BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases

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Abstract | Increasing evidence suggests that synaptic dysfunction is a key pathophysiological hallmark in neurodegenerative disorders, including Alzheimer's disease. Understanding the role of brain-derived neurotrophic factor (BDNF) in synaptic plasticity and synaptogenesis, the impact of the *BDNF* Val66Met polymorphism in Alzheimer's disease-relevant endophenotypes — including episodic memory and hippocampal volume — and the technological progress in measuring synaptic changes in humans all pave the way for a 'synaptic repair' therapy for neurodegenerative diseases that targets pathophysiology rather than pathogenesis. This article reviews the key issues in translating BDNF biology into synaptic repair therapies.

Disease-modifying therapies
Medical therapies that address the cause of the disease either directly or indirectly and thereby modify the course of the disease: that is, slow down, halt or reverse disease progression.

Symptomatic treatment
A medical therapy that only relieves or controls the disease symptoms but not its cause per se.

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Over the past 15 years, there has been significant advancement in neuroscience research, as evidenced by the dramatic increase in the number of papers published. Despite great progress in understanding the molecular mechanisms underlying nervous system disorders, such findings have not been effectively translated into developing disease-modifying therapies for neurological and psychiatric diseases. As the average human life expectancy continues to increase[1], the prevalence of age-related neurodegenerative diseases, particularly Alzheimer's disease (AD)[2] and Parkinson's disease (PD)[3], increases concomitantly, affecting millions worldwide.

In the development of disease-modifying drugs (as opposed to symptomatic treatment) for neurodegenerative diseases, efforts have so far primarily focused on a 'toxin-reducing' approach. This approach is based on the idea that removing the causes — such as amyloid-β (Aβ) aggregates, amyloid plaques, tau aggregates and neurofibrillary tangles in AD[4] — would halt disease progression. Emerging data from several Phase III clinical studies targeting the amyloid cascade suggest that this approach is ineffective, at least in patients at an advanced stage of the disease[5–7].

In this Review, we highlight evidence that the progression of neurodegenerative disorders is more tightly associated with synapse degeneration rather than with 'toxin build-up'. This suggests that synapse loss is a major pathophysiological hallmark shared by all neurodegenerative diseases and leads to the proposal that effective therapies should target this pathophysiological feature of neurodegenerative diseases rather than their pathogenesis. We review data showing that brain-derived neurotrophic factor (BDNF), in addition to its pro-survival effects, has powerful synaptic effects — promoting synaptic transmission, synaptic plasticity and synaptic growth (also called synaptogenesis) — and we propose a paradigm-shifting, BDNF-based 'synaptic repair' strategy for neurodegenerative diseases. Thus, a combination of BDNF–neurotrophic receptor tyrosine kinase TRKB (also known as NTRK2) pathway modulators and more reliable and sensitive methods to measure synaptic changes in humans *in vivo* could pave the way for developing effective disease-modifying therapies.

Challenges in neurodegeneration therapy

Many factors have contributed to the lack of success in the development of disease-modifying medicines for neurodegenerative disorders. First, the underlying disease mechanisms are complex and poorly understood. Although several mechanisms and pathways have been implicated in neurodegenerative diseases — including accumulation of neurotoxic substances, inflammation, lipid metabolism, oxidative stress, autophagy, protein degradation and mitochondrial dysfunction[8] — it remains unclear whether they are the cause of the disease or the consequence of the primary and/or secondary damage. Consequently, therapies based on some of these individual mechanisms have not been clinically successful. Second, given that accumulation of misfolded toxic proteins in the brain is considered

Phase III
A trial conducted to gather new information about the safety and effectiveness of a particular therapy in a larger group of patients than that used in Phase II clinical trials.

Synaptic plasticity
Activity-dependent modulation of synaptic structure and/or function.

Synaptogenesis
The formation of new synapses between neurons, and the maturation and stabilization of existing synapses. It is also termed synaptic growth.

to be a key pathogenic factor for neurodegenerative diseases, efforts to develop disease-modifying therapies for AD and other neurodegenerative diseases have thus far followed a toxin-reducing approach[9]. However, clinical studies to date have shown that lowering toxic proteins, such as Aβ in AD, reduced amyloid plaque pathology but failed to improve the clinical outcome[5,6]. Third, although many pharmacological agents showed beneficial effects in various animal models[10–13] of AD, few have translated into clinical efficacy[14,15]. It therefore remains a challenge to develop animal models with predictive value. Last, the lack of qualified biomarkers and robust clinical measurements hampers accurate and early diagnosis, patient stratification and early evaluation of therapeutic efficacy of new medications. Thus, clinical trials designed to evaluate disease modification are usually lengthy and involve a large number of patients.

