF levels in the CNS would be particularly desirable including depression and old age. It is conceivable that in the future further developments of noninvasive brain stimulation methods may represent a useful alternative to physical exercise. With regard to more traditional, pharmacological approaches, success with positive allosteric modulators of AMPA receptors has been limited thus far (for review, see Ref. 584). However, excitatory neurotransmission can be modulated in multiple ways in the CNS, and, as discussed in sect. 8, most antidepressants seem to act by potentiating the TrkB/BDNF pathway. There is renewed hope then that traditional pharmacological approaches targeting endogenous receptors may be used to increase BDNF levels and/or enhance BDNF/TrkB signaling.

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ACKNOWLEDGMENTS

The comments of Peter DiStefano, Ron Lindsay, Neil McDonald, and Michael Sendtner on early versions of this review are gratefully acknowledged. The unfailing support of Hans Thoenen was key during the decade-long effort leading to the purification and molecular cloning of BDNF, work that was also greatly facilitated by the environment and working conditions provided by the Max Planck Society.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

Y.-A.B. prepared figures; drafted manuscript; edited and revised manuscript; and approved final version of manuscript.

REFERENCES

1. Hamburger V**.** The history of the discovery of the nerve growth factor. **J Neurobiol** 24: 893–897, 1993. doi:10.1002/neu.480240702.

2. Bueker ED**.** Implantation of tumors in the hind limb field of the embryonic chick and the developmental response of the lumbosacral nervous system. **Anat Rec** 102: 369–389, 1948. doi:10.1002/ar.1091020309.

3. Cohen S, Levi-Montalcini R**.** Purification and properties of a nerve growth-promoting factor isolated from mouse sarcoma 180. **Cancer Res** 17: 15–20, 1957.

4. Barde YA, Lindsay RM, Monard D, Thoenen H**.** New factor released by cultured glioma cells supporting survival and

growth of sensory neurones. **Nature** 274: 818, 1978. doi:10.1038/274818a0.

5. Lindsay RM, Thoenen H, Barde YA**.** Placode and neural crest-derived sensory neurons are responsive at early developmental stages to brain-derived neurotrophic factor. **Dev Biol** 112: 319–328, 1985. doi:10.1016/0012-1606(85)90402-6.

6. Morita K, Her S**.** Progesterone pretreatment enhances serotonin-stimulated BDNF gene expression in rat c6 glioma cells through production of 5alpha-reduced neurosteroids. **J Mol Neurosci** 34: 193–200, 2008. doi:10.1007/s12031-007-9034-6.

7. Keasey MP, Kang SS, Lovins C, Hagg T**.** Inhibition of a novel specific neuroglial integrin signaling pathway increases STAT3-mediated CNTF expression. **Cell Commun Signal** 11: 35, 2013. doi:10.1186/1478-811X-11-35.

8. Martin-Zanca D, Hughes SH, Barbacid M**.** A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. **Nature** 319: 743–748, 1986. doi:10.1038/319743a0.

9. Levi-Montalcini R, Booker B**.** Destruction of the sympathetic ganglia in mammals by an antiserum to a nerve-growth protein. **Proc Natl Acad Sci USA** 46: 384–391, 1960. doi:10.1073/pnas.46.3.384.

10. Cohen S**.** Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum. **Proc Natl Acad Sci USA** 46: 302–311, 1960. doi:10.1073/pnas.46.3.302.

11. Ellis RE, Yuan JY, Horvitz HR**.** Mechanisms and functions of cell death. **Annu Rev Cell Biol** 7: 663–698, 1991. doi:10.1146/annurev.cb.07.110191.003311.

12. Jacobson MD, Weil M, Raff MC**.** Programmed cell death in animal development. **Cell** 88: 347–354, 1997. doi:10.1016/s0092-8674(00)81873-5.

13. Bothwell M**.** NGF, BDNF, NT3, and NT4. **Handb Exp Pharmacol** 220: 3–15, 2014. doi:10.1007/978-3-642-45106-5_1.

14. Hamburger V**.** History of the discovery of neuronal death in embryos. **J Neurobiol** 23: 1116–1123, 1992. doi:10.1002/neu.480230904.

15. Hamburger V, Brunso-Bechtold JK, Yip JW**.** Neuronal death in the spinal ganglia of the chick embryo and its reduction by nerve growth factor. **J Neurosci** 1: 60–71, 1981. doi:10.1523/JNEUROSCI.01-01-00060.1981.

16. McEwan AR, Hing B, Erickson JC, Hutchings G, Urama C, Norton-Hughes E, D'Ippolito M, Berry S, Delibegovic M, Grassmann F, MacKenzie A**.** An ancient polymorphic regulatory region within the BDNF gene associated with obesity modulates anxiety-like behaviour in mice and humans. **Mol Psychiatry** 29: 660–670, 2024. doi:10.1038/s41380-023-02359-7.

17. Esvald EE, Tuvikene J, Sirp A, Patil S, Bramham CR, Timmusk T**.** CREB family transcription factors are major mediators of BDNF transcriptional autoregulation in cortical neurons. **J Neurosci** 40: 1405–1426, 2020. doi:10.1523/JNEUROSCI.0367-19.2019.

18. Gorski JA, Zeiler SR, Tamowski S, Jones KR**.** Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites. **J Neurosci** 23: 6856–6865, 2003. doi:10.1523/JNEUROSCI.23-17-06856.2003.

19. Purves D**. Body and Brain Trophic Theory of Neural Connections**. Harvard University Press, 1988, p. 231.

20. Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F**.** GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. **Science** 260: 1130–1132, 1993. doi:10.1126science/.8493557.

21. Hofer MM, Barde YA**.** Brain-derived neurotrophic factor prevents neuronal death in vivo. **Nature** 331: 261–262, 1988. doi:10.1038/331261a0.

22. Leibrock J, Lottspeich F, Hohn A, Hofer M, Hengerer B, Masiakowski P, Thoenen H, Barde YA**.** Molecular cloning and expression of brain-derived neurotrophic factor. **Nature** 341: 149–152, 1989. doi:10.1038/341149a0.

23. Barde YA, Edgar D, Thoenen H**.** Purification of a new neurotrophic factor from mammalian brain. **EMBO J** 1: 549–553, 1982. doi:10.1002/j.1460-2075.1982.tb01207.x.

24. Kolbeck R, Jungbluth S, Barde YA**.** Characterisation of neurotrophin dimers and monomers. **Eur J Biochem** 225: 995–1003, 1994. doi:10.1111/j.1432-1033.1994.0995b.x.

25. Kaisho Y, Yoshimura K, Nakahama K**.** Cloning and expression of a cDNA encoding a novel human neurotrophic factor. **FEBS Lett** 266: 187–191, 1990. doi:10.1016/0014-5793(90)81536-w.

26. Barker RA, Bjorklund A, Gash DM, Whone A, Van Laar A, Kordower JH, et al**.** GDNF and Parkinson's disease: where next? A summary from a recent workshop. **J Parkinsons Dis** 10: 875–891, 2020. doi:10.3233/JPD-202004.

27. Hohn A, Leibrock J, Bailey K, Barde YA**.** Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. **Nature** 344: 339–341, 1990. doi:10.1038/344339a0.

28. Maisonpierre PC, Belluscio L, Squinto S, Ip NY, Furth ME, Lindsay RM, Yancopoulos GD**.** Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. **Science** 247: 1446–1451, 1990. doi:10.1126/science.247.4949.1446.

29. Jones KR, Reichardt LF**.** Molecular cloning of a human gene that is a member of the nerve growth factor family. **Proc Natl Acad Sci USA** 87: 8060–8064, 1990. doi:10.1073/pnas.87.20.8060.

30. Ernfors P, Ibáñez CF, Ebendal T, Olson L, Persson H**.** Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: developmental and topographical expression in the brain. **Proc Natl Acad Sci USA** 87: 5454–5458, 1990. doi:10.1073/pnas.87.14.5454.

31. Rosenthal A, Goeddel DV, Nguyen T, Lewis M, Shih A, Laramee GR, Nikolics K, Winslow JW**.** Primary structure and biological activity of a novel human neurotrophic factor. **Neuron** 4: 767–773, 1990. doi:10.1016/0896-6273(90)90203-r.

32. Hallböök F, Ibáñez CF, Persson H**.** Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in *Xenopus* ovary. **Neuron** 6: 845–858, 1991. doi:10.1016/0896-6273(91)90180-8.

33. Ip NY, Ibáñez CF, Nye SH, McClain J, Jones PF, Gies DR, Belluscio L, Le Beau MM, Espinosa R 3rd, Squinto SP**.** Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity. **Proc Natl Acad Sci USA** 89: 3060–3064, 1992. doi:10.1073/pnas.89.7.3060.

34. Lucaci AG, Notaras MJ, Kosakovsky Pond SL, Colak D**.** The evolution of BDNF is defined by strict purifying selection and prodomain spatial coevolution, but what does it mean for human brain disease? **Transl Psychiatry** 12: 258, 2022. doi:10.1038/s41398-022-02021-w.

35. Oppenheim RW. Cell death during development of the nervous system. **Annu Rev Neurosci** 14: 453–501, 1991. doi:10.1146/annurev.ne.14.030191.002321.

36. Davies AM, Thoenen H, Barde YA. The response of chick sensory neurons to brain-derived neurotrophic factor. **J Neurosci** 6: 1897–1904, 1986. doi:10.1523/JNEUROSCI.06-07-01897.1986.

37. Hefti F. Nerve growth factor promotes survival of septal cholinergic neurons after fimbrial transections. **J Neurosci** 6: 2155–2162, 1986. doi:10.1523/JNEUROSCI.06-08-02155.1986.

38. Ernfors P, Lee KF, Jaenisch R. Mice lacking brain-derived neurotrophic factor develop with sensory deficits. **Nature** 368: 147–150, 1994. doi:10.1038/368147a0.

39. Jones KR, Fariñas I, Backus C, Reichardt LF. Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. **Cell** 76: 989–999, 1994. doi:10.1016/0092-8674(94)90377-8.

40. Baquet ZC, Gorski JA, Jones KR. 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. doi:10.1523/JNEUROSCI.3920-03.2004.

41. Rauskolb S, Zagrebelsky M, Dreznjak A, Deogracias R, Matsumoto T, Wiese S, Erne B, Sendtner M, Schaeren-Wiemers N, Korte M, Barde YA. Global deprivation of brain-derived neurotrophic factor in the CNS reveals an area-specific requirement for dendritic growth. **J Neurosci** 30: 1739–1749, 2010. doi:10.1523/JNEUROSCI.5100-09.2010.

42. Liu X, Ernfors P, Wu H, Jaenisch R. Sensory but not motor neuron deficits in mice lacking NT4 and BDNF. **Nature** 375: 238–241, 1995. doi:10.1038/375238a0.

43. Sendtner M, Holtmann B, Kolbeck R, Thoenen H, Barde YA. Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. **Nature** 360: 757–759, 1992. doi:10.1038/360757a0.

44. Yan Q, Elliott J, Snider WD. Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. **Nature** 360: 753–755, 1992. doi:10.1038/360753a0.

45. Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ. Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. **Proc Natl Acad Sci USA** 91: 1632–1636, 1994. doi:10.1073/pnas.91.5.1632.

46. Johnson JE, Barde YA, Schwab M, Thoenen H. Brain-derived neurotrophic factor supports the survival of cultured rat retinal ganglion cells. **J Neurosci** 6: 3031–3038, 1986. doi:10.1523/JNEUROSCI.06-10-03031.1986.

47. Baquet ZC, Bickford PC, Jones KR. Brain-derived neurotrophic factor is required for the establishment of the proper number of dopaminergic neurons in the substantia nigra pars compacta. **J Neurosci** 25: 6251–6259, 2005. doi:10.1523/JNEUROSCI.4601-04.2005.

48. Esvald EE, Tuvikene J, Kiir CS, Avarlaid A, Tamberg L, Sirp A, Shubina A, Cabrera-Cabrera F, Pihlak A, Koppel I, Palm K, Timmusk T. Revisiting the expression of BDNF and its receptors in mammalian development. **Front Mol Neurosci** 16: 1182499, 2023. doi:10.3389/fnmol.2023.1182499.

49. Koppel I, Tuvikene J, Lekk I, Timmusk T. Efficient use of a translation start codon in BDNF exon I. **J Neurochem** 134: 1015–1025, 2015. doi:10.1111/jnc.13124.

50. Liu QR, Lu L, Zhu XG, Gong JP, Shaham Y, Uhl GR. Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. **Brain Res** 1067: 1–12, 2006. doi:10.1016/j.brainres.2005.10.004.

51. 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. doi:10.1002/jnr.21139.

52. 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. doi:10.1016/j.ygeno.2007.05.004.

53. Modarresi F, Pedram Fatemi R, Razavipour SF, Ricciardi N, Makhmutova M, Khoury N, Magistri M, Volmar CH, Wahlestedt C, Faghihi MA. A novel knockout mouse model of the noncoding antisense Brain-Derived Neurotrophic Factor (Bdnf) gene displays increased endogenous Bdnf protein and improved memory function following exercise. **Heliyon** 7: e07570, 2021. doi:10.1016/j.heliyon.2021.e07570.

54. Zafra F, Hengerer B, Leibrock J, Thoenen H, Lindholm D. Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. **EMBO J** 9: 3545–3550, 1990. doi:10.1002/j.1460-2075.1990.tb07564.x.

55. Isackson PJ, Huntsman MM, Murray KD, Gall CM. BDNF mRNA expression is increased in adult rat forebrain after limbic seizures: temporal patterns of induction distinct from NGF. **Neuron** 6: 937–948, 1991. doi:10.1016/0896-6273(91)90234-q.

56. Castrén E, Zafra F, Thoenen H, Lindholm D. Light regulates expression of brain-derived neurotrophic factor mRNA in rat visual cortex. **Proc Natl Acad Sci USA** 89: 9444–9448, 1992. doi:10.1073/pnas.89.20.9444.

57. Herzog KH, Bailey K, Barde YA. Expression of the BDNF gene in the developing visual system of the chick. **Development** 120: 1643–1649, 1994. doi:10.1242/dev.120.6.1643.

58. Hong EJ, McCord AE, Greenberg ME. A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. **Neuron** 60: 610–624, 2008. doi:10.1016/j.neuron.2008.09.024.

59. West AE, Pruunsild P, Timmusk T. Neurotrophins: transcription and translation. **Handb Exp Pharmacol** 220: 67–100, 2014. doi:10.1007/978-3-642-45106-5_4.

60. You H, Lu B. Diverse functions of multiple Bdnf transcripts driven by distinct Bdnf promoters. **Biomolecules** 13: 655, 2023. doi:10.3390/biom13040655.

61. Tuvikene J, Pruunsild P, Orav E, Esvald EE, Timmusk T. AP-1 transcription factors mediate BDNF-positive feedback loop in cortical neurons. **J Neurosci** 36: 1290–1305, 2016. doi:10.1523/JNEUROSCI.3360-15.2016.

62. Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, Cataudella T, Leavitt BR, Hayden MR, Timmusk T, Rigamonti D, Cattaneo E. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. **Nat Genet** 35: 76–83, 2003. doi:10.1038/ng1219.

63. 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. doi:10.1073/pnas.90.19.8802.

64. Tao X, Finkbeiner S, Arnold DB, Shaywitz AJ, Greenberg ME. Ca^{2+} influx regulates BDNF transcription by a CREB family transcription

factor-dependent mechanism. **Neuron** 20: 709–726, 1998. doi:10.1016/s0896-6273(00)81010-7.

65. Shieh PB, Hu SC, Bobb K, Timmusk T, Ghosh A. Identification of a signaling pathway involved in calcium regulation of BDNF expression. **Neuron** 20: 727–740, 1998. doi:10.1016/s0896-6273(00)81011-9.

66. Tao X, West AE, Chen WG, Corfas G, Greenberg ME. A calcium-responsive transcription factor, CaRF, that regulates neuronal activity-dependent expression of BDNF. **Neuron** 33: 383–395, 2002. doi:10.1016/s0896-6273(01)00561-x.

67. Flavell SW, Greenberg ME. Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. **Annu Rev Neurosci** 31: 563–590, 2008. doi:10.1146/annurev.neuro.31.060407.125631.

68. McDowell KA, Hutchinson AN, Wong-Goodrich SJ, Presby MM, Su D, Rodriguiz RM, Law KC, Williams CL, Wetsel WC, West AE. Reduced cortical BDNF expression and aberrant memory in Carf knock-out mice. **J Neurosci** 30: 7453–7465, 2010. doi:10.1523/JNEUROSCI.3997-09.2010.

69. Lyons MR, Chen LF, Deng JV, Finn C, Pfenning AR, Sabhlok A, Wilson KM, West AE. The transcription factor calcium-response factor limits NMDA receptor-dependent transcription in the developing brain. **J Neurochem** 137: 164–176, 2016. doi:10.1111/jnc.13556.

70. Esvald EE, Tuvikene J, Moistus A, Rannaste K, Kõomägi S, Timmusk T. Differential regulation of the BDNF gene in cortical and hippocampal neurons. **J Neurosci** 42: 9110–9128, 2022. doi:10.1523/JNEUROSCI.2535-21.2022.

71. Bach SV, Bauman AJ, Hosein D, Tuscher JJ, Ianov L, Greathouse KM, Henderson BW, Herskowitz JH, Martinowich K, Day JJ. Distinct roles of Bdnf I and Bdnf IV transcript variant expression in hippocampal neurons. **Hippocampus** 34: 218–229, 2024. doi:10.1002/hipo.23600.

72. Hill JL, Martinowich K. Activity-dependent signaling: influence on plasticity in circuits controlling fear-related behavior. **Curr Opin Neurobiol** 36: 59–65, 2016. doi:10.1016/j.conb.2015.10.001.

73. Hallock HL, Quillian HM, Maynard KR, Mai Y, Chen HY, Hamersky GR, Shin JH, Maher BJ, Jaffe AE, Martinowich K. Molecularly defined hippocampal inputs regulate population dynamics in the prelimbic cortex to suppress context fear memory retrieval. **Biol Psychiatry** 88: 554–565, 2020. doi:10.1016/j.biopsych.2020.04.014.

74. Maynard KR, Hill JL, Calcaterra NE, Palko ME, Kardian A, Paredes D, Sukumar M, Adler BD, Jimenez DV, Schloesser RJ, Tessarollo L, Lu B, Martinowich K. Functional Role of BDNF production from unique promoters in aggression and serotonin signaling. **Neuropsychopharmacology** 41: 1943–1955, 2016. doi:10.1038/npp.2015.349.

75. Maynard KR, Hobbs JW, Phan BN, Gupta A, Rajpurohit S, Williams C, Rajpurohit A, Shin JH, Jaffe AE, Martinowich K. BDNF-TrkB signaling in oxytocin neurons contributes to maternal behavior. **eLife** 7: e33676, 2018. doi:10.7554/eLife.33676.

76. McAllan L, Maynard KR, Kardian AS, Stayton AS, Fox SL, Stephenson EJ, Kinney CE, Alshibli NK, Gomes CK, Pierre JF, Puchowicz MA, Bridges D, Martinowich K, Han JC. Disruption of brain-derived neurotrophic factor production from individual promoters generates distinct body composition phenotypes in mice. **Am J Physiol Endocrinol Metab** 315: E1168–E1184, 2018. doi:10.1152/ajpendo.00205.2018.

77. Lin Y, Bloodgood BL, Hauser JL, Lapan AD, Koon AC, Kim TK, Hu LS, Malik AN, Greenberg ME. Activity-dependent regulation of inhibitory synapse development by Npas4. **Nature** 455: 1198–1204, 2008. doi:10.1038/nature07319.

78. Bloodgood BL, Sharma N, Browne HA, Trepman AZ, Greenberg ME. The activity-dependent transcription factor NPAS4 regulates domain-specific inhibition. **Nature** 503: 121–125, 2013. doi:10.1038/nature12743.

79. Pollina EA, Gilliam DT, Landau AT, Lin C, Pajarillo N, Davis CP, Harmin DA, Yap EL, Vogel IR, Griffith EC, Nagy MA, Ling E, Duffy EE, Sabatini BL, Weitz CJ, Greenberg ME. A NPAS4-NuA4 complex couples synaptic activity to DNA repair. **Nature** 614: 732–741, 2023. doi:10.1038/s41586-023-05711-7.

80. Lubin FD, Sweatt JD. The IkappaB kinase regulates chromatin structure during reconsolidation of conditioned fear memories. **Neuron** 55: 942–957, 2007. doi:10.1016/j.neuron.2007.07.039.

81. Tuvikene J, Esvald EE, Rähni A, Uustalu K, Zhuravskaya A, Avarlaid A, Makeyev EV, Timmusk T. Intronic enhancer region governs transcript-specific Bdnf expression in rodent neurons. **eLife** 10, 2021. doi:10.7554/eLife.65161.

82. Acheson A, Conover JC, Fandl JP, DeChiara TM, Russell M, Thadani A, Squinto SP, Yancopoulos GD, Lindsay RM. A BDNF autocrine loop in adult sensory neurons prevents cell death. **Nature** 374: 450–453, 1995. doi:10.1038/374450a0.

83. Huber K, Kuehnel F, Wyatt S, Davies AM. TrkB expression and early sensory neuron survival are independent of endogenous BDNF. **J Neurosci Res** 59: 372–378, 2000. doi:10.1002/(SICI)1097-4547(20000201)59:3<372::AID-JNR11>3.3.CO;2-6.

84. English CN, Vigers AJ, Jones KR. Genetic evidence that brain-derived neurotrophic factor mediates competitive interactions between individual cortical neurons. **Proc Natl Acad Sci USA** 109: 19456–19461, 2012. doi:10.1073/pnas.1206492109.

85. Yasuda M, Fukuchi M, Tabuchi A, Kawahara M, Tsuneki H, Azuma Y, Chiba Y, Tsuda M. Robust stimulation of TrkB induces delayed increases in BDNF and Arc mRNA expressions in cultured rat cortical neurons via distinct mechanisms. **J Neurochem** 103: 626–636, 2007. doi:10.1111/j.1471-4159.2007.04851.x.

86. Ibarra IL, Ratnu VS, Gordillo L, Hwang IY, Mariani L, Weinand K, Hammarén HM, Heck J, Bulyk ML, Savitski MM, Zaugg JB, Noh KM. Comparative chromatin accessibility upon BDNF stimulation delineates neuronal regulatory elements. **Mol Syst Biol** 18: e10473, 2022. doi:10.15252/msb.202110473.

87. Buenrostro JD, Wu B, Chang HY, Greenleaf WJ. ATAC-seq: a method for assaying chromatin accessibility genome-wide. **Curr Protoc Mol Biol** 109: 21.29.1–21.29.9, 2015. doi:10.1002/0471142727.mb2129s109.

88. Carullo NVN, Phillips RA, Simon RC, Soto SAR, Hinds JE, Salisbury AJ, Revanna JS, Bunner KD, Ianov L, Sultan FA, Savell KE, Gersbach CA, Day JJ. Enhancer RNAs predict enhancer-gene regulatory links and are critical for enhancer function in neuronal systems. **Nucleic Acids Res** 48: 9550–9570, 2020. doi:10.1093/nar/gkaa671.

89. Hofer M, Pagliusi SR, Hohn A, Leibrock J, Barde YA. Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. **EMBO J** 9: 2459–2464, 1990. doi:10.1002/j.1460-2075.1990.tb07423.x.

90. An JJ, Gharami K, Liao GY, Woo NH, Lau AG, Vanevski F, Torre ER, Jones KR, Feng Y, Lu B, Xu B. Distinct role of long 3' UTR BDNF

mRNA in spine morphology and synaptic plasticity in hippocampal neurons. **Cell** 134: 175–187, 2008. doi:10.1016/j.cell.2008.05.045.

91. Baj G, Leone E, Chao MV, Tongiorgi E**.** Spatial segregation of BDNF transcripts enables BDNF to differentially shape distinct dendritic compartments. **Proc Natl Acad Sci USA** 108: 16813–16818, 2011. doi:10.1073/pnas.1014168108.

92. Will TJ, Tushev G, Kochen L, Nassim-Assir B, Cajigas IJ, Tom Dieck S, Schuman EM**.** Deep sequencing and high-resolution imaging reveal compartment-specific localization of Bdnf mRNA in hippocampal neurons. **Sci Signal** 6: rs16, 2013. doi:10.1126/scisignal.2004520.

93. Lekk I, Cabrera-Cabrera F, Turconi G, Tuvikene J, Esvald EE, Rähni A, Casserly L, Garton DR, Andressoo JO, Timmusk T, Koppel I**.** Untranslated regions of brain-derived neurotrophic factor mRNA control its translatability and subcellular localization. **J Biol Chem** 299: 102897, 2023. doi:10.1016/j.jbc.2023.102897.

94. Hing B, Sathyaputri L, Potash JB**.** A comprehensive review of genetic and epigenetic mechanisms that regulate BDNF expression and function with relevance to major depressive disorder. **Am J Med Genet B Neuropsychiatr Genet** 177: 143–167, 2018. doi:10.1002/ajmg.b.32616.

95. Nan X, Campoy FJ, Bird A**.** MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. **Cell** 88: 471–481, 1997. doi:10.1016/s0092-8674(00)81887-5.

96. Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A**.** Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. **Nature** 393: 386–389, 1998. doi:10.1038/30764.

97. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY**.** Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. **Nat Genet** 23: 185–188, 1999. doi:10.1038/13810.

98. Chen RZ, Akbarian S, Tudor M, Jaenisch R**.** Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. **Nat Genet** 27: 327–331, 2001. doi:10.1038/85906.

99. Guy J, Hendrich B, Holmes M, Martin JE, Bird A**.** A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. **Nat Genet** 27: 322–326, 2001. doi:10.1038/85899.

100. Guy J, Gan J, Selfridge J, Cobb S, Bird A**.** Reversal of neurological defects in a mouse model of Rett syndrome. **Science** 315: 1143–1147, 2007. doi:10.1126/science.1138389.

101. Chen WG, Chang Q, Lin Y, Meissner A, West AE, Griffith EC, Jaenisch R, Greenberg ME**.** Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. **Science** 302: 885–889, 2003. doi:10.1126/science.1086446.

102. Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y, Fan G, Sun YE**.** DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. **Science** 302: 890–893, 2003. doi:10.1126/science.1090842.

103. Dani VS, Chang Q, Maffei A, Turrigiano GG, Jaenisch R, Nelson SB**.** Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. **Proc Natl Acad Sci USA** 102: 12560–12565, 2005. doi:10.1073/pnas.0506071102.

104. Chang Q, Khare G, Dani V, Nelson S, Jaenisch R**.** The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. **Neuron** 49: 341–348, 2006. doi:10.1016/j.neuron.2005.12.027.

105. Palmieri M, Pozzer D, Landsberger N**.** Advanced genetic therapies for the treatment of Rett syndrome: state of the art and future perspectives. **Front Neurosci** 17: 1172805, 2023. doi:10.3389/fnins.2023.1172805.

106. Katz DM**.** Brain-derived neurotrophic factor and Rett syndrome. **Handb Exp Pharmacol** 220: 481–495, 2014. doi:10.1007/978-3-642-45106-5_18.

107. Deogracias R, Yazdani M, Dekkers MP, Guy J, Ionescu MC, Vogt KE, Barde YA**.** Fingolimod, a sphingosine-1 phosphate receptor modulator, increases BDNF levels and improves symptoms of a mouse model of Rett syndrome. **Proc Natl Acad Sci USA** 109: 14230–14235, 2012. doi:10.1073/pnas.1206093109.

108. Andrés ME, Burger C, Peral-Rubio MJ, Battaglioli E, Anderson ME, Grimes J, Dallman J, Ballas N, Mandel G**.** CoREST: a functional corepressor required for regulation of neural-specific gene expression. **Proc Natl Acad Sci USA** 96: 9873–9878, 1999. doi:10.1073/pnas.96.17.9873.

109. Huang Y, Myers SJ, Dingledine R**.** Transcriptional repression by REST: recruitment of Sin3A and histone deacetylase to neuronal genes. **Nat Neurosci** 2: 867–872, 1999. doi:10.1038/13165.

110. Hara D, Fukuchi M, Miyashita T, Tabuchi A, Takasaki I, Naruse Y, Mori N, Kondo T, Tsuda M**.** Remote control of activity-dependent BDNF gene promoter-I transcription mediated by REST/NRSF. **Biochem Biophys Res Commun** 384: 506–511, 2009. doi:10.1016/j.bbrc.2009.05.007.

111. Prestigio C, Ferrante D, Marte A, Romei A, Lignani G, Onofri F, Valente P, Benfenati F, Baldelli P**.** REST/NRSF drives homeostatic plasticity of inhibitory synapses in a target-dependent fashion. **eLife** 10: e69058, 2021. doi:10.7554/eLife.69058.

112. Conforti P, Zuccato C, Gaudenzi G, Ieraci A, Camnasio S, Buckley NJ, Mutti C, Cotelli F, Contini A, Cattaneo E**.** Binding of the repressor complex REST-mSIN3b by small molecules restores neuronal gene transcription in Huntington's disease models. **J Neurochem** 127: 22–35, 2013. doi:10.1111/jnc.12348.

113. Modarresi F, Faghihi MA, Lopez-Toledano MA, Fatemi RP, Magistri M, Brothers SP, van der Brug MP, Wahlestedt C**.** Inhibition of natural antisense transcripts in vivo results in gene-specific transcriptional upregulation. **Nat Biotechnol** 30: 453–459, 2012. doi:10.1038/nbt.2158.

114. Coda DM, Gräff J**.** From cellular to fear memory: an epigenetic toolbox to remember. **Curr Opin Neurobiol** 84: 102829, 2024. doi:10.1016/j.conb.2023.102829.

115. Bredy TW, Wu H, Crego C, Zellhoefer J, Sun YE, Barad M**.** Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. **Learn Mem** 14: 268–276, 2007. doi:10.1101/lm.500907.

116. Lubin FD, Roth TL, Sweatt JD**.** Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. **J Neurosci** 28: 10576–10586, 2008. doi:10.1523/JNEUROSCI.1786-08.2008.

117. Gräff J, Rei D, Guan JS, Wang WY, Seo J, Hennig KM, Nieland TJ, Fass DM, Kao PF, Kahn M, Su SC, Samiei A, Joseph N, Haggarty SJ, Delalle I, Tsai LH**.** An epigenetic blockade of cognitive functions in the neurodegenerating brain. **Nature** 483: 222–226, 2012. doi:10.1038/nature10849.

118. Koppel I, Timmusk T**.** Differential regulation of Bdnf expression in cortical neurons by class-selective histone deacetylase inhibitors. **Neuropharmacology** 75: 106–115, 2013. doi:10.1016/j.neuropharm.2013.07.015.

119. Boersma GJ, Lee RS, Cordner ZA, Ewald ER, Purcell RH, Moghadam AA, Tamashiro KL**.** Prenatal stress decreases Bdnf expression and increases methylation of Bdnf exon IV in rats. **Epigenetics** 9: 437–447, 2014. doi:10.4161/epi.27558.

120. Sada N, Fujita Y, Mizuta N, Ueno M, Furukawa T, Yamashita T**.** Inhibition of HDAC increases BDNF expression and promotes neuronal rewiring and functional recovery after brain injury. **Cell Death Dis** 11: 655, 2020. doi:10.1038/s41419-020-02897-w.

121. Sleiman SF, Henry J, Al-Haddad R, El Hayek L, Abou Haidar E, Stringer T, Ulja D, Karuppagounder SS, Holson EB, Ratan RR, Ninan I, Chao MV**.** Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body beta-hydroxybutyrate. **eLife** 5: e15092, 2016. doi:10.7554/eLife.15092.

122. Santoni G, Astori S, Leleu M, Glauser L, Zamora SA, Schioppa M, Tarulli I, Sandi C, Gräff J**.** Chromatin plasticity predetermines neuronal eligibility for memory trace formation. **Science** 385: eadg9982, 2024. doi:10.1126/science.adg9982.

123. Ribeiro-Davis A, Al Saeedy DY, Jahr FM, Hawkins E, McClay JL, Deshpande LS**.** Ketamine produces antidepressant effects by inhibiting histone deacetylases and upregulating hippocampal brain-derived neurotrophic factor levels in a diisopropyl fluorophosphate-based rat model of Gulf War illness. **J Pharmacol Exp Ther** 388: 647–654, 2024. doi:10.1124/jpet.123.001824.

124. McDonald NQ, Hendrickson WA**.** A structural superfamily of growth factors containing a cystine knot motif. **Cell** 73: 421–424, 1993. doi:10.1016/0092-8674(93)90127-c.

125. Suter U, Heymach JV Jr, Shooter EM**.** Two conserved domains in the NGF propeptide are necessary and sufficient for the biosynthesis of correctly processed and biologically active NGF. **EMBO J** 10: 2395–2400, 1991. doi:10.1002/j.1460-2075.1991.tb07778.x.

126. McDonald NQ, Lapatto R, Murray-Rust J, Gunning J, Wlodawer A, Blundell TL**.** New protein fold revealed by a 2.3-A resolution crystal structure of nerve growth factor. **Nature** 354: 411–414, 1991. doi:10.1038/354411a0.

127. Jungbluth S, Bailey K, Barde YA**.** Purification and characterisation of a brain-derived neurotrophic factor/neurotrophin-3 (BDNF/NT-3) heterodimer. **Eur J Biochem** 221: 677–685, 1994. doi:10.1111/j.1432-1033.1994.tb18780.x.

128. Negro A, Corsa V, Moretto C, Skaper SD, Callegaro L**.** Synthesis and purification of biologically active rat brain-derived neurotrophic factor from *Escherichia coli*. **Biochem Biophys Res Commun** 186: 1553–1559, 1992. doi:10.1016/s0006-291x(05)81584-0.

129. Coulie B, Szarka LA, Camilleri M, Burton DD, McKinzie S, Stambler N, Cedarbaum JM**.** Recombinant human neurotrophic factors accelerate colonic transit and relieve constipation in humans. **Gastroenterology** 119: 41–50, 2000. doi:10.1053/gast.2000.8553.

130. Matsumoto T, Rauskolb S, Polack M, Klose J, Kolbeck R, Korte M, Barde YA**.** Biosynthesis and processing of endogenous BDNF: CNS neurons store and secrete BDNF, not pro-BDNF. **Nat Neurosci** 11: 131–133, 2008. doi:10.1038/nn2038.

131. Uegaki K, Kumanogoh H, Mizui T, Hirokawa T, Ishikawa Y, Kojima M**.** BDNF binds its pro-peptide with high affinity and the common Val66Met polymorphism attenuates the interaction. **Int J Mol Sci** 18, 2017. doi:10.3390/ijms18051042.

132. Dieni S, Matsumoto T, Dekkers M, Rauskolb S, Ionescu MS, Deogracias R, Gundelfinger ED, Kojima M, Nestel S, Frotscher M, Barde YA**.** BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. **J Cell Biol** 196: 775–788, 2012. doi:10.1083/jcb.201201038.

133. Bothwell MA, Shooter EM**.** Dissociation equilibrium constant of beta nerve growth factor. **J Biol Chem** 252: 8532–8536, 1977. doi:10.1016/S0021-9258(19)75252-1.

134. Radziejewski C, Robinson RC**.** Heterodimers of the neurotrophic factors: formation, isolation, and differential stability. **Biochemistry** 32: 13350–13356, 1993. doi:10.1021/bi00211a049.

135. Arakawa T, Haniu M, Narhi LO, Miller JA, Talvenheimo J, Philo JS, Chute HT, Matheson C, Carnahan J, Louis JC**.** Formation of heterodimers from three neurotrophins, nerve growth factor, neurotrophin-3, and brain-derived neurotrophic factor. **J Biol Chem** 269: 27833–27839, 1994. doi:10.1016/S0021-9258(18)46862-7.

136. Robinson RC, Radziejewski C, Stuart DI, Jones EY**.** Structure of the brain-derived neurotrophic factor/neurotrophin 3 heterodimer. **Biochemistry** 34: 4139–4146, 1995. doi:10.1021/bi00013a001.

137. Robinson RC, Radziejewski C, Spraggon G, Greenwald J, Kostura MR, Burtnick LD, Stuart DI, Choe S, Jones EY**.** The structures of the neurotrophin 4 homodimer and the brain-derived neurotrophic factor/neurotrophin 4 heterodimer reveal a common Trk-binding site. **Protein Sci** 8: 2589–2597, 1999. doi:10.1110/ps.8.12.2589.

138. Benicky J, Sanda M, Brnakova Kennedy Z, Goldman R**.** N-Glycosylation is required for secretion of the precursor to brain-derived neurotrophic factor (proBDNF) carrying sulfated LacdiNAc structures. **J Biol Chem** 294: 16816–16830, 2019. doi:10.1074/jbc.RA119.009989.

139. Seidah NG, Benjannet S, Pareek S, Savaria D, Hamelin J, Goulet B, Laliberte J, Lazure C, Chretien M, Murphy RA**.** Cellular processing of the nerve growth factor precursor by the mammalian pro-protein convertases. **Biochem J** 314 (Pt 3): 951–960, 1996. doi:10.1042/bj3140951.

140. Duran-Romaña R, Houben B, De Vleeschouwer M, Louros N, Wilson MP, Matthijs G, Schymkowitz J, Rousseau F**.** N-glycosylation as a eukaryotic protective mechanism against protein aggregation. **Sci Adv** 10: eadk8173, 2024. doi:10.1126/sciadv.adk8173.

141. Sachan V, Susan-Resiga D, Lam K, Seidah NG**.** The biology and clinical implications of PCSK7. **Endocr Rev** 46: 281–299, 2025. doi:10.1210/endrev/bnae031.

142. Wetsel WC, Rodriguiz RM, Guillemot J, Rousselet E, Essalmani R, Kim IH, Bryant JC, Marcinkiewicz J, Desjardins R, Day R, Constam DB, Prat A, Seidah NG**.** Disruption of the expression of the proprotein convertase PC7 reduces BDNF production and affects learning and memory in mice. **Proc Natl Acad Sci USA** 110: 17362–17367, 2013. doi:10.1073/pnas.1314698110.

143. Blondiaux A, Jia S, Annamneedi A, Çalışkan G, Nebel J, Montenegro-Venegas C, Wykes RC, Fejtova A, Walker MC, Stork O, Gundelfinger ED, Dityatev A, Seidenbecher CI**.** Linking epileptic phenotypes and neural extracellular matrix remodeling signatures in mouse models of epilepsy. **Neurobiol Dis** 188: 106324, 2023. doi:10.1016/j.nbd.2023.106324.

144. Heyden A, Ionescu MC, Romorini S, Kracht B, Ghiglieri V, Calabresi P, Seidenbecher C, Angenstein F, Gundelfinger ED**.** Hippocampal enlargement in Bassoon-mutant mice is associated with enhanced neurogenesis, reduced apoptosis, and abnormal BDNF levels. **Cell Tissue Res** 346: 11–26, 2011. doi:10.1007/s00441-011-1233-3.

145. Rohli KE, Boyer CK, Blom SE, Stephens SB**.** Nutrient regulation of pancreatic islet beta-cell secretory capacity and insulin production. **Biomolecules** 12: 2022. doi:10.3390/biom12020335.

146. Hoek JM, Hepkema WM, Halffman W**.** The effect of journal guidelines on the reporting of antibody validation. **PeerJ** 8: e9300, 2020. doi:10.7717/peerj.9300.

147. Chen ZY, Ieraci A, Teng H, Dall H, Meng CX, Herrera DG, Nykjaer A, Hempstead BL, Lee FS. Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. **J Neurosci** 25: 6156–6166, 2005. doi:10.1523/JNEUROSCI.1017-05.2005.

148. Wosnitzka E, Nan X, Nan J, Chacón-Fernández P, Kussmaul L, Schuler M, Hengerer B, Barde YA. A new mouse line reporting the translation of brain-derived neurotrophic factor using green fluorescent protein. **eNeuro** 7: ENEURO.0462-19.2019, 2020. doi:10.1523/ENEURO.0462-19.2019.

149. Andreska T, Rauskolb S, Schukraft N, Lüningschrör P, Sasi M, Signoret-Genest J, Behringer M, Blum R, Sauer M, Tovote P, Sendtner M. Induction of BDNF expression in layer II/III and layer V neurons of the motor cortex is essential for motor learning. **J Neurosci** 40: 6289–6308, 2020. doi:10.1523/JNEUROSCI.0288-20.2020.

150. Yang J, Siao CJ, Nagappan G, Marinic T, Jing D, McGrath K, Chen ZY, Mark W, Tessarollo L, Lee FS, Lu B, Hempstead BL. Neuronal release of proBDNF. **Nat Neurosci** 12: 113–115, 2009. doi:10.1038/nn.2244.

151. Ma Q, Yang J, Li T, Milner TA, Hempstead BL. Selective reduction of striatal mature BDNF without induction of proBDNF in the zQ175 mouse model of Huntington's disease. **Neurobiol Dis** 82: 466–477, 2015. doi:10.1016/j.nbd.2015.08.008.

152. Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, Lindsay RM, Wiegand SJ. Anterograde transport of brain-derived neurotrophic factor and its role in the brain. **Nature** 389: 856–860, 1997. doi:10.1038/39885.

153. Anastasia A, Deinhardt K, Chao MV, Will NE, Irmady K, Lee FS, Hempstead BL, Bracken C. Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. **Nat Commun** 4: 2490, 2013. doi:10.1038/ncomms3490.

154. Giza JI, Kim J, Meyer HC, Anastasia A, Dincheva I, Zheng CI, Lopez K, Bains H, Yang J, Bracken C, Liston C, Jing D, Hempstead BL, Lee FS. The BDNF Val66Met prodomain disassembles dendritic spines altering fear extinction circuitry and behavior. **Neuron** 99: 163–178.e6, 2018. doi:10.1016/j.neuron.2018.05.024.

155. Kojima M, Otabi H, Kumanogoh H, Toyoda A, Ikawa M, Okabe M, Mizui T. Reduction in BDNF from inefficient precursor conversion influences nest building and promotes depressive-like behavior in mice. **Int J Mol Sci** 21: 3984, 2020. doi:10.3390/ijms21113984.