An emerging idea in the toxin-reducing approach is to lower the levels of pathological toxins much earlier in the disease or to prevent the formation of protein aggregates that are thought to cause the disease in order to halt neuronal loss (BOX 1). This idea is based on the premise that genetic and/or environmental factors may trigger pathological mechanisms very early in the disease process that lead to sequential and/or parallel primary and secondary damage. At the time of diagnosis, the accumulation of molecular and cellular disturbances in the brain may have already led to profound pathophysiological changes — which may or may not be dependent on the continued presence of pathological factors — that give rise to distinct components of the clinical syndrome[16] (FIG. 1a). A significant hurdle in this 'early toxin-reducing' approach is that changes in brain function in the asymptomatic stage of the disease often take years to occur and are small and highly variable[17]. The lack of sensitive measures and qualified biomarkers of such changes makes it extremely challenging to identify patient cohorts and to demonstrate clinical efficacy (FIG. 1b). Therefore, a clinical trial that can truly measure the efficacy of a treatment that prevents toxin generation and accumulation will undoubtedly be long and require large patient cohorts, which are both expensive and difficult to manage.

Box 1 | **Prevention trials in Alzheimer's disease**

In an attempt to ultimately test the therapeutic value of treatment strategies based on the amyloid hypothesis, three new trials are underway to investigate the effectiveness of anti-amyloid therapies in patients before they exhibit clinical symptoms[199]. The Alzheimer's Prevention Initiative (API) and the Dominantly Inherited Alzheimer Network (DIAN) trials focus on cohorts carrying mutations associated with early-onset Alzheimer's disease, whereas the Anti-Amyloid Treatment of Asymptomatic Alzheimer's (A4) trial will enrol healthy subjects who display positive amyloid brain scans. These studies will offer, for the first time, the opportunity to test amyloid-based therapies in asymptomatic subjects. Substantial hurdles and limitations continue to exist, although there is optimism for such early prevention or intervention trials. For example, the lack of qualified biomarkers for early diagnosis makes it challenging to select cohorts of asymptomatic individuals for trials. Also, it is unclear how early a 'prevention' therapy has to begin to halt neuron loss. For instance, in subjects who carry an autosomal dominant mutation that increases the risk of Alzheimer's disease, deposition of amyloid-β in the brain or changes in the levels of amyloid-β in cerebrospinal fluid could happen as early as 15–25 years before any clinical symptoms become evident[17,200,201]. Nevertheless, results from these preventive trials will help to determine the direction of future Alzheimer's disease drug research and development.

Synaptic deficits: a pathophysiological hallmark

Given the current lack of success in the development of drugs that target the pathogenesis of a neurodegenerative disease, one could consider a different approach: to identify drugs that target the pathophysiology (FIG. 1a). This idea is based on the proposal by Lewis and Sweet regarding therapeutic approaches for schizophrenia[16]. These authors suggested that although targeting pathogenesis may be a suitable approach for prophylactic treatment, targeting the pathophysiology may be a better approach for therapeutic intervention in a disease that is already present. In the case of neurodegenerative diseases, targeting the pathophysiology would involve identifying and targeting the pathophysiological mechanisms that underlie the clinical syndromes.

Synapse degeneration is a major pathophysiological hallmark in neurodegenerative diseases. For example, substantial evidence indicates that in AD, there is a decrease in the number of synapses, which occurs later than Aβ accumulation and correlates with disease progression[18–20]. Several mechanistic mouse models[21] of AD show age-dependent deficits in hippocampal long-term potentiation (LTP)[22,23], which correlate with the impairment in hippocampus-dependent memory[22]. *In vivo* two-photon imaging in the triple transgenic 3xTg-AD mice[21] expressing yellow fluorescent protein revealed that the progressive loss of dendritic spines in the somatosensory cortex coincided with the accumulation of soluble Aβ and hyperphosphorylated tau (at 13 months of age), whereas a reduction in dendritic spine density in the hippocampus became evident only when amyloid plaques and hyperphosphorylated tau were abundant (at 15 months of age)[24]. Light and electron microscopic assessment of synaptic density in another transgenic model, Tg2576 (based on the human amyloid precursor protein (APP) 695 isoform with K670N and M671L mutations[25]), revealed region- and age-dependent synapse loss[26]. Furthermore, application of synthetically prepared Aβ oligomers to hippocampal slices induces LTP deficits[27] and spine loss[28], and synaptic deficits often occur in the proximity of Aβ plaques[26]. Together, these data provide a link between the pathogenic trigger (Aβ accumulation) and pathophysiological manifestation (synapse loss) of the disease.

Electron microscopic analysis of autopsied brain tissue from patients with mild to moderate AD[29–31] within 2–4 years after clinical onset demonstrated progressive synapse loss in the hippocampus, the frontal and inferior parietal cortex and the entorhinal cortex[32,33]. In the hippocampal CA1 region, progressive synapse loss has been reported (18% in subjects with mild cognitive impairment (MCI) to 55% in subjects with mild AD)[33]. Consistent with synapse loss, multiple studies using 2-deoxyglucose or fluorodeoxyglucose (FDG) positron emission tomography (PET) have reported an antecedent decline in cerebral glucose use decades before the diagnosis of AD[34]. Unbiased stereological counting of

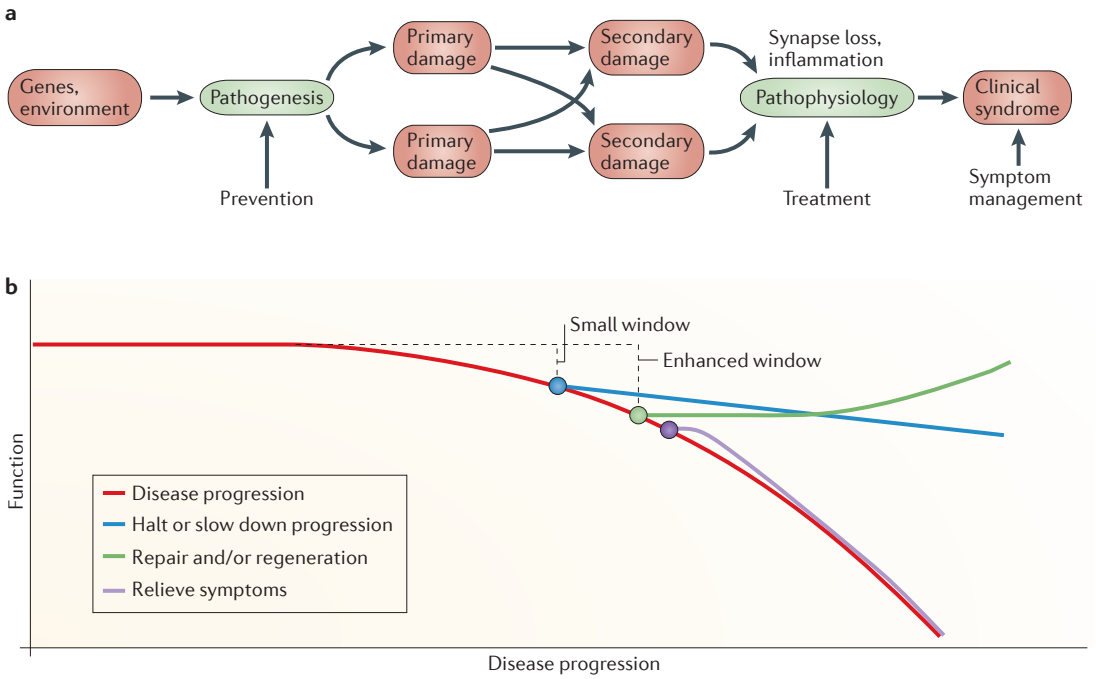

Figure 1 | **Disease progression and its underlying pathogenic and pathophysiological processes.** Advantages of focusing on pathophysiology rather than pathogenesis for the treatment of neurodegenerative diseases. **a** | Pathogenesis is the primary event (that is, it is the cause of or a contributor to the disease initiation process) triggered by genetic and/or environmental factors. Preventive therapies are expected to be efficacious if they are aimed at controlling pathogenesis (for example, active or passive amyloid-targeting therapies for Alzheimer's disease). The pathogenic process leads to a cascade of primary and secondary damage over a long period of time, ultimately resulting in pathophysiological changes that are manifested as the clinical syndrome. Targeting these pathophysiological changes may be an effective strategy for the treatment of neurodegenerative diseases; here, the changes to be targeted include synapse loss and inflammation. **b** | Graphical representation of disease progression (specifically, the functional deficits associated with disease progression). Different modes of intervention may alter disease progression: disease-modifying treatments can prevent, slow down or halt disease progression if they target either the causal pathogenic mechanisms early (blue line, with small window to demonstrate efficacy) or the driver pathophysiological mechanisms (green line, with larger window to show efficacy). Treatments that only relieve clinical symptoms do not halt or slow disease progression (purple line). It is important to note that early intervention is critical for targeting pathogenesis, whereas repair or regeneration therapies could start relatively later. Part **a** is modified, with permission, from REF. 16 © (2009) American Society for Clinical Investigation.

synapses also revealed that synapse degeneration was evident at the MCI stage and was strongly correlated with deficits in episodic memory (that is, delayed recall)[32]. In Huntington's disease, synapse degeneration occurs shortly after the build-up of the aggregated mutant huntingtin protein in the striatum but before neuronal loss, correlating with the progression of cognitive deficits[35]. Similarly, an impairment of synaptic plasticity (such as LTP and long-term depression (LTD)) in the striatum could account for the onset and the progression of motor and cognitive symptoms of PD[36].