156. Yang F, You H, Mizui T, Ishikawa Y, Takao K, Miyakawa T, Li X, Bai T, Xia K, Zhang L, Pang D, Xu Y, Zhu C, Kojima M, Lu B. Inhibiting proBDNF to mature BDNF conversion leads to ASD-like phenotypes in vivo. **Mol Psychiatry** 29: 3462–3474, 2024. doi:10.1038/s41380-024-02595-5.

157. Yang J, Harte-Hargrove LC, Siao CJ, Marinic T, Clarke R, Ma Q, Jing D, Lafrancois JJ, Bath KG, Mark W, Ballon D, Lee FS, Scharfman HE, Hempstead BL. proBDNF negatively regulates neuronal remodeling, synaptic transmission, and synaptic plasticity in hippocampus. **Cell Rep** 7: 796–806, 2014. doi:10.1016/j.celrep.2014.03.040.

158. Cozza A, Melissari E, Iacopetti P, Mariotti V, Tedde A, Nacmias B, Conte A, Sorbi S, Pellegrini S. SNPs in neurotrophin system genes and Alzheimer's disease in an Italian population. **J Alzheimers Dis** 15: 61–70, 2008. doi:10.3233/jad-2008-15105.

159. Aldoghachi AF, Tor YS, Redzun SZ, Lokman KA, Razaq NA, Shahbudin AF, Badamasi IM, Cheah PS, Stanslas J, Veerakumarasivam A, Rosli R, Ibrahim N, Lye MS, Ling KH. Screening of brain-derived neurotrophic factor (BDNF) single nucleotide polymorphisms and plasma BDNF levels among Malaysian major depressive disorder patients. **PLoS One** 14: e0211241, 2019. doi:10.1371/journal.pone.0211241.

160. Turrigiano G. Homeostatic signaling: the positive side of negative feedback. **Curr Opin Neurobiol** 17: 318–324, 2007. doi:10.1016/j.conb.2007.04.004.

161. Rutherford LC, DeWan A, Lauer HM, Turrigiano GG. Brain-derived neurotrophic factor mediates the activity-dependent regulation of inhibition in neocortical cultures. **J Neurosci** 17: 4527–4535, 1997. doi:10.1523/JNEUROSCI.17-12-04527.1997.

162. Xiao J. Thirty years of BDNF study in central myelination: from biology to therapy. **J Neurochem** 167: 321–336, 2023. doi:10.1111/jnc.15968.

163. Mizuno K, Carnahan J, Nawa H. Brain-derived neurotrophic factor promotes differentiation of striatal GABAergic neurons. **Dev Biol** 165: 243–256, 1994. doi:10.1006/dbio.1994.1250.

164. Ventimiglia R, Mather PE, Jones BE, Lindsay RM. The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. **Eur J Neurosci** 7: 213–222, 1995. doi:10.1111/j.1460-9568.1995.tb01057.x.

165. Widmer HR, Hefti F. Stimulation of GABAergic neuron differentiation by NT-4/5 in cultures of rat cerebral cortex. **Brain Res Dev Brain Res** 80: 279–284, 1994. doi:10.1016/0165-3806(94)90114-7.

166. Alderson RF, Alterman AL, Barde YA, Lindsay RM. Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. **Neuron** 5: 297–306, 1990. doi:10.1016/0896-6273(90)90166-d.

167. Marty S, Berninger B, Carroll P, Thoenen H. GABAergic stimulation regulates the phenotype of hippocampal interneurons through the regulation of brain-derived neurotrophic factor. **Neuron** 16: 565–570, 1996. doi:10.1016/s0896-6273(00)80075-6.

168. Marty S, Wehrlé R, Sotelo C. Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. **J Neurosci** 20: 8087–8095, 2000. doi:10.1523/JNEUROSCI.20-21-08087.2000.

169. Tan CL, Cooke EK, Leib DE, Lin YC, Daly GE, Zimmerman CA, Knight ZA. Warm-sensitive neurons that control body temperature. **Cell** 167: 47–59.e15, 2016. doi:10.1016/j.cell.2016.08.028.

170. Salio C, Averill S, Priestley JV, Merighi A. Costorage of BDNF and neuropeptides within individual dense-core vesicles in central and peripheral neurons. **Dev Neurobiol** 67: 326–338, 2007. doi:10.1002/dneu.20358.

171. Michael GJ, Averill S, Nitkunan A, Rattray M, Bennett DL, Yan Q, Priestley JV. Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. **J Neurosci** 17: 8476–8490, 1997. doi:10.1523/JNEUROSCI.17-21-08476.1997.

172. Berg EA, Johnson RJ, Leeman SE, Boyd N, Kimerer L, Fine RE. Isolation and characterization of substance P-containing dense core vesicles from rabbit optic nerve and termini. **J Neurosci Res** 62: 830–839, 2000. doi:10.1002/1097-4547.

173. Matsuda N, Lu H, Fukata Y, Noritake J, Gao H, Mukherjee S, Nemoto T, Fukata M, Poo M-MM. Differential activity-dependent secretion of brain-derived neurotrophic factor from axon and dendrite. **J Neurosci** 29: 14185–14198, 2009. doi:10.1523/JNEUROSCI.1863-09.2009.

174. Leschik J, Eckenstaler R, Endres T, Munsch T, Edelmann E, Richter K, Kobler O, Fischer KD, Zuschratter W, Brigadski T, Lutz B, Lessmann V**.** Prominent postsynaptic and dendritic exocytosis of endogenous BDNF vesicles in BDNF-GFP knock-in mice. **Mol Neurobiol** 56: 6833–6855, 2019. doi:10.1007/s12035-019-1551-0.

175. Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S**.** Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. **J Neurosci** 17: 2295–2313, 1997. doi:10.1523/JNEUROSCI.17-07-02295.1997.

176. Rudge JS, Mather PE, Pasnikowski EM, Cai N, Corcoran T, Acheson A, Anderson K, Lindsay RM, Wiegand SJ**.** Endogenous BDNF protein is increased in adult rat hippocampus after a kainic acid induced excitotoxic insult but exogenous BDNF is not neuroprotective. **Exp Neurol** 149: 398–410, 1998. doi:10.1006/exnr.1997.6737.

177. Harward SC, Hedrick NG, Hall CE, Parra-Bueno P, Milner TA, Pan E, Laviv T, Hempstead BL, Yasuda R, McNamara JO**.** Autocrine BDNF-TrkB signalling within a single dendritic spine. **Nature** 538: 99–103, 2016. doi:10.1038/nature19766.

178. Dean C, Liu H, Dunning FM, Chang PY, Jackson MB, Chapman ER**.** Synaptotagmin-IV modulates synaptic function and long-term potentiation by regulating BDNF release. **Nat Neurosci** 12: 767–776, 2009. doi:10.1038/nn.2315.

179. Glock C, Biever A, Tushev G, Nassim-Assir B, Kao A, Bartnik I, Tom Dieck S, Schuman EM**.** The translatome of neuronal cell bodies, dendrites, and axons. **Proc Natl Acad Sci USA** 118: e2113929118, 2021. doi:10.1073/pnas.2113929118.

180. Ehinger Y, Soneja D, Phamluong K, Salvi A, Ron D**.** Identification of novel BDNF-specific corticostriatal circuitries. **eNeuro** 10: ENEURO.0238-21.2023, 2023. doi:10.1523/ENEURO.0238-21.2023.

181. Kosse C, Ivanov J, Knight Z, Pellegrino K, Friedman J**.** A subcortical feeding circuit linking an interoceptive node to jaw movement. **Nature** 636: 151–161, 2024. doi:10.1038/s41586-024-08098-1.

182. Niu C, Yue X, An JJ, Bass R, Xu H, Xu B**.** Genetic dissection of BDNF and TrkB expression in glial cells. **Biomolecules** 14: 91, 2024. doi:10.3390/biom14010091.

183. Geraghty AC, Gibson EM, Ghanem RA, Greene JJ, Ocampo A, Goldstein AK, Ni L, Yang T, Marton RM, Paşca SP, Greenberg ME, Longo FM, Monje M**.** Loss of adaptive myelination contributes to methotrexate chemotherapy-related cognitive impairment. **Neuron** 103: 250–265.e8, 2019. doi:10.1016/j.neuron.2019.04.032.

184. Barakat-Walter I**.** Brain-derived neurotrophic factor-like immunoreactivity is localized mainly in small sensory neurons of rat dorsal root ganglia. **J Neurosci Methods** 68: 281–288, 1996. doi:10.1016/0165-0270(96)00093-3.

185. Zhou XF, Rush RA**.** Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons. **Neuroscience** 74: 945–953, 1996. doi:10.1016/0306-4522(96)00237-0.

186. von Bartheld CS, Byers MR, Williams R, Bothwell M**.** Anterograde transport of neurotrophins and axodendritic transfer in the developing visual system. **Nature** 379: 830–833, 1996. doi:10.1038/379830a0.

187. Korsching S**.** The neurotrophic factor concept: a reexamination. **J Neurosci** 13: 2739–2748, 1993. doi:10.1523/JNEUROSCI.13-07-02739.1993.

188. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR 3rd, Lafaille JJ, Hempstead BL, Littman DR, Gan WB**.** Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. **Cell** 155: 1596–1609, 2013. doi:10.1016/j.cell.2013.11.030.

189. Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y**.** BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. **Nature** 438: 1017–1021, 2005. doi:10.1038/nature04223.

190. Urabe H, Kojima H, Chan L, Terashima T, Ogawa N, Katagi M, Fujino K, Kumagai A, Kawai H, Asakawa A, Inui A, Yasuda H, Eguchi Y, Oka K, Maegawa H, Kashiwagi A, Kimura H**.** Haematopoietic cells produce BDNF and regulate appetite upon migration to the hypothalamus. **Nat Commun** 4: 1526, 2013. doi:10.1038/ncomms2536.

191. Kerschensteiner M, Gallmeier E, Behrens L, Leal VV, Misgeld T, Klinkert WE, Kolbeck R, Hoppe E, Oropeza-Wekerle RL, Bartke I, Stadelmann C, Lassmann H, Wekerle H, Hohlfeld R**.** Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? **J Exp Med** 189: 865–870, 1999. doi:10.1084/jem.189.5.865.

192. Honey D, Wosnitzka E, Klann E, Weinhard L**.** Analysis of microglial BDNF function and expression in the motor cortex. **Front Cell Neurosci** 16: 961276, 2022. doi:10.3389/fncel.2022.961276.

193. Chacón-Fernández P, Sauberli K, Colzani M, Moreau T, Ghevaert C, Barde YA**.** Brain-derived neurotrophic factor in megakaryocytes. **J Biol Chem** 291: 9872–9881, 2016. doi:10.1074/jbc.M116.720029.

194. Rutlin M, Ho CY, Abraira VE, Cassidy C, Bai L, Woodbury CJ, Ginty DD**.** The cellular and molecular basis of direction selectivity of Adelta-LTMRs. **Cell** 159: 1640–1651, 2014. doi:10.1016/j.cell.2014.11.038.

195. Lommatzsch M, Braun A, Mannsfeldt A, Botchkarev VA, Botchkareva NV, Paus R, Fischer A, Lewin GR, Renz H**.** Abundant production of brain-derived neurotrophic factor by adult visceral epithelia. Implications for paracrine and target-derived neurotrophic functions. **Am J Pathol** 155: 1183–1193, 1999. doi:10.1016/S0002-9440(10)65221-2.

196. Johnson D, Lanahan A, Buck CR, Sehgal A, Morgan C, Mercer E, Bothwell M, Chao M**.** Expression and structure of the human NGF receptor. **Cell** 47: 545–554, 1986. doi:10.1016/0092-8674(86)90619-7.

197. Radeke MJ, Misko TP, Hsu C, Herzenberg LA, Shooter EM**.** Gene transfer and molecular cloning of the rat nerve growth factor receptor. **Nature** 325: 593–597, 1987. doi:10.1038/325593a0.

198. Vilar M, Charalampopoulos I, Kenchappa RS, Simi A, Karaca E, Reversi A, Choi S, Bothwell M, Mingarro I, Friedman WJ, Schiavo G, Bastiaens PI, Verveer PJ, Carter BD, Ibáñez CF**.** Activation of the p75 neurotrophin receptor through conformational rearrangement of disulphide-linked receptor dimers. **Neuron** 62: 72–83, 2009. doi:10.1016/j.neuron.2009.02.020.

199. Coulson EJ, Reid K, Baca M, Shipham KA, Hulett SM, Kilpatrick TJ, Bartlett PF**.** Chopper, a new death domain of the p75 neurotrophin receptor that mediates rapid neuronal cell death. **J Biol Chem** 275: 30537–30545, 2000. doi:10.1074/jbc.M005214200.

200. Gluska S, Zahavi EE, Chein M, Gradus T, Bauer A, Finke S, Perlson E**.** Rabies virus hijacks and accelerates the p75NTR retrograde axonal transport machinery. **PLoS Pathog** 10: e1004348, 2014. doi:10.1371/journal.ppat.1004348.

201. Ceni C, Unsain N, Zeinieh MP, Barker PA**.** Neurotrophins in the regulation of cellular survival and death. **Handb Exp Pharmacol** 220: 193–221, 2014. doi:10.1007/978-3-642-45106-5_8.

202. Dechant G, Barde YA. The neurotrophin receptor p75^{NTR}: novel functions and implications for diseases of the nervous system. **Nat Neurosci** 5: 1131–1136, 2002. doi:10.1038/nn1102-1131.

203. Roux PP, Barker PA. Neurotrophin signaling through the p75 neurotrophin receptor. **Prog Neurobiol** 67: 203–233, 2002. doi:10.1016/s0301-0082(02)00016-3.

204. Skeldal S, Sykes AM, Glerup S, Matusica D, Palstra N, Autio H, Boskovic Z, Madsen P, Castrén E, Nykjaer A, Coulson EJ. Mapping of the interaction site between sortilin and the p75 neurotrophin receptor reveals a regulatory role for the sortilin intracellular domain in p75 neurotrophin receptor shedding and apoptosis. **J Biol Chem** 287: 43798–43809, 2012. doi:10.1074/jbc.M112.374710.

205. Kraemer BR, Yoon SO, Carter BD. The biological functions and signaling mechanisms of the p75 neurotrophin receptor. **Handb Exp Pharmacol** 220: 121–164, 2014. doi:10.1007/978-3-642-45106-5_6.

206. Conroy JN, Coulson EJ. High-affinity TrkA and p75 neurotrophin receptor complexes: a twisted affair. **J Biol Chem** 298: 101568, 2022. doi:10.1016/j.jbc.2022.101568.

207. Ramanujan A, Li Z, Ma Y, Lin Z, Ibáñez CF. RhoGDI phosphorylation by PKC promotes its interaction with death receptor p75^{NTR} to gate axon growth and neuron survival. **EMBO Rep** 25: 1490–1512, 2024. doi:10.1038/s44319-024-00064-2.

208. Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD, Kermani P, Torkin R, Chen ZY, Lee FS, Kraemer RT, Nykjaer A, Hempstead BL. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. **J Neurosci** 25: 5455–5463, 2005. doi:10.1523/JNEUROSCI.5123-04.2005.

209. Liepinsh E, Ilag LL, Otting G, Ibáñez CF. NMR structure of the death domain of the p75 neurotrophin receptor. **EMBO J** 16: 4999–5005, 1997. doi:10.1093/emboj/16.16.4999.

210. Carter BD, Zirrgiebel U, Barde YA. Differential regulation of p21ras activation in neurons by nerve growth factor and brain-derived neurotrophic factor. **J Biol Chem** 270: 21751–21757, 1995. doi:10.1074/jbc.270.37.21751.

211. Kisiswa L, Fernández-Suárez D, Sergaki MC, Ibáñez CF. RIP2 gates TRAF6 interaction with death receptor p75^{NTR} to regulate cerebellar granule neuron survival. **Cell Rep** 24: 1013–1024, 2018. doi:10.1016/j.celrep.2018.06.098.

212. Lin Z, Tann JY, Goh ET, Kelly C, Lim KB, Gao JF, Ibanez CF. Structural basis of death domain signaling in the p75 neurotrophin receptor. **eLife** 4: e11692, 2015. doi:10.7554/eLife.11692.

213. Sutter A, Riopelle RJ, Harris-Warrick RM, Shooter EM. Nerve growth factor receptors. Characterization of two distinct classes of binding sites on chick embryo sensory ganglia cells. **J Biol Chem** 254: 5972–5982, 1979. doi:10.1016/S0021-9258(18)50507-X.

214. Rodriguez-Tébar A, Dechant G, Barde YA. Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. **Neuron** 4: 487–492, 1990. doi:10.1016/0896-6273(90)90107-q.

215. Rodríguez-Tébar A, Dechant G, Gotz R, Barde YA. Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. **EMBO J** 11: 917–922, 1992. doi:10.1002/j.1460-2075.1992.tb05130.x.

216. Bothwell M. Functional interactions of neurotrophins and neurotrophin receptors. **Annu Rev Neurosci** 18: 223–253, 1995. doi:10.1146/annurev.ne.18.030195.001255.

217. Lee KF, Li E, Huber LJ, Landis SC, Sharpe AH, Chao MV, Jaenisch R. Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. **Cell** 69: 737–749, 1992. doi:10.1016/0092-8674(92)90286-l.

218. Davies AM, Lee KF, Jaenisch R. p75-deficient trigeminal sensory neurons have an altered response to NGF but not to other neurotrophins. **Neuron** 11: 565–574, 1993. doi:10.1016/0896-6273(93)90069-4.

219. Hempstead BL, Martin-Zanca D, Kaplan DR, Parada LF, Chao MV. High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. **Nature** 350: 678–683, 1991. doi:10.1038/350678a0.

220. Bibel M, Hoppe E, Barde YA. Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR. **EMBO J** 18: 616–622, 1999. doi:10.1093/emboj/18.3.616.

221. Baho E, Chattopadhyaya B, Lavertu-Jolin M, Mazziotti R, Awad PN, Chehrazi P, Groleau M, Jahannault-Talignani C, Vaucher E, Ango F, Pizzorusso T, Baroncelli L, Di Cristo G. p75 neurotrophin receptor activation regulates the timing of the maturation of cortical parvalbumin interneuron connectivity and promotes juvenile-like plasticity in adult visual cortex. **J Neurosci** 39: 4489–4510, 2019. doi:10.1523/JNEUROSCI.2881-18.2019.

222. Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted proneurotrophins. **Science** 294: 1945–1948, 2001. doi:10.1126/science.1065057.

223. Frade JM, Rodríguez-Tébar A, Barde YA. Induction of cell death by endogenous nerve growth factor through its p75 receptor. **Nature** 383: 166–168, 1996. doi:10.1038/383166a0.

224. Casaccia-Bonnefil P, Carter BD, Dobrowsky RT, Chao MV. Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. **Nature** 383: 716–719, 1996. doi:10.1038/383716a0.

225. Bamji SX, Majdan M, Pozniak CD, Belliveau DJ, Aloyz R, Kohn J, Causing CG, Miller FD. The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. **J Cell Biol** 140: 911–923, 1998. doi:10.1083/jcb.140.4.911.

226. Kasemeier-Kulesa JC, Morrison JA, Lefcort F, Kulesa PM. TrkB/BDNF signalling patterns the sympathetic nervous system. **Nat Commun** 6: 8281, 2015. doi:10.1038/ncomms9281.

227. Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, Jacobsen C, Kliemannel M, Schwarz E, Willnow TE, Hempstead BL, Petersen CM. Sortilin is essential for proNGF-induced neuronal cell death. **Nature** 427: 843–848, 2004. doi:10.1038/nature02319.

228. Cosgaya JM, Chan JR, Shooter EM. The neurotrophin receptor p75NTR as a positive modulator of myelination. **Science** 298: 1245–1248, 2002. doi:10.1126/science.1076595.

229. Follis RM, Tep C, Genaro-Mattos TC, Kim ML, Ryu JC, Morrison VE, Chan JR, Porter N, Carter BD, Yoon SO. metabolic control of sensory neuron survival by the p75 neurotrophin receptor in Schwann cells. **J Neurosci** 41: 8710–8724, 2021. doi:10.1523/JNEUROSCI.3243-20.2021.

230. Young KM, Merson TD, Sotthibundhu A, Coulson EJ, Bartlett PF. p75 neurotrophin receptor expression defines a population of BDNF-responsive neurogenic precursor cells. **J Neurosci** 27: 5146–5155, 2007. doi:10.1523/JNEUROSCI.0654-07.2007.

231. Kirschenbaum B, Goldman SA. Brain-derived neurotrophic factor promotes the survival of neurons arising from the adult rat forebrain

subependymal zone. **Proc Natl Acad Sci USA** 92: 210–214, 1995. doi:10.1073/pnas.92.1.210.

232. Zigova T, Pencea V, Wiegand SJ, Luskin MB. Intraventricular administration of BDNF increases the number of newly generated neurons in the adult olfactory bulb. **Mol Cell Neurosci** 11: 234–245, 1998. doi:10.1006/mcne.1998.0684.

233. Rösch H, Schweigreiter R, Bonhoeffer T, Barde YA, Korte M. The neurotrophin receptor p75NTR modulates long-term depression and regulates the expression of AMPA receptor subunits in the hippocampus. **Proc Natl Acad Sci USA** 102: 7362–7367, 2005. doi:10.1073/pnas.0502460102.

234. Woo NH, Teng HK, Siao CJ, Chiaruttini C, Pang PT, Milner TA, Hempstead BL, Lu B. Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. **Nat Neurosci** 8: 1069–1077, 2005. doi:10.1038/nn1510.

235. Hall A. Rho GTPases and the actin cytoskeleton. **Science** 279: 509–514, 1998. doi:10.1126/science.279.5350.509.

236. Yamashita T, Tucker KL, Barde YA. Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. **Neuron** 24: 585–593, 1999. doi:10.1016/s0896-6273(00)81114-9.

237. Yamashita T, Tohyama M. The p75 receptor acts as a displacement factor that releases Rho from Rho-GDI. **Nat Neurosci** 6: 461–467, 2003. doi:10.1038/nn1045.

238. Collins F. An effect of nerve growth factor on the parasympathetic ciliary ganglion. **J Neurosci** 4: 1281–1288, 1984. doi:10.1523/JNEUROSCI.04-05-01281.1984.

239. Carter BD, Kaltschmidt C, Kaltschmidt B, Offenhäuser N, Böhm-Matthaei R, Baeuerle PA, Barde YA. Selective activation of NF-kappa B by nerve growth factor through the neurotrophin receptor p75. **Science** 272: 542–545, 1996. doi:10.1126/science.272.5261.542.

240. Charalampopoulos I, Vicario A, Pediaditakis I, Gravanis A, Simi A, Ibáñez CF. Genetic dissection of neurotrophin signaling through the p75 neurotrophin receptor. **Cell Rep** 2: 1563–1570, 2012. doi:10.1016/j.celrep.2012.11.009.

241. Cheng HC, Sun Y, Lai LC, Chen SY, Lee WC, Chen JH, Chen TF, Chen HH, Wen LL, Yip PK, Chu YM, Chen WJ, Chen YC. Genetic polymorphisms of nerve growth factor receptor (NGFR) and the risk of Alzheimer's disease. **J Negat Results Biomed** 11: 5, 2012. doi:10.1186/1477-5751-11-5.

242. Klein R, Parada LF, Coulier F, Barbacid M. trkB, a novel tyrosine protein kinase receptor expressed during mouse neural development. **EMBO J** 8: 3701–3709, 1989. doi:10.1002/j.1460-2075.1989.tb08545.x.

243. Barbacid M. The Trk family of neurotrophin receptors. **J Neurobiol** 25: 1386–1403, 1994. doi:10.1002/neu.480251107.

244. Klein R, Smeyne RJ, Wurst W, Long LK, Auerbach BA, Joyner AL, Barbacid M. Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. **Cell** 75: 113–122, 1993.

245. Silos-Santiago I, Fagan AM, Garber M, Fritzsch B, Barbacid M. Severe sensory deficits but normal CNS development in newborn mice lacking TrkB and TrkC tyrosine protein kinase receptors. **Eur J Neurosci** 9: 2045–2056, 1997. doi:10.1111/j.1460-9568.1997.tb01372.x.

246. Deinhardt K, Chao MV. Trk receptors. **Handb Exp Pharmacol** 220: 103–119, 2014. doi:10.1007/978-3-642-45106-5_5.

247. Minichiello L. TrkB signalling pathways in LTP and learning. **Nat Rev Neurosci** 10: 850–860, 2009. doi:10.1038/nrn2738.

248. Ohira K, Kumanogoh H, Sahara Y, Homma KJ, Hirai H, Nakamura S, Hayashi M. A truncated tropomyosin-related kinase B receptor, T1, regulates glial cell morphology via Rho GDP dissociation inhibitor 1. **J Neurosci** 25: 1343–1353, 2005. doi:10.1523/JNEUROSCI.4436-04.2005.

249. Hanus C, Geptin H, Tushev G, Garg S, Alvarez-Castelao B, Sambandan S, Kochen L, Hafner AS, Langer JD, Schuman EM. Unconventional secretory processing diversifies neuronal ion channel properties. **eLife** 5: e20609, 2016. doi:10.7554/eLife.20609.

250. Merkouris S, Barde YA, Binley KE, Allen ND, Stepanov AV, Wu NC, Grande G, Lin CW, Li M, Nan X, Chacon-Fernandez P, DiStefano PS, Lindsay RM, Lerner RA, Xie J. Fully human agonist antibodies to TrkB using autocrine cell-based selection from a combinatorial antibody library. **Proc Natl Acad Sci USA** 115: E7023–E7032, 2018. doi:10.1073/pnas.1806660115.

251. Ateaque S, Merkouris S, Wyatt S, Allen ND, Xie J, DiStefano PS, Lindsay RM, Barde YA. Selective activation and down-regulation of Trk receptors by neurotrophins in human neurons co-expressing TrkB and TrkC. **J Neurochem** 161: 463–477, 2022. doi:10.1111/jnc.15617.

252. Cardoso-Moreira M, Halbert J, Valloton D, Velten B, Chen C, Shao Y, et al. Gene expression across mammalian organ development. **Nature** 571: 505–509, 2019. doi:10.1038/s41586-019-1338-5.

253. Stoilov P, Castren E, Stamm S. Analysis of the human TrkB gene genomic organization reveals novel TrkB isoforms, unusual gene length, and splicing mechanism. **Biochem Biophys Res Commun** 290: 1054–1065, 2002. doi:10.1006/bbrc.2001.6301.

254. Luberg K, Wong J, Weickert CS, Timmusk T. Human TrkB gene: novel alternative transcripts, protein isoforms and expression pattern in the prefrontal cerebral cortex during postnatal development. **J Neurochem** 113: 952–964, 2010. doi:10.1111/j.1471-4159.2010.06662.x.

255. Tessarollo L, Yanpallewar S. TrkB truncated isoform receptors as transducers and determinants of BDNF functions. **Front Neurosci** 16: 847572, 2022. doi:10.3389/fnins.2022.847572.

256. Carim-Todd L, Bath KG, Fulgenzi G, Yanpallewar S, Jing D, Barrick CA, Becker J, Buckley H, Dorsey SG, Lee FS, Tessarollo L. Endogenous truncated TrkB.T1 receptor regulates neuronal complexity and TrkB kinase receptor function in vivo. **J Neurosci** 29: 678–685, 2009. doi:10.1523/JNEUROSCI.5060-08.2009.

257. Ameroso D, Meng A, Chen S, Felsted J, Dulla CG, Rios M. Astrocytic BDNF signaling within the ventromedial hypothalamus regulates energy homeostasis. **Nat Metab** 4: 627–643, 2022. doi:10.1038/s42255-022-00566-0.

258. Fulgenzi G, Tomassoni-Ardori F, Babini L, Becker J, Barrick C, Puverel S, Tessarollo L. BDNF modulates heart contraction force and long-term homeostasis through truncated TrkB.T1 receptor activation. **J Cell Biol** 210: 1003–1012, 2015. doi:10.1083/jcb.201502100.

259. Fulgenzi G, Hong Z, Tomassoni-Ardori F, Barella LF, Becker J, Barrick C, Swing D, Yanpallewar S, Croix BS, Wess J, Gavrilova O, Tessarollo L. Novel metabolic role for BDNF in pancreatic beta-cell insulin secretion. **Nat Commun** 11: 1950, 2020. doi:10.1038/s41467-020-15833-5.

260. Nauck MA, Muller TD. Incretin hormones and type 2 diabetes. **Diabetologia** 66: 1780–1795, 2023. doi:10.1007/s00125-023-05956-x.

261. Udit S, Blake K, Chiu IM. Somatosensory and autonomic neuronal regulation of the immune response. **Nat Rev Neurosci** 23: 157–171, 2022. doi:10.1038/s41583-021-00555-4.

262. Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. **Nat Rev Neurosci** 4: 299–309, 2003. doi:10.1038/nrn1078.

263. Drake CT, Milner TA, Patterson SL. Ultrastructural localization of full-length trkB immunoreactivity in rat hippocampus suggests multiple roles in modulating activity-dependent synaptic plasticity. **J Neurosci** 19: 8009–8026, 1999. doi:10.1523/JNEUROSCI.19-18-08009.1999.

264. Andreska T, Luningschrör P, Sendtner M. Regulation of TrkB cell surface expression-a mechanism for modulation of neuronal responsiveness to brain-derived neurotrophic factor. **Cell Tissue Res** 382: 5–14, 2020. doi:10.1007/s00441-020-03224-7.

265. Luningschrör P, Andreska T, Veh A, Wolf D, Giridhar NJ, Moradi M, Denzel A, Sendtner M. Calnexin controls TrkB cell surface transport and ER-phagy in mouse cerebral cortex development. **Dev Cell** 58: 1733–1747.e6, 2023. doi:10.1016/j.devcel.2023.07.004.

266. Sommerfeld MT, Schweigreiter R, Barde YA, Hoppe E. Down-regulation of the neurotrophin receptor TrkB following ligand binding. Evidence for an involvement of the proteasome and differential regulation of TrkA and TrkB. **J Biol Chem** 275: 8982–8990, 2000. doi:10.1074/jbc.275.12.8982.

267. Arévalo JC, Waite J, Rajagopal R, Beyna M, Chen ZY, Lee FS, Chao MV. Cell survival through Trk neurotrophin receptors is differentially regulated by ubiquitination. **Neuron** 50: 549–559, 2006. doi:10.1016/j.neuron.2006.03.044.

268. Frank L, Ventimiglia R, Anderson K, Lindsay RM, Rudge JS. BDNF down-regulates neurotrophin responsiveness, TrkB protein and TrkB mRNA levels in cultured rat hippocampal neurons. **Eur J Neurosci** 8: 1220–1230, 1996. doi:10.1111/j.1460-9568.1996.tb01290.x.

269. Allen SJ, Wilcock GK, Dawbarn D. Profound and selective loss of catalytic TrkB immunoreactivity in Alzheimer's disease. **Biochem Biophys Res Commun** 264: 648–651, 1999. doi:10.1006/bbrc.1999.1561.

270. Nikoletopoulou V, Lickert H, Frade JM, Rencurel C, Giallonardo P, Zhang L, Bibel M, Barde YA. Neurotrophin receptors TrkA and TrkC cause neuronal death whereas TrkB does not. **Nature** 467: 59–63, 2010. doi:10.1038/nature09336.

271. Bredesen DE, Mehlen P, Rabizadeh S. Apoptosis and dependence receptors: a molecular basis for cellular addiction. **Physiol Rev** 84: 411–430, 2004. doi:10.1152/physrev.00027.2003.

272. Ménard M, Costechareyre C, Coelho-Aguiar JM, Jarrosson-Wuilleme L, Rama N, Blachier J, Kindbeiter K, Bozon M, Cabrera JR, Dupin E, Le Douarin N, Mehlen P, Tauszig-Delamasure S. The dependence receptor TrkC regulates the number of sensory neurons during DRG development. **Dev Biol** 442: 249–261, 2018. doi:10.1016/j.ydbio.2018.07.022.

273. Lohof AM, Ip NY, Poo MM. Potentiation of developing neuromuscular synapses by the neurotrophins NT-3 and BDNF. **Nature** 363: 350–353, 1993. doi:10.1038/363350a0.

274. Kang H, Schuman EM. Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. **Science** 267: 1658–1662, 1995. doi:10.1126/science.7886457.

275. Schuman EM. Neurotrophin regulation of synaptic transmission. **Curr Opin Neurobiol** 9: 105–109, 1999. doi:10.1016/s0959-4388(99)80013-0.

276. Frerking M, Malenka RC, Nicoll RA. Brain-derived neurotrophic factor (BDNF) modulates inhibitory, but not excitatory, transmission in the CA1 region of the hippocampus. **J Neurophysiol** 80: 3383–3386, 1998. doi:10.1152/jn.1998.80.6.3383.

277. Kafitz KW, Rose CR, Thoenen H, Konnerth A. Neurotrophin-evoked rapid excitation through TrkB receptors. **Nature** 401: 918–921, 1999. doi:10.1038/44847.

278. Blum R, Kafitz KW, Konnerth A. Neurotrophin-evoked depolarization requires the sodium channel $Na_V1.9$. **Nature** 419: 687–693, 2002. doi:10.1038/nature01085.

279. Rose CR, Blum R, Pichler B, Lepier A, Kafitz KW, Konnerth A. Truncated TrkB-T1 mediates neurotrophin-evoked calcium signalling in glia cells. **Nature** 426: 74–78, 2003. doi:10.1038/nature01983.

280. Blum R, Konnerth A. Neurotrophin-mediated rapid signaling in the central nervous system: mechanisms and functions. **Physiology (Bethesda)** 20: 70–78, 2005. doi:10.1152/physiol.00042.2004.

281. Merighi A, Bardoni R, Salio C, Lossi L, Ferrini F, Prandini M, Zonta M, Gustincich S, Carmignoto G. Presynaptic functional trkB receptors mediate the release of excitatory neurotransmitters from primary afferent terminals in lamina II (substantia gelatinosa) of postnatal rat spinal cord. **Dev Neurobiol** 68: 457–475, 2008. doi:10.1002/dneu.20605.

282. Bolton MM, Pittman AJ, Lo DC. Brain-derived neurotrophic factor differentially regulates excitatory and inhibitory synaptic transmission in hippocampal cultures. **J Neurosci** 20: 3221–3232, 2000. doi:10.1523/JNEUROSCI.20-09-03221.2000.

283. Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. **Proc Natl Acad Sci USA** 92: 8856–8860, 1995. doi:10.1073/pnas.92.19.8856.

284. Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. **Neuron** 16: 1137–1145, 1996. doi:10.1016/s0896-6273(00)80140-3.

285. Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. **Nature** 381: 706–709, 1996. doi:10.1038/381706a0.

286. Ying SW, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TV, Bramham CR. Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. **J Neurosci** 22: 1532–1540, 2002. doi:10.1523/JNEUROSCI.22-05-01532.2002.

287. Abidin I, Köhler T, Weiler E, Zoidl G, Eysel UT, Lessmann V, Mittmann T. Reduced presynaptic efficiency of excitatory synaptic transmission impairs LTP in the visual cortex of BDNF-heterozygous mice. **Eur J Neurosci** 24: 3519–3531, 2006. doi:10.1111/j.1460-9568.2006.05242.x.

288. Kossel AH, Cambridge SB, Wagner U, Bonhoeffer T. A caged Ab reveals an immediate/instructive effect of BDNF during hippocampal synaptic potentiation. **Proc Natl Acad Sci USA** 98: 14702–14707, 2001. doi:10.1073/pnas.251326998.

289. Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, Teng KK, Yung WH, Hempstead BL, Lu B. Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. **Science** 306: 487–491, 2004. doi:10.1126/science.1100135.

290. Zakharenko SS, Patterson SL, Dragatsis I, Zeitlin SO, Siegelbaum SA, Kandel ER, Morozov A. Presynaptic BDNF required for a presynaptic but not postsynaptic component of LTP at hippocampal CA1-CA3 synapses. **Neuron** 39: 975–990, 2003. doi:10.1016/s0896-6273(03)00543-9.

291. Tanaka J, Horiike Y, Matsuzaki M, Miyazaki T, Ellis-Davies GC, Kasai H. Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. **Science** 319: 1683–1687, 2008. doi:10.1126/science.1152864.

292. Tongiorgi E, Righi M, Cattaneo A. Activity-dependent dendritic targeting of BDNF and TrkB mRNAs in hippocampal neurons. **J Neurosci** 17: 9492–9505, 1997. doi:10.1523/JNEUROSCI.17-24-09492.1997.

293. Ikegaya Y, Ishizaka Y, Matsuki N. BDNF attenuates hippocampal LTD via activation of phospholipase C: implications for a vertical shift in the frequency-response curve of synaptic plasticity. **Eur J Neurosci** 16: 145–148, 2002. doi:10.1046/j.1460-9568.2002.02051.x.

294. Mizui T, Ishikawa Y, Kumanogoh H, Lume M, Matsumoto T, Hara T, Yamawaki S, Takahashi M, Shiosaka S, Itami C, Uegaki K, Saarma M, Kojima M. BDNF pro-peptide actions facilitate hippocampal LTD and are altered by the common BDNF polymorphism Val66Met. **Proc Natl Acad Sci USA** 112: E3067–E3074, 2015. doi:10.1073/pnas.1422336112.

295. Balkowiec A, Katz DM. Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA in situ. **J Neurosci** 20: 7417–7423, 2000. doi:10.1523/JNEUROSCI.20-19-07417.2000.

296. Balkowiec A, Katz DM. Cellular mechanisms regulating activity-dependent release of native brain-derived neurotrophic factor from hippocampal neurons. **J Neurosci** 22: 10399–10407, 2002. doi:10.1523/JNEUROSCI.22-23-10399.2002.

297. Lever IJ, Bradbury EJ, Cunningham JR, Adelson DW, Jones MG, McMahon SB, Marvizón JC, Malcangio M. Brain-derived neurotrophic factor is released in the dorsal horn by distinctive patterns of afferent fiber stimulation. **J Neurosci** 21: 4469–4477, 2001. doi:10.1523/JNEUROSCI.21-12-04469.2001.

298. Pezet S, Malcangio M, McMahon SB. BDNF: a neuromodulator in nociceptive pathways? **Brain Res Brain Res Rev** 40: 240–249, 2002. doi:10.1016/s0165-0173(02)00206-0.

299. Park H, Popescu A, Poo MM. Essential role of presynaptic NMDA receptors in activity-dependent BDNF secretion and corticostriatal LTP. **Neuron** 84: 1009–1022, 2014. doi:10.1016/j.neuron.2014.10.045.

300. Aicardi G, Argilli E, Cappello S, Santi S, Riccio M, Thoenen H, Canossa M. 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. doi:10.1073/pnas.0406960101.

301. Heppenstall PA, Lewin GR. BDNF but not NT-4 is required for normal flexion reflex plasticity and function. **Proc Natl Acad Sci USA** 98: 8107–8112, 2001. doi:10.1073/pnas.141015098.

302. Kang H, Schuman EM. A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. **Science** 273: 1402–1406, 1996. doi:10.1126/science.273.5280.1402.

303. Takei N, Inamura N, Kawamura M, Namba H, Hara K, Yonezawa K, Nawa H. 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. doi:10.1523/JNEUROSCI.1427-04.2004.

304. Caldeira MV, Melo CV, Pereira DB, Carvalho R, Correia SS, Backos DS, Carvalho AL, Esteban JA, Duarte CB. Brain-derived neurotrophic factor regulates the expression and synaptic delivery of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunits in hippocampal neurons. **J Biol Chem** 282: 12619–12628, 2007. doi:10.1074/jbc.M700607200.

305. Rana S, Fusco AF, Witkin JM, Radin DP, Cerne R, Lippa A, Fuller DD. Pharmacological modulation of respiratory control: ampakines as a therapeutic strategy. **Pharmacol Ther** 265: 108744, 2025. doi:10.1016/j.pharmthera.2024.108744.

306. Jourdi H, Hsu YT, Zhou M, Qin Q, Bi X, Baudry M. Positive AMPA receptor modulation rapidly stimulates BDNF release and increases dendritic mRNA translation. **J Neurosci** 29: 8688–8697, 2009. doi:10.1523/JNEUROSCI.6078-08.2009.

307. Bramham CR, Wells DG. Dendritic mRNA: transport, translation and function. **Nat Rev Neurosci** 8: 776–789, 2007. doi:10.1038/nrn2150.

308. Hirokawa N. mRNA transport in dendrites: RNA granules, motors, and tracks. **J Neurosci** 26: 7139–7142, 2006. doi:10.1523/JNEUROSCI.1821-06.2006.

309. Kiebler MA, DesGroseillers L. Molecular insights into mRNA transport and local translation in the mammalian nervous system. **Neuron** 25: 19–28, 2000. doi:10.1016/s0896-6273(00)80868-5.

310. Fonkeu Y, Kraynyukova N, Hafner AS, Kochen L, Sartori F, Schuman EM, Tchumatchenko T. How mRNA localization and protein synthesis sites influence dendritic protein distribution and dynamics. **Neuron** 103: 1109–1122.e7, 2019. doi:10.1016/j.neuron.2019.06.022.

311. Dalla Costa I, Buchanan CN, Zdradzinski MD, Sahoo PK, Smith TP, Thames E, Kar AN, Twiss JL. The functional organization of axonal mRNA transport and translation. **Nat Rev Neurosci** 22: 77–91, 2021. doi:10.1038/s41583-020-00407-7.

312. Kanai Y, Dohmae N, Hirokawa N. Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. **Neuron** 43: 513–525, 2004. doi:10.1016/j.neuron.2004.07.022.

313. Schratt GM, Nigh EA, Chen WG, Hu L, Greenberg ME. BDNF regulates the translation of a select group of mRNAs by a mammalian target of rapamycin-phosphatidylinositol 3-kinase-dependent pathway during neuronal development. **J Neurosci** 24: 7366–7377, 2004. doi:10.1523/JNEUROSCI.1739-04.2004.