Synaptic repair therapy

We suggest that this pathophysiological hallmark of neurodegenerative diseases — synapse degeneration — should be considered as a target for disease-modifying treatments for these diseases. Specifically, such treatments could aim for neuronal repair, especially synaptic repair and regeneration. This proposal is based on several facts. First, synaptic dysfunction and synapse loss, unlike neuronal loss, are reversible (FIG. 2). Indeed, synapses are highly dynamic and plastic[37–39], such that dysfunctional synapses can be repaired and new synapses can be formed. This is important, as synaptic integrity and function are the foundation of neuronal circuits and are essential for maintaining physiological and behavioural functions[40]. Second, as synaptic dysfunction and loss are a point of convergence in most complex neurological diseases[35,41–44], therapeutic intervention at the level of synaptic structure and function may be beneficial in multiple neurological diseases regardless of the type or origin of the toxic insult. Last, and most importantly, the window for therapeutic intervention based on synaptic repair and regeneration is longer than that for toxin-clearance approaches, and such interventions can thus be applied at a relatively late stage of the disease to slow or halt progression (FIG. 2) when early diagnosis remains a challenge.

Synapses are regulated at the level of synaptic transmission, synaptic plasticity and synaptic growth. Accordingly, therapeutics targeting synaptic dysfunction could involve three different approaches (BOX 2).

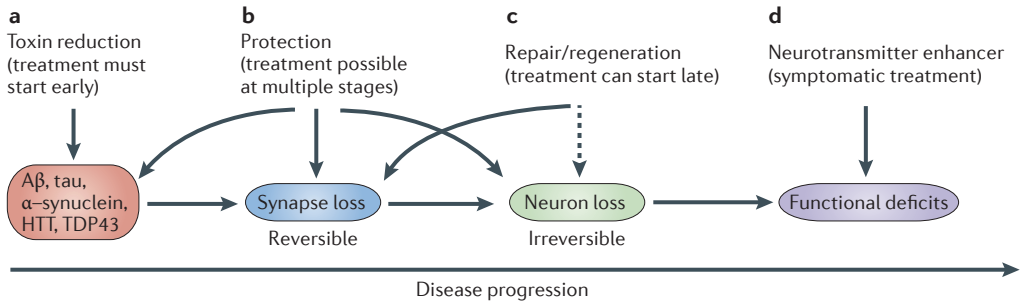

Figure 2 | **Schematic representation of temporal events in neurodegenerative diseases.** The formation and/or accumulation of toxic protein aggregates may occur even in asymptomatic and prodromal conditions. Along with environmental factors, they may trigger the initiation of a disease process. Loss of synapses (which is reversible) and loss of neurons (which is irreversible) are likely to be the pathophysiological consequences of various forms of damage caused by toxin-accumulation mechanisms. Functional deficits, for example, in terms of cognitive function, are the clinical manifestations of the resulting alterations in synaptic connections and neuronal networks. Therapies aimed at reducing toxic protein aggregates (the primary event) are most likely to have an effect on the disease if they are started early in disease progression (part **a**). Strategies that target pathophysiological events such as synapse loss (part **b**) and/or neuron loss (part **c**) are more likely to modify disease progression. Synapse loss is a reversible process, and targeting such loss may provide therapeutic benefits even at later stages of neurodegenerative diseases. Treatments targeting the clinical syndromes (for example, neurotransmitter enhancers) are likely to offer only symptomatic benefits (part **d**). Aβ, amyloid-β; HTT, huntingtin, TDP43, TAR DNA-binding protein 43.

Conceptually, it is important to distinguish short-term processes that last from seconds to minutes, such as synaptic transmission and short-term plasticity, from long-term processes that last for hours, days or even longer, such as long-term plasticity and synaptogenesis[45]. Although therapies aimed at enhancing synaptic transmission may be useful for certain disease indications, for neurodegenerative diseases, where synapse loss and synaptic dysfunction are progressive, simple enhancement of synaptic transmission would probably not be able to halt the deterioration of neuronal circuits. Such an approach could at best provide symptomatic management. A disease-modifying agent should induce long-lasting functional changes (for example, LTP) and/or structural changes (for example, synaptogenesis, which involves increasing the number of synapses or enlargement of existing synapses) at synapses to slow, halt or reverse disease progression. Of note, substantial evidence suggests that long-term plasticity, such as LTP, leads to synaptic growth[46–48].