314. Elvira G, Wasiak S, Blandford V, Tong XK, Serrano A, Fan X, Sánchez-Carbente M, Servant F, Bell AW, Boismenu D, Lacaille JC, McPherson PS, DesGroseillers L, Sossin WS. Characterization of an RNA granule from developing brain. **Mol Cell Proteomics** 5: 635–651, 2006. doi:10.1074/mcp.M500255-MCP200.

315. Courtney KD, Grove M, Vandongen H, Vandongen A, LaMantia AS, Pendergast AM. Localization and phosphorylation of Abl-interactor proteins, Abi-1 and Abi-2, in the developing nervous system. **Mol Cell Neurosci** 16: 244–257, 2000. doi:10.1006/mcne.2000.0865.

316. Leal G, Comprido D, de Luca P, Morais E, Rodrigues L, Mele M, Santos AR, Costa RO, Pinto MJ, Patil S, Berentsen B, Afonso P, Carreto L, Li KW, Pinheiro P, Almeida RD, Santos MA, Bramham CR, Duarte CB. The RNA-binding protein hnRNP K mediates the effect of BDNF on dendritic mRNA metabolism and regulates synaptic NMDA receptors in hippocampal neurons. **eNeuro** 4:

ENEURO.0268-17.2017, 2017. doi:10.1523/ENEURO.0268-17.2017.

317. Hafner AS, Donlin-Asp PG, Leitch B, Herzog E, Schuman EM. Local protein synthesis is a ubiquitous feature of neuronal pre- and postsynaptic compartments. **Science** 364: eaau3644, 2019. doi:10.1126/science.aau3644.

318. Deng C, Moradi M, Reinhard S, Ji C, Jablonka S, Hennlein L, Lüningschrör P, Doose S, Sauer M, Sendtner M. Dynamic remodeling of ribosomes and endoplasmic reticulum in axon terminals of motoneurons. **J Cell Sci** 134: jcs.258785, 2021. doi:10.1242/jcs.258785.

319. Messaoudi E, Ying SW, Kanhema T, Croll SD, Bramham CR. Brain-derived neurotrophic factor triggers transcription-dependent, late phase long-term potentiation in vivo. **J Neurosci** 22: 7453–7461, 2002. doi:10.1523/JNEUROSCI.22-17-07453.2002.

320. Xu B, Gottschalk W, Chow A, Wilson RI, Schnell E, Zang K, Wang D, Nicoll RA, Lu B, Reichardt LF. 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. doi:10.1523/JNEUROSCI.20-18-06888.2000.

321. Panja D, Kenney JW, D'Andrea L, Zalfa F, Vedeler A, Wibrand K, Fukunaga R, Bagni C, Proud CG, Bramham CR. Two-stage translational control of dentate gyrus LTP consolidation is mediated by sustained BDNF-TrkB signaling to MNK. **Cell Rep** 9: 1430–1445, 2014. doi:10.1016/j.celrep.2014.10.016.

322. Nikolaienko O, Patil S, Eriksen MS, Bramham CR. Arc protein: a flexible hub for synaptic plasticity and cognition. **Semin Cell Dev Biol** 77: 33–42, 2018. doi:10.1016/j.semcdb.2017.09.006.

323. Link W, Konietzko U, Kauselmann G, Krug M, Schwanke B, Frey U, Kuhl D. Somatodendritic expression of an immediate early gene is regulated by synaptic activity. **Proc Natl Acad Sci USA** 92: 5734–5738, 1995. doi:10.1073/pnas.92.12.5734.

324. Lyford GL, Yamagata K, Kaufmann WE, Barnes CA, Sanders LK, Copeland NG, Gilbert DJ, Jenkins NA, Lanahan AA, Worley PF. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. **Neuron** 14: 433–445, 1995. doi:10.1016/0896-6273(95)90299-6.

325. Farris S, Lewandowski G, Cox CD, Steward O. Selective localization of arc mRNA in dendrites involves activity- and translation-dependent mRNA degradation. **J Neurosci** 34: 4481–4493, 2014. doi:10.1523/JNEUROSCI.4944-13.2014.

326. Pastuzyn ED, Day CE, Kearns RB, Kyrke-Smith M, Taibi AV, McCormick J, Yoder N, Belnap DM, Erlendsson S, Morado DR, Briggs JA, Feschotte C, Shepherd JD. The neuronal gene Arc encodes a repurposed retrotransposon gag protein that mediates intercellular RNA transfer. **Cell** 172: 275–288.e18, 2018. doi:10.1016/j.cell.2018.03.024.

327. Oliveira MM, Mohamed M, Elder MK, Banegas-Morales K, Mamcarz M, Lu EH, Golhan EA, Navrange N, Chatterjee S, Abel T, Klann E. The integrated stress response effector GADD34 is repurposed by neurons to promote stimulus-induced translation. **Cell Rep** 43: 113670, 2024. doi:10.1016/j.celrep.2023.113670.

328. Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. **Nature** 397: 251–255, 1999. doi:10.1038/16697.

329. McMoneagle E, Zhou J, Zhang S, Huang W, Josiah SS, Ding K, Wang Y, Zhang J. Neuronal K^+-Cl^- cotransporter KCC2 as a promising drug target for epilepsy treatment. **Acta Pharmacol Sin** 45: 1–22, 2024. doi:10.1038/s41401-023-01149-9.

330. Ludwig A, Uvarov P, Soni S, Thomas-Crusells J, Airaksinen MS, Rivera C. Early growth response 4 mediates BDNF induction of potassium chloride cotransporter 2 transcription. **J Neurosci** 31: 644–649, 2011. doi:10.1523/JNEUROSCI.2006-10.2011.

331. Yeo M, Berglund K, Augustine G, Liedtke W. Novel repression of Kcc2 transcription by REST-RE-1 controls developmental switch in neuronal chloride. **J Neurosci** 29: 14652–14662, 2009. doi:10.1523/JNEUROSCI.2934-09.2009.

332. Rivera C, Voipio J, Thomas-Crusells J, Li H, Emri Z, Sipilä S, Payne JA, Minichiello L, Saarma M, Kaila K. Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. **J Neurosci** 24: 4683–4691, 2004. doi:10.1523/JNEUROSCI.5265-03.2004.

333. Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, Kokaia Z, Airaksinen MS, Voipio J, Kaila K, Saarma M. BDNF-induced TrkB activation down-regulates the K^+-Cl^- cotransporter KCC2 and impairs neuronal Cl^- extrusion. **J Cell Biol** 159: 747–752, 2002. doi:10.1083/jcb.200209011.

334. Lee-Hotta S, Uchiyama Y, Kametaka S. Role of the BDNF-TrkB pathway in KCC2 regulation and rehabilitation following neuronal injury: a mini review. **Neurochem Int** 128: 32–38, 2019. doi:10.1016/j.neuint.2019.04.003.

335. Cho N, Kontou G, Smalley JL, Bope C, Dengler J, Montrose K, Deeb TZ, Brandon NJ, Yamamoto T, Davies PA, Giamas G, Moss SJ. The brain-specific kinase LMTK3 regulates neuronal excitability by decreasing KCC2-dependent neuronal Cl^- extrusion. **iScience** 27: 109512, 2024. doi:10.1016/j.isci.2024.109512.

336. Li H, Khirug S, Cai C, Ludwig A, Blaesse P, Kolikova J, Afzalov R, Coleman SK, Lauri S, Airaksinen MS, Keinänen K, Khiroug L, Saarma M, Kaila K, Rivera C. KCC2 interacts with the dendritic cytoskeleton to promote spine development. **Neuron** 56: 1019–1033, 2007. doi:10.1016/j.neuron.2007.10.039.

337. Sala C, Segal M. Dendritic spines: the locus of structural and functional plasticity. **Physiol Rev** 94: 141–188, 2014. doi:10.1152/physrev.00012.2013.

338. Engert F, Bonhoeffer T. Dendritic spine changes associated with hippocampal long-term synaptic plasticity. **Nature** 399: 66–70, 1999. doi:10.1038/19978.

339. Toni N, Buchs PA, Nikonenko I, Bron CR, Muller D. LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. **Nature** 402: 421–425, 1999. doi:10.1038/46574.

340. Rex CS, Lin CY, Kramár EA, Chen LY, Gall CM, Lynch G. Brain-derived neurotrophic factor promotes long-term potentiation-related cytoskeletal changes in adult hippocampus. **J Neurosci** 27: 3017–3029, 2007. doi:10.1523/JNEUROSCI.4037-06.2007.

341. Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, Greenberg ME. A brain-specific microRNA regulates dendritic spine development. **Nature** 439: 283–289, 2006. doi:10.1038/nature04367.

342. Antoniou A, Khudayberdiev S, Idziak A, Bicker S, Jacob R, Schratt G. The dynamic recruitment of TRBP to neuronal membranes mediates dendritogenesis during development. **EMBO Rep** 19: e44853, 2018. doi:10.15252/embr.201744853.

343. Amen AM, Ruiz-Garzon CR, Shi J, Subramanian M, Pham DL, Meffert MK**.** A rapid induction mechanism for Lin28a in trophic responses. **Mol Cell** 65: 490–503.e7, 2017. doi:10.1016/j.molcel.2016.12.025.

344. Sambandan S, Akbalik G, Kochen L, Rinne J, Kahlstatt J, Glock C, Tushev G, Alvarez-Castelao B, Heckel A, Schuman EM**.** Activity-dependent spatially localized miRNA maturation in neuronal dendrites. **Science** 355: 634–637, 2017. doi:10.1126/science.aaf8995.

345. Antoniou A, Auderset L, Kaurani L, Sebastian E, Zeng Y, Allahham M, Cases-Cunillera S, Schoch S, Gründemann J, Fischer A, Schneider A**.** Neuronal extracellular vesicles and associated microRNAs induce circuit connectivity downstream BDNF. **Cell Rep** 42: 112063, 2023. doi:10.1016/j.celrep.2023.112063.

346. Andreska T, Lüningschrör P, Wolf D, McFleder RL, Ayon-Olivas M, Rattka M, Drechsler C, Perschin V, Blum R, Aufmkolk S, Granado N, Moratalla R, Sauer M, Monoranu C, Volkmann J, Ip CW, Stigloher C, Sendtner M**.** DRD1 signaling modulates TrkB turnover and BDNF sensitivity in direct pathway striatal medium spiny neurons. **Cell Rep** 42: 112575, 2023. doi:10.1016/j.celrep.2023.112575.

347. Kohara K, Kitamura A, Adachi N, Nishida M, Itami C, Nakamura S, Tsumoto T**.** Inhibitory but not excitatory cortical neurons require presynaptic brain-derived neurotrophic factor for dendritic development, as revealed by chimera cell culture. **J Neurosci** 23: 6123–6131, 2003. doi:10.1523/JNEUROSCI.23-14-06123.2003.

348. Zagrebelsky M, Gödecke N, Remus A, Korte M**.** Cell type-specific effects of BDNF in modulating dendritic architecture of hippocampal neurons. **Brain Struct Funct** 223: 3689–3709, 2018. doi:10.1007/s00429-018-1715-0.

349. Nikoletopoulou V, Sidiropoulou K, Kallergi E, Dalezios Y, Tavernarakis N**.** Modulation of autophagy by BDNF underlies synaptic plasticity. **Cell Metab** 26: 230–242.e5, 2017. doi:10.1016/j.cmet.2017.06.005.

350. Kallergi E, Daskalaki AD, Kolaxi A, Camus C, Ioannou E, Mercaldo V, Haberkant P, Stein F, Sidiropoulou K, Dalezios Y, Savitski MM, Bagni C, Choquet D, Hosy E, Nikoletopoulou V**.** Dendritic autophagy degrades postsynaptic proteins and is required for long-term synaptic depression in mice. **Nat Commun** 13: 680, 2022. doi:10.1038/s41467-022-28301-z.

351. Neeper SA, Gómez-Pinilla F, Choi J, Cotman C**.** Exercise and brain neurotrophins. **Nature** 373: 109, 1995. doi:10.1038/373109a0.

352. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR**.** The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. **Cell** 112: 257–269, 2003. doi:10.1016/s0092-8674(03)00035-7.

353. Morris R**.** Developments of a water-maze procedure for studying spatial learning in the rat. **J Neurosci Methods** 11: 47–60, 1984. doi:10.1016/0165-0270(84)90007-4.

354. Kesslak JP, So V, Choi J, Cotman CW, Gomez-Pinilla F**.** Learning upregulates brain-derived neurotrophic factor messenger ribonucleic acid: a mechanism to facilitate encoding and circuit maintenance? **Behav Neurosci** 112: 1012–1019, 1998. doi:10.1037//0735-7044.112.4.1012.

355. Russo-Neustadt A, Beard RC, Cotman CW**.** Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. **Neuropsychopharmacology** 21: 679–682, 1999. doi:10.1016/S0893-133X(99)00059-7.

356. Hall J, Thomas KL, Everitt BJ**.** Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. **Nat Neurosci** 3: 533–535, 2000. doi:10.1038/75698.

357. Linnarsson S, Björklund A, Ernfors P**.** Learning deficit in BDNF mutant mice. **Eur J Neurosci** 9: 2581–2587, 1997. doi:10.1111/j.1460-9568.1997.tb01687.x.

358. Montkowski A, Holsboer F**.** Intact spatial learning and memory in transgenic mice with reduced BDNF. **Neuroreport** 8: 779–782, 1997. doi:10.1097/00001756-199702100-00040.

359. Minichiello L, Korte M, Wolfer D, Kühn R, Unsicker K, Cestari V, Rossi-Arnaud C, Lipp HP, Bonhoeffer T, Klein R**.** Essential role for TrkB receptors in hippocampus-mediated learning. **Neuron** 24: 401–414, 1999. doi:10.1016/s0896-6273(00)80853-3.

360. Adlard PA, Cotman CW**.** Voluntary exercise protects against stress-induced decreases in brain-derived neurotrophic factor protein expression. **Neuroscience** 124: 985–992, 2004. doi:10.1016/j.neuroscience.2003.12.039.

361. Berchtold NC, Castello N, Cotman CW**.** Exercise and time-dependent benefits to learning and memory. **Neuroscience** 167: 588–597, 2010. doi:10.1016/j.neuroscience.2010.02.050.

362. Cirulli F, Berry A, Chiarotti F, Alleva E**.** Intrahippocampal administration of BDNF in adult rats affects short-term behavioral plasticity in the Morris water maze and performance in the elevated plus-maze. **Hippocampus** 14: 802–807, 2004. doi:10.1002/hipo.10220.

363. Mu JS, Li WP, Yao ZB, Zhou XF**.** Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. **Brain Res** 835: 259–265, 1999. doi:10.1016/s0006-8993(99)01592-9.

364. Rattiner LM, Davis M, French CT, Ressler KJ**.** Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. **J Neurosci** 24: 4796–4806, 2004. doi:10.1523/JNEUROSCI.5654-03.2004.

365. Cathomas F, Vogler C, Euler-Sigmund JC, de Quervain DJ, Papassotiropoulos A**.** Fine-mapping of the brain-derived neurotrophic factor (BDNF) gene supports an association of the Val66Met polymorphism with episodic memory. **Int J Neuropsychopharmacol** 13: 975–980, 2010. doi:10.1017/S1461145710000519.

366. Bueller JA, Aftab M, Sen S, Gomez-Hassan D, Burmeister M, Zubieta JK**.** BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. **Biol Psychiatry** 59: 812–815, 2006. doi:10.1016/j.biopsych.2005.09.022.

367. Petryshen TL, Sabeti PC, Aldinger KA, Fry B, Fan JB, Schaffner SF, Waggoner SG, Tahl AR, Sklar P**.** Population genetic study of the brain-derived neurotrophic factor (BDNF) gene. **Mol Psychiatry** 15: 810–815, 2010. doi:10.1038/mp.2009.24.

368. Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS**.** Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. **Science** 314: 140–143, 2006. doi:10.1126/science.1129663.

369. Sotres-Bayon F, Cain CK, LeDoux JE**.** Brain mechanisms of fear extinction: historical perspectives on the contribution of prefrontal cortex. **Biol Psychiatry** 60: 329–336, 2006. doi:10.1016/j.biopsych.2005.10.012.

370. Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ**.** Induction of fear extinction with hippocampal-infralimbic BDNF. **Science** 328: 1288–1290, 2010. doi:10.1126/science.1186909.

371. Soliman F, Glatt CE, Bath KG, Levita L, Jones RM, Pattwell SS, Jing D, Tottenham N, Amso D, Somerville LH, Voss HU, Glover G, Ballon DJ, Liston C, Teslovich T, Van Kempen T, Lee FS, Casey BJ**.** A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. **Science** 327: 863–866, 2010. doi:10.1126/science.1181886.

372. Zhang L, Benedek DM, Fullerton CS, Forsten RD, Naifeh JA, Li XX, Hu XZ, Li H, Jia M, Xing GQ, Benevides KN, Ursano RJ**.** PTSD risk is associated with BDNF Val66Met and BDNF overexpression. **Mol Psychiatry** 19: 8–10, 2014. doi:10.1038/mp.2012.180.

373. Felmingham KL, Dobson-Stone C, Schofield PR, Quirk GJ, Bryant RA**.** The brain-derived neurotrophic factor Val66Met polymorphism predicts response to exposure therapy in posttraumatic stress disorder. **Biol Psychiatry** 73: 1059–1063, 2013. doi:10.1016/j.biopsych.2012.10.033.

374. Bath KG, Jing DQ, Dincheva I, Neeb CC, Pattwell SS, Chao MV, Lee FS, Ninan I**.** BDNF Val66Met impairs fluoxetine-induced enhancement of adult hippocampus plasticity. **Neuropsychopharmacology** 37: 1297–1304, 2012. doi:10.1038/npp.2011.318.

375. Baj G, Carlino D, Gardossi L, Tongiorgi E**.** Toward a unified biological hypothesis for the BDNF Val66Met-associated memory deficits in humans: a model of impaired dendritic mRNA trafficking. **Front Neurosci** 7: 188, 2013. doi:10.3389/fnins.2013.00188.

376. Xie X, Houtz J, Liao GY, Chen Y, Xu B**.** Genetic Val66Met BDNF variant increases hyperphagia on fat-rich diets in mice. **Endocrinology** 164: 2023. doi:10.1210/endocr/bqad008.

377. Warnault V, Darcq E, Morisot N, Phamluong K, Wilbrecht L, Massa SM, Longo FM, Ron D**.** The BDNF valine 68 to methionine polymorphism increases compulsive alcohol drinking in mice that is reversed by tropomyosin receptor kinase B activation. **Biol Psychiatry** 79: 463–473, 2016. doi:10.1016/j.biopsych.2015.06.007.

378. Jaehne EJ, Kent JN, Antolasic EJ, Wright BJ, Spiers JG, Creutzberg KC, De Rosa F, Riva MA, Sortwell CE, Collier TJ, van den Buuse M**.** Behavioral phenotyping of a rat model of the BDNF Val66Met polymorphism reveals selective impairment of fear memory. **Transl Psychiatry** 12: 93, 2022. doi:10.1038/s41398-022-01858-5.

379. Hunter LE, Freudenberg-Hua Y, Davies P, Kim M, Fleysher R, Stewart WF, Lipton RB, Lipton ML**.** BDNF Val^{66}Met positive players demonstrate diffusion tensor imaging consistent with impaired myelination associated with high levels of soccer heading: indication of a potential gene-environment interaction mechanism. **Front Neurol** 10: 1297, 2019. doi:10.3389/fneur.2019.01297.

380. Brown BM, Bourgeat P, Peiffer JJ, Burnham S, Laws SM, Rainey-Smith SR, Bartrés-Faz D, Villemagne VL, Taddei K, Rembach A, Bush A, Ellis KA, Macaulay SL, Rowe CC, Ames D, Masters CL, Maruff P, Martins RN; AIBL Research Group**.** Influence of BDNF Val66Met on the relationship between physical activity and brain volume. **Neurology** 83: 1345–1352, 2014. doi:10.1212/WNL.0000000000000867.

381. Cellerino A, Carroll P, Thoenen H, Barde YA**.** Reduced size of retinal ganglion cell axons and hypomyelination in mice lacking brain-derived neurotrophic factor. **Mol Cell Neurosci** 9: 397–408, 1997. doi:10.1006/mcne.1997.0641.

382. Xiao J, Wong AW, Willingham MM, van den Buuse M, Kilpatrick TJ, Murray SS**.** Brain-derived neurotrophic factor promotes central nervous system myelination via a direct effect upon oligodendrocytes. **Neurosignals** 18: 186–202, 2010. doi:10.1159/000323170.

383. Vondran MW, Clinton-Luke P, Honeywell JZ, Dreyfus CF**.** BDNF+/− mice exhibit deficits in oligodendrocyte lineage cells of the basal forebrain. **Glia** 58: 848–856, 2010. doi:10.1002/glia.20969.

384. Yalcin B, Pomrenze MB, Malacon K, Drexler R, Rogers AE, Shamardani K, Chau IJ, Taylor KR, Ni L, Contreras-Esquivel D, Malenka RC, Monje M**.** Myelin plasticity in the ventral tegmental area is required for opioid reward. **Nature** 630: 677–685, 2024. doi:10.1038/s41586-024-07525-7.

385. Scholz J, Woolf CJ**.** The neuropathic pain triad: neurons, immune cells and glia. **Nat Neurosci** 10: 1361–1368, 2007. doi:10.1038/nn1992.

386. Fatt MP, Zhang MD, Kupari J, Altinkök M, Yang Y, Hu Y, Svenningsson P, Ernfors P**.** Morphine-responsive neurons that regulate mechanical antinociception. **Science** 385: eado6593, 2024. doi:10.1126/science.ado6593.

387. Brady R, Zaidi SI, Mayer C, Katz DM**.** BDNF is a target-derived survival factor for arterial baroreceptor and chemoafferent primary sensory neurons. **J Neurosci** 19: 2131–2142, 1999. doi:10.1523/JNEUROSCI.19-06-02131.1999.

388. Bianchi LM, Conover JC, Fritzsch B, DeChiara T, Lindsay RM, Yancopoulos GD**.** Degeneration of vestibular neurons in late embryogenesis of both heterozygous and homozygous BDNF null mutant mice. **Development** 122: 1965–1973, 1996. doi:10.1242/dev.122.6.1965.

389. Xu B, Xie X**.** Neurotrophic factor control of satiety and body weight. **Nat Rev Neurosci** 17: 282–292, 2016. doi:10.1038/nrn.2016.24.

390. D'Amico F, Lugarà C, Luppino G, Giuffrida C, Giorgianni Y, Patanè EM, Manti S, Gambadauro A, La Rocca M, Abbate T**.** The influence of neurotrophins on the brain-lung axis: conception, pregnancy, and neonatal period. **Curr Issues Mol Biol** 46: 2528–2543, 2024. doi:10.3390/cimb46030160.

391. Meerschaert KA, Chiu IM**.** The gut-brain axis and pain signalling mechanisms in the gastrointestinal tract. **Nat Rev Gastroenterol Hepatol** 22: 206–221, 2025. doi:10.1038/s41575-024-01017-9.

392. Ohara TE, Hsiao EY**.** Microbiota-neuroepithelial signalling across the gut-brain axis. **Nat Rev Microbiol** 23: 371–384, 2025. doi:10.1038/s41579-024-01136-9.

393. Kupari J, Häring M, Agirre E, Castelo-Branco G, Ernfors P**.** An atlas of vagal sensory neurons and their molecular specialization. **Cell Rep** 27: 2508–2523.e4, 2019. doi:10.1016/j.celrep.2019.04.096.

394. Carroll P, Lewin GR, Koltzenburg M, Toyka KV, Thoenen H**.** A role for BDNF in mechanosensation. **Nat Neurosci** 1: 42–46, 1998. doi:10.1038/242.

395. Yan Q, Rosenfeld RD, Matheson CR, Hawkins N, Lopez OT, Bennett L, Welcher AA**.** Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. **Neuroscience** 78: 431–448, 1997. doi:10.1016/s0306-4522(96)00613-6.

396. Kermani P, Hempstead B**.** BDNF actions in the cardiovascular system: roles in development, adulthood and response to injury. **Front Physiol** 10: 455, 2019. doi:10.3389/fphys.2019.00455.

397. Lu YZ, Nayer B, Singh SK, Alshoubaki YK, Yuan E, Park AJ, Maruyama K, Akira S, Martino MM**.** CGRP sensory neurons promote tissue healing via neutrophils and macrophages. **Nature** 628: 604–611, 2024. doi:10.1038/s41586-024-07237-y.

398. Padmanaban V, Keller I, Seltzer ES, Ostendorf BN, Kerner Z, Tavazoie SF**.** Neuronal substance P drives metastasis through an

extracellular RNA-TLR7 axis. **Nature** 633: 207–215, 2024. doi:10.1038/s41586-024-07767-5.

399. Mu X, Silos-Santiago I, Carroll SL, Snider WD**.** Neurotrophin receptor genes are expressed in distinct patterns in developing dorsal root ganglia. **J Neurosci** 13: 4029–4041, 1993. doi:10.1523/JNEUROSCI.13-09-04029.1993.

400. Jang K, Garraway SM**.** A review of dorsal root ganglia and primary sensory neuron plasticity mediating inflammatory and chronic neuropathic pain. **Neurobiol Pain** 15: 100151, 2024. doi:10.1016/j.ynpai.2024.100151.

401. Siuciak JA, Altar CA, Wiegand SJ, Lindsay RM**.** Antinociceptive effect of brain-derived neurotrophic factor and neurotrophin-3. **Brain Res** 633: 326–330, 1994. doi:10.1016/0006-8993(94)91556-3.

402. Siuciak JA, Wong V, Pearsall D, Wiegand SJ, Lindsay RM**.** BDNF produces analgesia in the formalin test and modifies neuropeptide levels in rat brain and spinal cord areas associated with nociception. **Eur J Neurosci** 7: 663–670, 1995. doi:10.1111/j.1460-9568.1995.tb00670.x.

403. Mannion RJ, Costigan M, Decosterd I, Amaya F, Ma QP, Holstege JC, Ji RR, Acheson A, Lindsay RM, Wilkinson GA, Woolf CJ**.** Neurotrophins: peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity. **Proc Natl Acad Sci USA** 96: 9385–9390, 1999. doi:10.1073/pnas.96.16.9385.

404. Chen JT, Guo D, Campanelli D, Frattini F, Mayer F, Zhou L, Kuner R, Heppenstall PA, Knipper M, Hu J**.** Presynaptic GABAergic inhibition regulated by BDNF contributes to neuropathic pain induction. **Nat Commun** 5: 5331, 2014. doi:10.1038/ncomms6331.

405. Ha SO, Kim JK, Hong HS, Kim DS, Cho HJ**.** Expression of brain-derived neurotrophic factor in rat dorsal root ganglia, spinal cord and gracile nuclei in experimental models of neuropathic pain. **Neuroscience** 107: 301–309, 2001. doi:10.1016/s0306-4522(01)00353-0.

406. Kolbeck R, Bartke I, Eberle W, Barde YA**.** Brain-derived neurotrophic factor levels in the nervous system of wild-type and neurotrophin gene mutant mice. **J Neurochem** 72: 1930–1938, 1999. doi:10.1046/j.1471-4159.1999.0721930.x.

407. Sikandar S, Minett MS, Millet Q, Santana-Varela S, Lau J, Wood JN, Zhao J**.** Brain-derived neurotrophic factor derived from sensory neurons plays a critical role in chronic pain. **Brain** 141: 1028–1039, 2018. doi:10.1093/brain/awy009.

408. Pulskamp TG, Johnson LM, Berlau DJ**.** Novel non-opioid analgesics in pain management. **Pain Manag** 14: 641–651, 2024. doi:10.1080/17581869.2024.2442292.

409. Indo Y, Tsuruta M, Hayashida Y, Karim MA, Ohta K, Kawano T, Mitsubuchi H, Tonoki H, Awaya Y, Matsuda I**.** Mutations in the TRKA/NGF receptor gene in patients with congenital insensitivity to pain with anhidrosis. **Nat Genet** 13: 485–488, 1996. doi:10.1038/ng0896-485.

410. Einarsdottir E, Carlsson A, Minde J, Toolanen G, Svensson O, Solders G, Holmgren G, Holmberg D, Holmberg M**.** A mutation in the nerve growth factor beta gene (NGFB) causes loss of pain perception. **Hum Mol Genet** 13: 799–805, 2004. doi:10.1093/hmg/ddh096.

411. Indo Y**.** Neurobiology of pain, interoception and emotional response: lessons from nerve growth factor-dependent neurons. **Eur J Neurosci** 39: 375–391, 2014. doi:10.1111/ejn.12448.

412. Patel MK, Kaye AD, Urman RD**.** Tanezumab: Therapy targeting nerve growth factor in pain pathogenesis. **J Anaesthesiol Clin Pharmacol** 34: 111–116, 2018. doi:10.4103/joacp.JOACP_389_15.

413. Ferrini F, Salio C, Boggio EM, Merighi A**.** Interplay of BDNF and GDNF in the mature spinal somatosensory system and its potential therapeutic relevance. **Curr Neuropharmacol** 19: 1225–1245, 2021. doi:10.2174/1570159X18666201116143422.

414. Richner M, Pallesen LT, Ulrichsen M, Poulsen ET, Holm TH, Login H, Castonguay A, Lorenzo LE, Gonçalves NP, Andersen OM, Lykke-Hartmann K, Enghild JJ, Rønn LC, Malik IJ, De Koninck Y, Bjerrum OJ, Vaegter CB, Nykjaer A**.** Sortilin gates neurotensin and BDNF signaling to control peripheral neuropathic pain. **Sci Adv** 5: eaav9946, 2019. doi:10.1126/sciadv.aav9946.

415. Liedtke W**.** Long march toward safe and effective analgesia by enhancing gene expression of Kcc2: first steps taken. **Front Mol Neurosci** 15: 865600, 2022. doi:10.3389/fnmol.2022.865600.

416. Proudfit HK, Anderson EG**.** Morphine analgesia: blockade by raphe magnus lesions. **Brain Res** 98: 612–618, 1975. doi:10.1016/0006-8993(75)90380-7.

417. Basbaum AI, Clanton CH, Fields HL**.** Opiate and stimulus-produced analgesia: functional anatomy of a medullospinal pathway. **Proc Natl Acad Sci USA** 73: 4685–4688, 1976. doi:10.1073/pnas.73.12.4685.

418. Oliveras JL, Redjemi F, Guilbaud G, Besson JM**.** Analgesia induced by electrical stimulation of the inferior centralis nucleus of the raphe in the cat. **Pain** 1: 139–145, 1975. doi:10.1016/0304-3959(75)90098-6.

419. Hammen C**.** Stress and depression. **Annu Rev Clin Psychol** 1: 293–319, 2005. doi:10.1146/annurev.clinpsy.1.102803.143938.

420. Holsboer F**.** The corticosteroid receptor hypothesis of depression. **Neuropsychopharmacology** 23: 477–501, 2000. doi:10.1016/S0893-133X(00)00159-7.

421. Smith MA, Makino S, Kvetnansky R, Post RM**.** Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. **J Neurosci** 15: 1768–1777, 1995. doi:10.1523/JNEUROSCI.15-03-01768.1995.

422. Sahay A, Hen R**.** Adult hippocampal neurogenesis in depression. **Nat Neurosci** 10: 1110–1115, 2007. doi:10.1038/nn1969.

423. Woolley CS, Gould E, McEwen BS**.** Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. **Brain Res** 531: 225–231, 1990. doi:10.1016/0006-8993(90)90778-a.

424. Nibuya M, Morinobu S, Duman RS**.** Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. **J Neurosci** 15: 7539–7547, 1995. doi:10.1523/JNEUROSCI.15-11-07539.1995.

425. Hearing CM, Chang WC, Szuhany KL, Deckersbach T, Nierenberg AA, Sylvia LG**.** Physical exercise for treatment of mood disorders: a critical review. **Curr Behav Neurosci Rep** 3: 350–359, 2016. doi:10.1007/s40473-016-0089-y.

426. Mamounas LA, Blue ME, Siuciak JA, Altar CA**.** Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. **J Neurosci** 15: 7929–7939, 1995. doi:10.1523/JNEUROSCI.15-12-07929.1995.

427. Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM**.** Antidepressant-like effect of brain-derived neurotrophic factor (BDNF).

Pharmacol Biochem Behav 56: 131–137, 1997. doi:10.1016/S0091-3057(96)00169-4.

428. Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E, Agerman K, Haapasalo A, Nawa H, Aloyz R, Ernfors P, Castrén E**.** Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. **J Neurosci** 23: 349–357, 2003. doi:10.1523/JNEUROSCI.23-01-00349.2003.

429. Rantamäki T, Hendolin P, Kankaanpää A, Mijatovic J, Piepponen P, Domenici E, Chao MV, Männistö PT, Castren E**.** Pharmacologically diverse antidepressants rapidly activate brain-derived neurotrophic factor receptor TrkB and induce phospholipase-Cgamma signaling pathways in mouse brain. **Neuropsychopharmacology** 32: 2152–2162, 2007. doi:10.1038/sj.npp.1301345.

430. Casarotto PC, Girych M, Fred SM, Kovaleva V, Moliner R, Enkavi G, Biojone C, Cannarozzo C, Sahu MP, Kaurinkoski K, Brunello CA, Steinzeig A, Winkel F, Patil S, Vestring S, Serchov T, Diniz C, Laukkanen L, Cardon I, Antila H, Rog T, Piepponen TP, Bramham CR, Normann C, Lauri SE, Saarma M, Vattulainen I, Castrén E**.** Antidepressant drugs act by directly binding to TRKB neurotrophin receptors. **Cell** 184: 1299–1313.e19, 2021. doi:10.1016/j.cell.2021.01.034.

431. Moliner R, Girych M, Brunello CA, Kovaleva V, Biojone C, Enkavi G, et al**.** Psychedelics promote plasticity by directly binding to BDNF receptor TrkB. **Nat Neurosci** 26: 1032–1041, 2023. doi:10.1038/s41593-023-01316-5.

432. Boltaev U, Meyer Y, Tolibzoda F, Jacques T, Gassaway M, Xu Q, Wagner F, Zhang YL, Palmer M, Holson E, Sames D**.** Multiplex quantitative assays indicate a need for reevaluating reported small-molecule TrkB agonists. **Sci Signal** 10: eaal1670, 2017. doi:10.1126/scisignal.aal1670.

433. Jang SW, Liu X, Yepes M, Shepherd KR, Miller GW, Liu Y, Wilson WD, Xiao G, Blanchi B, Sun YE, Ye K**.** A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. **Proc Natl Acad Sci USA** 107: 2687–2692, 2010. doi:10.1073/pnas.0913572107.

434. Li Y, Wang X, Meng Y, Hu T, Zhao J, Li R, Bai Q, Yuan P, Han J, Hao K, Wei Y, Qiu Y, Li N, Zhao Y**.** Dopamine reuptake and inhibitory mechanisms in human dopamine transporter. **Nature** 632: 686–694, 2024. doi:10.1038/s41586-024-07796-0.

435. Srivastava DK, Navratna V, Tosh DK, Chinn A, Sk MF, Tajkhorshid E, Jacobson KA, Gouaux E**.** Structure of the human dopamine transporter and mechanisms of inhibition. **Nature** 632: 672–677, 2024. doi:10.1038/s41586-024-07739-9.

436. Nielsen JC, Salomon K, Kalenderoglou IE, Bargmeyer S, Pape T, Shahsavar A, Loland CJ**.** Structure of the human dopamine transporter in complex with cocaine. **Nature** 632: 678–685, 2024. doi:10.1038/s41586-024-07804-3.

437. Gao AX, Xia TC, Lin LS, Dong TT, Tsim KW**.** The neurotrophic activities of brain-derived neurotrophic factor are potentiated by binding with apigenin, a common flavone in vegetables, in stimulating the receptor signaling. **CNS Neurosci Ther** 29: 2787–2799, 2023. doi:10.1111/cns.14230.

438. Diniz CR, Crestani AP, Casarotto PC, Biojone C, Cannarozzo C, Winkel F, Prozorov MA, Kot EF, Goncharuk SA, Benette Marques D, Rakauskas Zacharias L, Autio H, Sahu MP, Borges-Assis AB, Leite JP, Mineev KS, Castrén E, Resstel LB**.** Fluoxetine and ketamine enhance extinction memory and brain plasticity by triggering the p75 neurotrophin receptor proteolytic pathway. **Biol Psychiatry** 97: 248–260, 2025. doi:10.1016/j.biopsych.2024.06.021.

439. Castrén E, Hen R**.** Neuronal plasticity and antidepressant actions. **Trends Neurosci** 36: 259–267, 2013. doi:10.1016/j.tins.2012.12.010.

440. Simmons DA, Knowles JK, Belichenko NP, Banerjee G, Finkle C, Massa SM, Longo FM**.** A small molecule p75NTR ligand, LM11A-31, reverses cholinergic neurite dystrophy in Alzheimer's disease mouse models with mid- to late-stage disease progression. **PLoS One** 9: e102136, 2014. doi:10.1371/journal.pone.0102136.

441. Dwivedi Y, Rizavi HS, Conley RR, Roberts RC, Tamminga CA, Pandey GN**.** Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. **Arch Gen Psychiatry** 60: 804–815, 2003. doi:10.1001/archpsyc.60.8.804.

442. Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R**.** Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. **Brain Res Mol Brain Res** 136: 29–37, 2005. doi:10.1016/j.molbrainres.2004.12.020.

443. Kohli MA, Salyakina D, Pfennig A, Lucae S, Horstmann S, Menke A, Kloiber S, Hennings J, Bradley BB, Ressler KJ, Uhr M, Müller-Myhsok B, Holsboer F, Binder EB**.** Association of genetic variants in the neurotrophic receptor-encoding gene NTRK2 and a lifetime history of suicide attempts in depressed patients. **Arch Gen Psychiatry** 67: 348–359, 2010. doi:10.1001/archgenpsychiatry.2009.201.

444. Hartig J, Nemeş B**.** BDNF-related mutations in major depressive disorder: a systematic review. **Acta Neuropsychiatr** 35: 5–26, 2023. doi:10.1017/neu.2022.22.

445. Keller S, Sarchiapone M, Zarrilli F, Videtic A, Ferraro A, Carli V, Sacchetti S, Lembo F, Angiolillo A, Jovanovic N, Pisanti F, Tomaiuolo R, Monticelli A, Balazic J, Roy A, Marusic A, Cocozza S, Fusco A, Bruni CB, Castaldo G, Chiariotti L**.** Increased BDNF promoter methylation in the Wernicke area of suicide subjects. **Arch Gen Psychiatry** 67: 258–267, 2010. doi:10.1001/archgenpsychiatry.2010.9.

446. Craft LL, Perna FM**.** The benefits of exercise for the clinically depressed. **Prim Care Companion J Clin Psychiatry** 6: 104–111, 2004. doi:10.4088/pcc.v06n0301.

447. Davidson B, Bhattacharya A, Sarica C, Darmani G, Raies N, Chen R, Lozano AM**.** Neuromodulation techniques—from non-invasive brain stimulation to deep brain stimulation. **Neurotherapeutics** 21: e00330, 2024. doi:10.1016/j.neurot.2024.e00330.

448. Woodham RD, Selvaraj S, Lajmi N, Hobday H, Sheehan G, Ghazi-Noori AR, Lagerberg PJ, Rizvi M, Kwon SS, Orhii P, Maislin D, Hernandez L, Machado-Vieira R, Soares JC, Young AH, Fu CHY**.** Home-based transcranial direct current stimulation treatment for major depressive disorder: a fully remote phase 2 randomized sham-controlled trial. **Nat Med** 31: 87–95, 2025. doi:10.1038/s41591-024-03305-y.

449. Stagg CJ, Nitsche MA**.** Physiological basis of transcranial direct current stimulation. **Neuroscientist** 17: 37–53, 2011. doi:10.1177/1073858410386614.

450. Meinzer M, Shahbabaie A, Antonenko D, Blankenburg F, Fischer R, Hartwigsen G, Nitsche MA, Li SC, Thielscher A, Timmann D, Waltemath D, Abdelmotaleb M, Kocataş H, Caisachana Guevara LM, Batsikadze G, Grundei M, Cunha T, Hayek D, Turker S, Schlitt F, Shi Y, Khan A, Burke M, Riemann S, Niemann F, Flöel A**.** Investigating the neural mechanisms of transcranial direct current stimulation effects on human cognition: current issues and potential solutions. **Front Neurosci** 18: 1389651, 2024. doi:10.3389/fnins.2024.1389651.

451. Grossman N, Bono D, Dedic N, Kodandaramaiah SB, Rudenko A, Suk HJ, Cassara AM, Neufeld E, Kuster N, Tsai LH, Pascual-Leone A, Boyden ES**.** Noninvasive deep brain stimulation via temporally interfering electric fields. **Cell** 169: 1029–1041.e16, 2017. doi:10.1016/j.cell.2017.05.024.

452. Cassarà AM, Newton TH, Zhuang K, Regel SJ, Achermann P, Pascual-Leone A, Kuster N, Neufeld E**.** Recommendations for the safe application of temporal interference stimulation in the human brain Part II: biophysics, dosimetry, and safety recommendations. **Bioelectromagnetics** 46: e22536, 2025. doi:10.1002/bem.22536.

453. Lapchak PA, Hefti F**.** BDNF and NGF treatment in lesioned rats: effects on cholinergic function and weight gain. **Neuroreport** 3: 405–408, 1992. doi:10.1097/00001756-199205000-00007.

454. Lyons WE, Mamounas LA, Ricaurte GA, Coppola V, Reid SW, Bora SH, Wihler C, Koliatsos VE, Tessarollo L**.** Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. **Proc Natl Acad Sci USA** 96: 15239–15244, 1999. doi:10.1073/pnas.96.26.15239.

455. Kernie SG, Liebl DJ, Parada LF**.** BDNF regulates eating behavior and locomotor activity in mice. **EMBO J** 19: 1290–1300, 2000. doi:10.1093/emboj/19.6.1290.

456. Xu B, Goulding EH, Zang K, Cepoi D, Cone RD, Jones KR, Tecott LH, Reichardt LF**.** Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. **Nat Neurosci** 6: 736–742, 2003. doi:10.1038/nn1073.

457. Unger TJ, Calderon GA, Bradley LC, Sena-Esteves M, Rios M**.** Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. **J Neurosci** 27: 14265–14274, 2007. doi:10.1523/JNEUROSCI.3308-07.2007.

458. Friedman JM**.** Leptin and the endocrine control of energy balance. **Nat Metab** 1: 754–764, 2019. doi:10.1038/s42255-019-0095-y.

459. Nicholson JR, Peter JC, Lecourt AC, Barde YA, Hofbauer KG**.** Melanocortin-4 receptor activation stimulates hypothalamic brain-derived neurotrophic factor release to regulate food intake, body temperature and cardiovascular function. **J Neuroendocrinol** 19: 974–982, 2007. doi:10.1111/j.1365-2826.2007.01610.x.

460. Luo F, Mu Y, Gao C, Xiao Y, Zhou Q, Yang Y, Ni X, Shen WL, Yang J**.** Whole-brain patterns of the presynaptic inputs and axonal projections of BDNF neurons in the paraventricular nucleus. **J Genet Genomics** 46: 31–40, 2019. doi:10.1016/j.jgg.2018.11.004.

461. Balthasar N, Dalgaard LT, Lee CE, Yu J, Funahashi H, Williams T, Ferreira M, Tang V, McGovern RA, Kenny CD, Christiansen LM, Edelstein E, Choi B, Boss O, Aschkenasi C, Zhang CY, Mountjoy K, Kishi T, Elmquist JK, Lowell BB**.** Divergence of melanocortin pathways in the control of food intake and energy expenditure. **Cell** 123: 493–505, 2005. doi:10.1016/j.cell.2005.08.035.

462. Nonomura T, Tsuchida A, Ono-Kishino M, Nakagawa T, Taiji M, Noguchi H**.** Brain-derived neurotrophic factor regulates energy expenditure through the central nervous system in obese diabetic mice. **Int J Exp Diabetes Res** 2: 201–209, 2001. doi:10.1155/edr.2001.201.

463. Nakagawa T, Tsuchida A, Itakura Y, Nonomura T, Ono M, Hirota F, Inoue T, Nakayama C, Taiji M, Noguchi H**.** Brain-derived neurotrophic factor regulates glucose metabolism by modulating energy balance in diabetic mice. **Diabetes** 49: 436–444, 2000. doi:10.2337/diabetes.49.3.436.

464. Ono M, Ichihara J, Nonomura T, Itakura Y, Taiji M, Nakayama C, Noguchi H**.** Brain-derived neurotrophic factor reduces blood glucose level in obese diabetic mice but not in normal mice. **Biochem Biophys Res Commun** 238: 633–637, 1997. doi:10.1006/bbrc.1997.7220.

465. Cannon B, Nedergaard J**.** Brown adipose tissue: function and physiological significance. **Physiol Rev** 84: 277–359, 2004. doi:10.1152/physrev.00015.2003.

466. Barde YA, Chinet A, Girardier L**.** Potassium-induced increase in oxygen consumption of brown adipose tissue from the rat. **J Physiol** 252: 523–536, 1975. doi:10.1113/jphysiol.1975.sp011156.

467. Nicholls DG, Rial E**.** A history of the first uncoupling protein, UCP1. **J Bioenerg Biomembr** 31: 399–406, 1999. doi:10.1023/a:1005436121005.

468. Wang P, Loh KH, Wu M, Morgan DA, Schneeberger M, Yu X, Chi J, Kosse C, Kim D, Rahmouni K, Cohen P, Friedman J**.** A leptin-BDNF pathway regulating sympathetic innervation of adipose tissue. **Nature** 583: 839–844, 2020. doi:10.1038/s41586-020-2527-y.

469. Friedman JM, Halaas JL**.** Leptin and the regulation of body weight in mammals. **Nature** 395: 763–770, 1998. doi:10.1038/27376.

470. Gao X, Murphy MM, Peyer JG, Ni Y, Yang M, Zhang Y, Guo J, Kara N, Embree C, Tasdogan A, Ubellacker JM, Crane GM, Fang S, Zhao Z, Shen B, Morrison SJ**.** Leptin receptor^{+} cells promote bone marrow innervation and regeneration by synthesizing nerve growth factor. **Nat Cell Biol** 25: 1746–1757, 2023. doi:10.1038/s41556-023-01284-9.

471. An JJ, Liao GY, Kinney CE, Sahibzada N, Xu B**.** Discrete BDNF neurons in the paraventricular hypothalamus control feeding and energy expenditure. **Cell Metab** 22: 175–188, 2015. doi:10.1016/j.cmet.2015.05.008.

472. Ly T, Oh JY, Sivakumar N, Shehata S, La Santa Medina N, Huang H, Liu Z, Fang W, Barnes C, Dundar N, Jarvie BC, Ravi A, Barnhill OK, Li C, Lee GR, Choi J, Jang H, Knight ZA**.** Sequential appetite suppression by oral and visceral feedback to the brainstem. **Nature** 624: 130–137, 2023. doi:10.1038/s41586-023-06758-2.

473. Bai L, Mesgarzadeh S, Ramesh KS, Huey EL, Liu Y, Gray LA, Aitken TJ, Chen Y, Beutler LR, Ahn JS, Madisen L, Zeng H, Krasnow MA, Knight ZA**.** Genetic identification of vagal sensory neurons that control feeding. **Cell** 179: 1129–1143.e3, 2019. doi:10.1016/j.cell.2019.10.031.

474. Wu XY, Zhu JX, Gao J, Owyang C, Li Y**.** Neurochemical phenotype of vagal afferent neurons activated to express C-FOS in response to luminal stimulation in the rat. **Neuroscience** 130: 757–767, 2005. doi:10.1016/j.neuroscience.2004.09.060.

475. Vermehren-Schmaedick A, Khanjian RA, Balkowiec A**.** Cellular mechanisms of activity-dependent BDNF expression in primary sensory neurons. **Neuroscience** 310: 665–673, 2015. doi:10.1016/j.neuroscience.2015.10.007.

476. Balkowiec A, Katz DM**.** Brain-derived neurotrophic factor is required for normal development of the central respiratory rhythm in mice. **J Physiol** 510: 527–533, 1998. doi:10.1111/j.1469-7793.1998.527bk.x.

477. Feetham CH, Collabolletta V, Worth AA, Shoop R, Groom S, Harding C, Boutagouga Boudjadja M, Coskun T, Emmerson PJ, D'Agostino G, Luckman SM**.** Brainstem BDNF neurons are downstream of GFRAL/GLP1R signalling. **Nat Commun** 15: 10749, 2024. doi:10.1038/s41467-024-54367-y.

478. Elias A, Padinjakara N, Lautenschlager NT**.** Effects of intermittent fasting on cognitive health and Alzheimer's disease. **Nutr Rev** 81: 1225–1233, 2023. doi:10.1093/nutrit/nuad021.

479. Kumar A, Karuppagounder SS, Chen Y, Corona C, Kawaguchi R, Cheng Y, Balkaya M, Sagdullaev BT, Wen Z, Stuart C, Cho S, Ming GL, Tuvikene J, Timmusk T, Geschwind DH, Ratan RR**.** 2-Deoxyglucose drives plasticity via an adaptive ER stress-ATF4 pathway and elicits stroke recovery and Alzheimer's resilience. **Neuron** 111: 2831–2846.e10, 2023. doi:10.1016/j.neuron.2023.06.013.

480. Sonoyama T, Stadler LK, Zhu M, Keogh JM, Henning E, Hisama F, Kirwan P, Jura M, Blaszczyk BK, DeWitt DC, Brouwers B, Hyvönen M, Barroso I, Merkle FT, Appleyard SM, Wayman GA, Farooqi IS**.** Human BDNF/TrkB variants impair hippocampal synaptogenesis and associate with neurobehavioural abnormalities. **Sci Rep** 10: 9028, 2020. doi:10.1038/s41598-020-65531-x.

481. Yeo GS, Hung CC, Rochford J, Keogh J, Gray J, Sivaramakrishnan S, O'Rahilly S, Farooqi IS**.** A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. **Nat Neurosci** 7: 1187–1189, 2004. doi:10.1038/nn1336.

482. Gray J, Yeo GS, Cox JJ, Morton J, Adlam AL, Keogh JM, Yanovski JA, El Gharbawy A, Han JC, Tung YC, Hodges JR, Raymond FL, O'Rahilly S, Farooqi IS**.** Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. **Diabetes** 55: 3366–3371, 2006. doi:10.2337/db06-0550.

483. Han JC, Liu QR, Jones M, Levinn RL, Menzie CM, Jefferson-George KS, Adler-Wailes DC, Sanford EL, Lacbawan FL, Uhl GR, Rennert OM, Yanovski JA**.** Brain-derived neurotrophic factor and obesity in the WAGR syndrome. **N Engl J Med** 359: 918–927, 2008. doi:10.1056/NEJMoa0801119.

484. Han JC, Thurm A, Golden Williams C, Joseph LA, Zein WM, Brooks BP, Butman JA, Brady SM, Fuhr SR, Hicks MD, Huey AE, Hanish AE, Danley KM, Raygada MJ, Rennert OM, Martinowich K, Sharp SJ, Tsao JW, Swedo SE**.** Association of brain-derived neurotrophic factor (BDNF) haploinsufficiency with lower adaptive behaviour and reduced cognitive functioning in WAGR/11p13 deletion syndrome. **Cortex** 49: 2700–2710, 2013. doi:10.1016/j.cortex.2013.02.009.

485. Bochukova EG, Lawler K, Croizier S, Keogh JM, Patel N, Strohbehn G, Lo KK, Humphrey J, Hokken-Koelega A, Damen L, Donze S, Bouret SG, Plagnol V, Farooqi IS**.** A transcriptomic signature of the hypothalamic response to fasting and BDNF deficiency in Prader-Willi syndrome. **Cell Rep** 22: 3401–3408, 2018. doi:10.1016/j.celrep.2018.03.018.

486. Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al**.** Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. **Nat Genet** 42: 937–948, 2010. doi:10.1038/ng.686.

487. Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadottir A, et al**.** Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. **Nat Genet** 41: 18–24, 2009. doi:10.1038/ng.274.

488. Mou Z, Hyde TM, Lipska BK, Martinowich K, Wei P, Ong CJ, Hunter LA, Palaguachi GI, Morgun E, Teng R, Lai C, Condarco TA, Demidowich AP, Krause AJ, Marshall LJ, Haack K, Voruganti VS, Cole SA, Butte NF, Comuzzie AG, Nalls MA, Zonderman AB, Singleton AB, Evans MK, Martin B, Maudsley S, Tsao JW, Kleinman JE, Yanovski JA, Han JC**.** Human obesity associated with an intronic SNP in the brain-derived neurotrophic factor locus. **Cell Rep** 13: 1073–1080, 2015. doi:10.1016/j.celrep.2015.09.065.

489. Lin JC, Tsao D, Barras P, Bastarrachea RA, Boyd B, Chou J, Rosete R, Long H, Forgie A, Abdiche Y, Dilley J, Stratton J, Garcia C, Sloane DL, Comuzzie AG, Rosenthal A**.** Appetite enhancement and weight gain by peripheral administration of TrkB agonists in non-human primates. **PLoS One** 3: e1900, 2008. doi:10.1371/journal.pone.0001900.

490. Perreault M, Feng G, Will S, Gareski T, Kubasiak D, Marquette K, Vugmeyster Y, Unger TJ, Jones J, Qadri A, Hahm S, Sun Y, Rohde CM, Zwijnenberg R, Paulsen J, Gimeno RE**.** Activation of TrkB with TAM-163 results in opposite effects on body weight in rodents and non-human primates. **PLoS One** 8: e62616, 2013. doi:10.1371/journal.pone.0062616.

491. Erickson KI, Donofry SD, Sewell KR, Brown BM, Stillman CM**.** Cognitive aging and the promise of physical activity. **Annu Rev Clin Psychol** 18: 417–442, 2022. doi:10.1146/annurev-clinpsy-072720-014213.

492. MoTrPAC Study Group**. Lead Analysts, MoTrPAC Study Group.** Temporal dynamics of the multi-omic response to endurance exercise training. **Nature** 629: 174–183, 2024. doi:10.1038/s41586-023-06877-w.

493. Pruunsild P, Bengtson CP, Bading H**.** Networks of cultured iPSC-derived neurons reveal the human synaptic activity-regulated adaptive gene program. **Cell Rep** 18: 122–135, 2017. doi:10.1016/j.celrep.2016.12.018.

494. Siette J, Westbrook RF, Cotman C, Sidhu K, Zhu W, Sachdev P, Valenzuela MJ**.** Age-specific effects of voluntary exercise on memory and the older brain. **Biol Psychiatry** 73: 435–442, 2013. doi:10.1016/j.biopsych.2012.05.034.

495. Intlekofer KA, Berchtold NC, Malvaez M, Carlos AJ, McQuown SC, Cunningham MJ, Wood MA, Cotman CW**.** Exercise and sodium butyrate transform a subthreshold learning event into long-term memory via a brain-derived neurotrophic factor-dependent mechanism. **Neuropsychopharmacology** 38: 2027–2034, 2013. doi:10.1038/npp.2013.104.

496. Hayashi M, Mistunaga F, Ohira K, Shimizu K**.** Changes in BDNF-immunoreactive structures in the hippocampal formation of the aged macaque monkey. **Brain Res** 918: 191–196, 2001. doi:10.1016/s0006-8993(01)03002-5.

497. Livingston G, Huntley J, Liu KY, Costafreda SG, Selbæk G, Alladi S, Ames D, Banerjee S, Burns A, Brayne C, Fox NC, Ferri CP, Gitlin LN, Howard R, Kales HC, Kivimäki M, Larson EB, Nakasujja N, Rockwood K, Samus Q, Shirai K, Singh-Manoux A, Schneider LS, Walsh S, Yao Y, Sommerlad A, Mukadam N**.** Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. **Lancet** 404: 572–628, 2024. doi:10.1016/S0140-6736(24)01296-0.

498. Buchman AS, Yu L, Boyle PA, Schneider JA, De Jager PL, Bennett DA**.** Higher brain BDNF gene expression is associated with slower cognitive decline in older adults. **Neurology** 86: 735–741, 2016. doi:10.1212/WNL.0000000000002387.

499. Katz L, Rubin M**. Keep Your Brain Alive: 83 Neurobic Exercises to Help Prevent Memory Loss and Increase Mental Fitness**. Workman Publishing, Inc., 1999.

500. West MO, Carelli RM, Pomerantz M, Cohen SM, Gardner JP, Chapin JK, Woodward DJ**.** A region in the dorsolateral striatum of the rat exhibiting single-unit correlations with specific locomotor limb movements. **J Neurophysiol** 64: 1233–1246, 1990. doi:10.1152/jn.1990.64.4.1233.

501. Polacchini A, Metelli G, Francavilla R, Baj G, Florean M, Mascaretti LG, Tongiorgi E**.** A method for reproducible measurements of serum BDNF: comparison of the performance of six commercial assays. **Sci Rep** 5: 17989, 2015. doi:10.1038/srep17989.

502. Rasmussen P, Brassard P, Adser H, Pedersen MV, Leick L, Hart E, Secher NH, Pedersen BK, Pilegaard H**.** Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. **Exp Physiol** 94: 1062–1069, 2009. doi:10.1113/expphysiol.2009.048512.

503. Pardridge WM, Kang YS, Buciak JL**.** Transport of human recombinant brain-derived neurotrophic factor (BDNF) through the rat blood-brain barrier in vivo using vector-mediated peptide drug delivery. **Pharm Res** 11: 738–746, 1994. doi:10.1023/a:1018940732550.

504. Yamamoto H, Gurney ME**.** Human platelets contain brain-derived neurotrophic factor. **J Neurosci** 10: 3469–3478, 1990. doi:10.1523/JNEUROSCI.10-11-03469.1990.

505. Radka SF, Holst PA, Fritsche M, Altar CA**.** Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay. **Brain Res** 709: 122–301, 1996. doi:10.1016/0006-8993(95)01321-0.

506. Balkenhol J, Kaltdorf KV, Mammadova-Bach E, Braun A, Nieswandt B, Dittrich M, Dandekar T**.** Comparison of the central human and mouse platelet signaling cascade by systems biological analysis. **BMC Genomics** 21: 897, 2020. doi:10.1186/s12864-020-07215-4.

507. Naegelin Y, Dingsdale H, Säuberli K, Schadelin S, Kappos L, Barde YA**.** Measuring and validating the levels of brain-derived neurotrophic factor in human serum. **eNeuro** 5: ENEURO.0419-17.2018, 2018. doi:10.1523/ENEURO.0419-17.2018.

508. Zuccato C, Marullo M, Vitali B, Tarditi A, Mariotti C, Valenza M, Lahiri N, Wild EJ, Sassone J, Ciammola A, Bachoud-Lèvi AC, Tabrizi SJ, Di Donato S, Cattaneo E**.** Brain-derived neurotrophic factor in patients with Huntington's disease. **PLoS One** 6: e22966, 2011. doi:10.1371/journal.pone.0022966.

509. Serra-Millàs M**.** Are the changes in the peripheral brain-derived neurotrophic factor levels due to platelet activation? **World J Psychiatry** 6: 84–101, 2016. doi:10.5498/wjp.v6.i1.84.

510. Want A, Morgan JE, Barde YA**.** Brain-derived neurotrophic factor measurements in mouse serum and plasma using a sensitive and specific enzyme-linked immunosorbent assay. **Sci Rep** 13: 7740, 2023. doi:10.1038/s41598-023-34262-0.

511. Ikenouchi A, Okamoto N, Hamada S, Chibaatar E, Fujii R, Konishi Y, Igata R, Tesen H, Yoshimura R**.** Association between salivary mature brain-derived neurotrophic factor and psychological distress in healthcare workers. **Brain Behav** 13: e3278, 2023. doi:10.1002/brb3.3278.

512. Delezie J, Weihrauch M, Maier G, Tejero R, Ham DJ, Gill JF, Karrer-Cardel B, Rüegg MA, Tabares L, Handschin C**.** BDNF is a mediator of glycolytic fiber-type specification in mouse skeletal muscle. **Proc Natl Acad Sci USA** 116: 16111–16120, 2019. doi:10.1073/pnas.1900544116.

513. McGregor CE, English AW**.** The role of BDNF in peripheral nerve regeneration: activity-dependent treatments and Val66Met. **Front Cell Neurosci** 12: 522, 2018. doi:10.3389/fncel.2018.00522.

514. Aatonen MT, Ohman T, Nyman TA, Laitinen S, Grönholm M, Siljander PR**.** Isolation and characterization of platelet-derived extracellular vesicles. **J Extracell Vesicles** 6: 3, 2014. doi:10.3402/jev.v3.24692.

515. Dingsdale H, Nan X, Garay SM, Mueller A, Sumption LA, Chacón-Fernández P, Martinez-Garay I, Ghevaert C, Barde YA, John RM**.** The placenta protects the fetal circulation from anxiety-driven elevations in maternal serum levels of brain-derived neurotrophic factor. **Transl Psychiatry** 11: 62, 2021. doi:10.1038/s41398-020-01176-8.

516. Want A, Nan X, Kokkali E, Barde YA, Morgan JE**.** Brain-derived neurotrophic factor released from blood platelets prevents dendritic atrophy of lesioned adult central nervous system neurons. **Brain Commun** 5: fcad046, 2023. doi:10.1093/braincomms/fcad046.

517. Leiter O, Brici D, Fletcher SJ, Yong XL, Widagdo J, Matigian N, Schroer AB, Bieri G, Blackmore DG, Bartlett PF, Anggono V, Villeda SA, Walker TL**.** Platelet-derived exerkine CXCL4/platelet factor 4 rejuvenates hippocampal neurogenesis and restores cognitive function in aged mice. **Nat Commun** 14: 4375, 2023. doi:10.1038/s41467-023-39873-9.

518. Leiter O, Seidemann S, Overall RW, Ramasz B, Rund N, Schallenberg S, Grinenko T, Wielockx B, Kempermann G, Walker TL**.** Exercise-induced activated platelets increase adult hippocampal precursor proliferation and promote neuronal differentiation. **Stem Cell Reports** 12: 667–679, 2019. doi:10.1016/j.stemcr.2019.02.009.

519. Mitsumoto H, Ikeda K, Klinkosz B, Cedarbaum JM, Wong V, Lindsay RM**.** Arrest of motor neuron disease in wobbler mice cotreated with CNTF and BDNF. **Science** 265: 1107–1110, 1994. doi:10.1126/science.8066451.

520. The BDNF Study Group (Phase III)**.** A controlled trial of recombinant methionyl human BDNF in ALS. **Neurology** 52: 1427–1427, 1999. doi:10.1212/WNL.52.7.1427.

521. Ochs G, Penn RD, York M, Giess R, Beck M, Tonn J, Haigh J, Malta E, Traub M, Sendtner M, Toyka KV**.** A phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis. **Amyotroph Lateral Scler Other Motor Neuron Disord** 1: 201–206, 2000. doi:10.1080/14660820050515197.

522. Thoenen H, Sendtner M**.** Neurotrophins: from enthusiastic expectations through sobering experiences to rational therapeutic approaches. **Nat Neurosci** 5, *Suppl*: 1046–1050, 2002. doi:10.1038/nn938.

523. Knusel B, Gao H, Okazaki T, Yoshida T, Mori N, Hefti F, Kaplan DR**.** Ligand-induced down-regulation of Trk messenger RNA, protein and tyrosine phosphorylation in rat cortical neurons. **Neuroscience** 78: 851–862, 1997. doi:10.1016/s0306-4522(96)00616-1.

524. Xu B, Michalski B, Racine RJ, Fahnestock M**.** The effects of brain-derived neurotrophic factor (BDNF) administration on kindling induction, Trk expression and seizure-related morphological changes. **Neuroscience** 126: 521–531, 2004. doi:10.1016/j.neuroscience.2004.03.044.

525. Phillips HS, Hains JM, Armanini M, Laramee GR, Johnson SA, Winslow JW**.** BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. **Neuron** 7: 695–702, 1991. doi:10.1016/0896-6273(91)90273-3.

526. Goedert M, Fine A, Hunt SP, Ullrich A**.** Nerve growth factor mRNA in peripheral and central rat tissues and in the human central nervous system: lesion effects in the rat brain and levels in Alzheimer's disease. **Brain Res** 387: 85–92, 1986. doi:10.1016/0169-328x(86)90023-9.

527. Fischer W, Wictorin K, Björklund A, Williams LR, Varon S, Gage FH**.** Amelioration of cholinergic neuron atrophy and spatial memory impairment in aged rats by nerve growth factor. **Nature** 329: 65–68, 1987. doi:10.1038/329065a0.

528. Gnahn H, Hefti F, Heumann R, Schwab ME, Thoenen H**.** NGF-mediated increase of choline acetyltransferase (ChAT) in the neonatal rat forebrain: evidence for a physiological role of NGF in the brain? **Brain Res** 285: 45–52, 1983. doi:10.1016/0165-3806(83)90107-4.

529. Steininger TL, Wainer BH, Klein R, Barbacid M, Palfrey HC. High-affinity nerve growth factor receptor (Trk) immunoreactivity is localized in cholinergic neurons of the basal forebrain and striatum in the adult rat brain. **Brain Res** 612: 330–335, 1993. doi:10.1016/0006-8993(93)91681-h.

530. Yu W, Yu W, Yang Y, Lü Y. Exploring the key genes and identification of potential diagnosis biomarkers in Alzheimer's disease using bioinformatics analysis. **Front Aging Neurosci** 13: 602781, 2021. doi:10.3389/fnagi.2021.602781.

531. Jerónimo-Santos A, Vaz SH, Parreira S, Rapaz-Lérias S, Caetano AP, Buée-Scherrer V, Castren E, Valente CA, Blum D, Sebastiao AM, Diogenes MJ. Dysregulation of TrkB receptors and BDNF function by amyloid-beta peptide is mediated by calpain. **Cereb Cortex** 25: 3107–3121, 2015. doi:10.1093/cercor/bhu105.

532. He CY, Wang ZT, Shen YY, Shi AY, Li HY, Chen DW, Zeng GH, Tan CR, Yu JT, Zeng F, Wang YJ; Alzheimer's Disease Neuroimaging Initiative. Association of rs2072446 in the NGFR gene with the risk of Alzheimer's disease and amyloid-beta deposition in the brain. **CNS Neurosci Ther** 28: 2218–2229, 2022. doi:10.1111/cns.13965.

533. Li Y, Rowland C, Tacey K, Catanese J, Sninsky J, Hardy J, Powell J, Lovestone S, Morris JC, Thal L, Goate A, Owen M, Williams J, Grupe A. The BDNF Val66Met polymorphism is not associated with late onset Alzheimer's disease in three case-control samples. **Mol Psychiatry** 10: 809–810, 2005. doi:10.1038/sj.mp.4001702.

534. Lim YY, Villemagne VL, Laws SM, Ames D, Pietrzak RH, Ellis KA, Harrington KD, Bourgeat P, Salvado O, Darby D, Snyder PJ, Bush AI, Martins RN, Masters CL, Rowe CC, Nathan PJ, Maruff P; Australian Imaging, Biomarkers and Lifestyle (AIBL) Research Group. BDNF Val66Met, Abeta amyloid, and cognitive decline in preclinical Alzheimer's disease. **Neurobiol Aging** 34: 2457–2464, 2013. doi:10.1016/j.neurobiolaging.2013.05.006.

535. Lim YY, Hassenstab J, Goate A, Fagan AM, Benzinger TL, Cruchaga C, McDade E, Chhatwal J, Levin J, Farlow MR, Graff-Radford NR, Laske C, Masters CL, Salloway S, Schofield P, Morris JC, Maruff P, Bateman RJ; Dominantly Inherited Alzheimer Network. Effect of BDNFVal66Met on disease markers in dominantly inherited Alzheimer's disease. **Ann Neurol** 84: 424–435, 2018. doi:10.1002/ana.25299.

536. Lim YY, Maruff P, Barthélemy NR, Goate A, Hassenstab J, Sato C, Fagan AM, Benzinger TL, Xiong C, Cruchaga C, Levin J, Farlow MR, Graff-Radford NR, Laske C, Masters CL, Salloway S, Schofield PR, Morris JC, Bateman RJ, McDade E; Dominantly Inherited Alzheimer Network. Association of BDNF Val66Met with tau hyperphosphorylation and cognition in dominantly inherited Alzheimer disease. **JAMA Neurol** 79: 261–270, 2022. doi:10.1001/jamaneurol.2021.5181.

537. Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, Wang L, Blesch A, Kim A, Conner JM, Rockenstein E, Chao MV, Koo EH, Geschwind D, Masliah E, Chiba AA, Tuszynski MH. Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. **Nat Med** 15: 331–337, 2009. doi:10.1038/nm.1912.

538. Sotthibundhu A, Sykes AM, Fox B, Underwood CK, Thangnipon W, Coulson EJ. Beta-amyloid(1-42) induces neuronal death through the p75 neurotrophin receptor. **J Neurosci** 28: 3941–3946, 2008. doi:10.1523/JNEUROSCI.0350-08.2008.

539. Yaar M, Zhai S, Pilch PF, Doyle SM, Eisenhauer PB, Fine RE, Gilchrest BA. Binding of beta-amyloid to the p75 neurotrophin receptor induces apoptosis. A possible mechanism for Alzheimer's disease. **J Clin Invest** 100: 2333–2340, 1997. doi:10.1172/JCI119772.

540. Murphy M, Wilson YM, Vargas E, Munro KM, Smith B, Huang A, Li QX, Xiao J, Masters CL, Reid CA, Barrett GL. Reduction of p75 neurotrophin receptor ameliorates the cognitive deficits in a model of Alzheimer's disease. **Neurobiol Aging** 36: 740–752, 2015. doi:10.1016/j.neurobiolaging.2014.09.014.

541. Yi C, Goh KY, Wong LW, Ramanujan A, Tanaka K, Sajikumar S, Ibáñez CF. Inactive variants of death receptor $p75^{NTR}$ reduce Alzheimer's neuropathology by interfering with APP internalization. **EMBO J** 40: e104450, 2021. doi:10.15252/embj.2020104450.

542. Massa SM, Xie Y, Yang T, Harrington AW, Kim ML, Yoon SO, Kraemer R, Moore LA, Hempstead BL, Longo FM. Small, nonpeptide p75NTR ligands induce survival signaling and inhibit proNGF-induced death. **J Neurosci** 26: 5288–5300, 2006. doi:10.1523/JNEUROSCI.3547-05.2006.

543. Shanks HR, Chen K, Reiman EM, Blennow K, Cummings JL, Massa SM, Longo FM, Börjesson-Hanson A, Windisch M, Schmitz TW. p75 neurotrophin receptor modulation in mild to moderate Alzheimer disease: a randomized, placebo-controlled phase 2a trial. **Nat Med** 30: 1761–1770, 2024. doi:10.1038/s41591-024-02977-w.

544. Fonseca-Gomes J, Costa-Coelho T, Ferreira-Manso M, Inteiro-Oliveira S, Vaz SH, Alemãn-Serrano N, et al. A small TAT-TrkB peptide prevents BDNF receptor cleavage and restores synaptic physiology in Alzheimer's disease. **Mol Ther** 33: 421, 2025. doi:10.1016/j.ymthe.2024.08.022.

545. Gauthier LR, Charrin BC, Borrell-Pagès M, Dompierre JP, Rangone H, Cordelières FP, De Mey J, MacDonald ME, Lessmann V, Humbert S, Saudou F. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. **Cell** 118: 127–138, 2004. doi:10.1016/j.cell.2004.06.018.

546. Zuccato C, Cattaneo E. Brain-derived neurotrophic factor in neurodegenerative diseases. **Nat Rev Neurol** 5: 311–322, 2009. doi:10.1038/nrneurol.2009.54.

547. Ferrer I, Goutan E, Marín C, Rey MJ, Ribalta T. Brain-derived neurotrophic factor in Huntington disease. **Brain Res** 866: 257–261, 2000. doi:10.1016/s0006-8993(00)02237-x.

548. Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR, Timmusk T, Sipione S, Cattaneo E. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. **Science** 293: 493–498, 2001. doi:10.1126/science.1059581.

549. Zuccato C, Belyaev N, Conforti P, Ooi L, Tartari M, Papadimou E, MacDonald M, Fossale E, Zeitlin S, Buckley N, Cattaneo E. Widespread disruption of repressor element-1 silencing transcription factor/neuron-restrictive silencer factor occupancy at its target genes in Huntington's disease. **J Neurosci** 27: 6972–6983, 2007. doi:10.1523/JNEUROSCI.4278-06.2007.

550. Strand AD, Baquet ZC, Aragaki AK, Holmans P, Yang L, Cleren C, Beal MF, Jones L, Kooperberg C, Olson JM, Jones KR. Expression profiling of Huntington's disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. **J Neurosci** 27: 11758–11768, 2007. doi:10.1523/JNEUROSCI.2461-07.2007.

551. Buckley NJ, Johnson R, Zuccato C, Bithell A, Cattaneo E. The role of REST in transcriptional and epigenetic dysregulation in Huntington's disease. **Neurobiol Dis** 39: 28–39, 2010. doi:10.1016/j.nbd.2010.02.003.

552. Simmons DA, Rex CS, Palmer L, Pandyarajan V, Fedulov V, Gall CM, Lynch G. Up-regulating BDNF with an ampakine rescues synaptic

plasticity and memory in Huntington's disease knockin mice. **Proc Natl Acad Sci USA** 106: 4906–4911, 2009. doi:10.1073/pnas.0811228106.

553. Quinn L, Kegelmeyer D, Kloos A, Rao AK, Busse M, Fritz NE**.** Clinical recommendations to guide physical therapy practice for Huntington disease. **Neurology** 94: 217–228, 2020. doi:10.1212/WNL.0000000000008887.

554. Hyman C, Hofer M, Barde YA, Juhasz M, Yancopoulos GD, Squinto SP, Lindsay RM**.** BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. **Nature** 350: 230–232, 1991. doi:10.1038/350230a0.

555. Pelosi A, Nakamura Y, Girault JA, Hervé D**.** BDNF/TrkB pathway activation in D1 receptor-expressing striatal projection neurons plays a protective role against L-DOPA-induced dyskinesia. **Neurobiol Dis** 185: 106238, 2023. doi:10.1016/j.nbd.2023.106238.

556. Morgan JE, Bevan RJ, Cimaglia G**.** Retinal ganglion cell subtypes and their vulnerability in glaucoma. **Methods Mol Biol** 2858: 191–205, 2025. doi:10.1007/978-1-0716-4140-8_16.

557. Rodriguez-Tébar A, Jeffrey PL, Thoenen H, Barde YA**.** The survival of chick retinal ganglion cells in response to brain-derived neurotrophic factor depends on their embryonic age. **Dev Biol** 136: 296–303, 1989. doi:10.1016/0012-1606(89)90256-x.

558. Thanos S, Bähr M, Barde YA, Vanselow J**.** Survival and axonal elongation of adult rat retinal ganglion cells. **Eur J Neurosci** 1: 19–26, 1989. doi:10.1111/j.1460-9568.1989.tb00770.x.

559. Binley KE, Ng WS, Barde YA, Song B, Morgan JE**.** Brain-derived neurotrophic factor prevents dendritic retraction of adult mouse retinal ganglion cells. **Eur J Neurosci** 44: 2028–2039, 2016. doi:10.1111/ejn.13295.

560. Mey J, Thanos S**.** Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats in vivo. **Brain Res** 602: 304–317, 1993. doi:10.1016/0006-8993(93)90695-j.

561. Unoki K, LaVail MM**.** Protection of the rat retina from ischemic injury by brain-derived neurotrophic factor, ciliary neurotrophic factor, and basic fibroblast growth factor. **Invest Ophthalmol Vis Sci** 35: 907–915, 1994.

562. Perez MT, Caminos E**.** Expression of brain-derived neurotrophic factor and of its functional receptor in neonatal and adult rat retina. **Neurosci Lett** 183: 96–99, 1995. doi:10.1016/0304-3940(94)11123-z.

563. Morgan JE**.** Retina ganglion cell degeneration in glaucoma: an opportunity missed? A review. **Clin Exp Ophthalmol** 40: 364–368, 2012. doi:10.1111/j.1442-9071.2012.02789.x.

564. Adamis AP, Brittain CJ, Dandekar A, Hopkins JJ**.** Building on the success of anti-vascular endothelial growth factor therapy: a vision for the next decade. **Eye (Lond)** 34: 1966–1972, 2020. doi:10.1038/s41433-020-0895-z.

565. Todd D, Gowers I, Dowler SJ, Wall MD, McAllister G, Fischer DF, Dijkstra S, Fratantoni SA, van de Bospoort R, Veenman-Koepke J, Flynn G, Arjomand J, Dominguez C, Munoz-Sanjuan I, Wityak J, Bard JA**.** A monoclonal antibody TrkB receptor agonist as a potential therapeutic for Huntington's disease. **PLoS One** 9: e87923, 2014. doi:10.1371/journal.pone.0087923.

566. Traub S, Stahl H, Rosenbrock H, Simon E, Florin L, Hospach L, Hörer S, Heilker R**.** Pharmaceutical characterization of tropomyosin receptor kinase B-agonistic antibodies on human induced pluripotent stem (hiPS) cell-derived neurons. **J Pharmacol Exp Ther** 361: 355–365, 2017. doi:10.1124/jpet.117.240184.

567. Guo W, Pang K, Chen Y, Wang S, Li H, Xu Y, Han F, Yao H, Liu H, Lopes-Rodrigues V, Sun D, Shao J, Shen J, Dou Y, Zhang W, You H, Wu W, Lu B**.** TrkB agonistic antibodies superior to BDNF: utility in treating motoneuron degeneration. **Neurobiol Dis** 132: 104590, 2019. doi:10.1016/j.nbd.2019.104590.

568. Bai Y, Xu J, Brahimi F, Zhuo Y, Sarunic MV, Saragovi HU**.** An agonistic TrkB mAb causes sustained TrkB activation, delays RGC death, and protects the retinal structure in optic nerve axotomy and in glaucoma. **Invest Ophthalmol Vis Sci** 51: 4722–4731, 2010. doi:10.1167/iovs.09-5032.

569. Saraon P, Pathmanathan S, Snider J, Lyakisheva A, Wong V, Stagljar I**.** Receptor tyrosine kinases and cancer: oncogenic mechanisms and therapeutic approaches. **Oncogene** 40: 4079–4093, 2021. doi:10.1038/s41388-021-01841-2.

570. Taylor J, Pavlick D, Yoshimi A, Marcelus C, Chung SS, Hechtman JF, Benayed R, Cocco E, Durham BH, Bitner L, Inoue D, Chung YR, Mullaney K, Watts JM, Diamond EL, Albacker LA, Mughal TI, Ebata K, Tuch BB, Ku N, Scaltriti M, Roshal M, Arcila M, Ali S, Hyman DM, Park JH, Abdel-Wahab O**.** Oncogenic TRK fusions are amenable to inhibition in hematologic malignancies. **J Clin Invest** 128: 3819–3825, 2018. doi:10.1172/JCI120787.

571. Drilon A, Laetsch TW, Kummar S, DuBois SG, Lassen UN, Demetri GD, et al**.** Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. **N Engl J Med** 378: 731–739, 2018. doi:10.1056/NEJMoa1714448.

572. Dunn DB**.** Larotrectinib and entrectinib: TRK inhibitors for the treatment of pediatric and adult patients with NTRK gene fusion. **J Adv Pract Oncol** 11: 418–423, 2020. doi:10.6004/jadpro.2020.11.4.9.

573. Nakagawara A, Azar CG, Scavarda NJ, Brodeur GM**.** Expression and function of TRK-B and BDNF in human neuroblastomas. **Mol Cell Biol** 14: 759–767, 1994. doi:10.1128/MCB.14.1.759.

574. Nakagawara A**.** Trk receptor tyrosine kinases: a bridge between cancer and neural development. **Cancer Lett** 169: 107–114, 2001. doi:10.1016/s0304-3835(01)00530-4.

575. Zhu Y, Li Y, Haraguchi S, Yu M, Ohira M, Ozaki T, Nakagawa A, Ushijima T, Isogai E, Koseki H, Nakamura Y, Kong C, Mehlen P, Arakawa H, Nakagawara A**.** Dependence receptor UNC5D mediates nerve growth factor depletion-induced neuroblastoma regression. **J Clin Invest** 123: 2935–2947, 2013. doi:10.1172/JCI65988.

576. Tauszig-Delamasure S, Yu LY, Cabrera JR, Bouzas-Rodriguez J, Mermet-Bouvier C, Guix C, Bordeaux MC, Arumäe U, Mehlen P**.** The TrkC receptor induces apoptosis when the dependence receptor notion meets the neurotrophin paradigm. **Proc Natl Acad Sci USA** 104: 13361–13366, 2007. doi:10.1073/pnas.0701243104.

577. Negulescu AM, Mehlen P**.** Dependence receptors—the dark side awakens. **FEBS J** 285: 3909–3924, 2018. doi:10.1111/febs.14507.

578. Stroebe H**.** Ueber Entstehung und Bau der Gehirngliome. **Beit path Anat** 18: 405–486, 1895.

579. Osswald M, Jung E, Sahm F, Solecki G, Venkataramani V, Blaes J, et al**.** Brain tumour cells interconnect to a functional and resistant network. **Nature** 528: 93–98, 2015. doi:10.1038/nature16071.

580. Venkataramani V, Tanev DI, Strahle C, Studier-Fischer A, Fankhauser L, Kessler T, Körber C, Kardorff M, Ratliff M, Xie R, Horstmann H, Messer M, Paik SP, Knabbe J, Sahm F, Kurz FT, Acikgöz AA, Herrmannsdörfer F, Agarwal A, Bergles DE, Chalmers A, Miletic H, Turcan S, Mawrin C, Hanggi D, Liu HK, Wick W, Winkler

F, Kuner T**.** Glutamatergic synaptic input to glioma cells drives brain tumour progression. **Nature** 573: 532–538, 2019. doi:10.1038/s41586-019-1564-x.

581. Venkatesh HS, Johung TB, Caretti V, Noll A, Tang Y, Nagaraja S, Gibson EM, Mount CW, Polepalli J, Mitra SS, Woo PJ, Malenka RC, Vogel H, Bredel M, Mallick P, Monje M**.** Neuronal activity promotes glioma growth through Neuroligin-3 secretion. **Cell** 161: 803–816, 2015. doi:10.1016/j.cell.2015.04.012.

582. Taylor KR, Barron T, Hui A, Spitzer A, Yalçin B, Ivec AE, Geraghty AC, Hartmann GG, Arzt M, Gillespie SM, Kim YS, Maleki Jahan S, Zhang H, Shamardani K, Su M, Ni L, Du PP, Woo PJ, Silva-Torres A, Venkatesh HS, Mancusi R, Ponnuswami A, Mulinyawe S, Keough MB, Chau I, Aziz-Bose R, Tirosh I, Suvà ML, Monje M**.** Glioma synapses recruit mechanisms of adaptive plasticity. **Nature** 623: 366–374, 2023. doi:10.1038/s41586-023-06678-1.

583. Wang J, Guo YM, Wei J, Min J, Ye L**.** Comparative efficacy and safety of different anti-VEGF agents combined with different delivery methods for neovascular glaucoma: a systematic review and Bayesian network meta-analysis. **BMJ Open** 14: e080103, 2024. doi:10.1136/bmjopen-2023-080103.

584. Chang PK, Verbich D, McKinney RA**.** AMPA receptors as drug targets in neurological disease—advantages, caveats, and future outlook. **Eur J Neurosci** 35: 1908–1916, 2012. doi:10.1111/j.1460-9568.2012.08165.x.