BDNF: a potent synaptic repair molecule

Of all the molecules involved in synapse biology, BDNF (a member of the neurotrophin family), is by far the best studied and arguably the only one that has been associated with synaptic regulation in humans[49–51]. Substantial *in vitro* and *in vivo* evidence supports the pro-survival functions of BDNF on neurons under various pathological conditions (BOX 3).

BDNF is widely expressed in the CNS, and its expression is reduced in various pathological conditions[52–56]. The neurotrophic function of BDNF is primarily mediated by the TRKB receptor. In the adult brain, the main function of BDNF is to enhance synaptic transmission, facilitate synaptic plasticity and promote synaptic growth (FIG. 3). Given that these are exactly the three processes that could be targeted in synaptic repair therapy (BOX 2), the BDNF–TRKB pathway is a particularly suitable candidate to test the feasibility of the proposed 'synaptic repair' strategy.

BDNF effects on synaptic transmission and plasticity. An early study showed that fast perfusion of BDNF onto adult rat hippocampal slices rapidly potentiated basal synaptic transmission at CA1 synapses[57]. Similarly, intrahippocampal infusion of a high dose of BDNF induced a lasting potentiation of basal synaptic transmission in the hippocampal dentate gyrus *in vivo*[58]. However, replication of these results remained a significant challenge[59–63]. Some studies showed that slow perfusion of BDNF on hippocampal slices from neonatal rats facilitated classic early-phase LTP (E-LTP) at hippocampal synapses without affecting basal synaptic transmission[59,60,64,65]. In parallel, in adult rat hippocampal slices, slow perfusion of BDNF converted short-term synaptic potentiation induced by a weak tetanic stimulation into LTP. This LTP-promoting effect of BDNF was further validated in studies using BDNF knockout mice[66,67] and TRKB knockout mice[68,69]. A recent study not only resolved the long-standing debate on whether BDNF facilitates basal synaptic transmission but also provided new insights into the mechanism of BDNF signalling[70]. Specifically, acute BDNF application results in a rapid increase in BDNF levels and a transient activation of the TRKB receptor and its downstream signalling pathways, leading to a rapid enhancement in synaptic transmission. By contrast, slow perfusion of BDNF elicits a gradual increase in BDNF levels and a sustained activation of TRKB signalling, resulting in an increase in the magnitude of LTP[70].

BDNF also plays a crucial part in late-phase LTP (L-LTP)[71,72]. First, L-LTP-inducing, strong theta-burst stimulation triggers not only the secretion[73–75] but also the synthesis of BDNF in hippocampal and cortical neurons[76]. Second, BDNF is required for L-LTP induction, as genetically[66] or pharmacologically[77,78] reducing the levels of BDNF blocks L-LTP. Third, an increase in BDNF levels is sufficient for the maintenance of L-LTP:

Early-phase LTP
(E-LTP). Early-phase long-term potentiation (LTP) is a sustained increase in synaptic efficacy that is induced by brief, high-frequency tetanic stimulation and lasts for 1–2 hours. It does not require gene transcription or protein synthesis.

Late-phase LTP
(L-LTP). Late-phase long-term potentiation (LTP) is a long-lasting increase in synaptic efficacy that is induced by strong, multiple tetanic stimuli and lasts for several hours or even days. It is both transcription- and translation-dependent and is often accompanied by morphological changes at the stimulated synapses.

Box 2 | **Three aspects of synaptic modulation**

There are three aspects to the modulation of synapse function. The first is enhancement of synaptic transmission. One way to do this is to increase neurotransmitter concentrations at the synaptic cleft by enhancing transmitter release or blocking transmitter degradation and/or reuptake. For example, donepezil, an acetylcholinesterase inhibitor that blocks the metabolism of acetylcholine, enhances transmission at the cholinergic synapses. It is a front-line drug for managing cognitive symptoms in patients with mild to moderate Alzheimer's disease[15]. Levodopa, a dopamine precursor that can be taken up by neurons and converted to dopamine and that is the first-line treatment for Parkinson's disease, is another example. An alternative approach is to activate and/or enhance postsynaptic function by activating postsynaptic receptors directly with an agonist or by modulating receptor signalling or function. Indeed, several dopamine receptor agonists are used to treat parkinsonian symptoms[202]. In both cases, the enhancement of synaptic function is transient, and synaptic deficits ensue after cessation of the treatment. Furthermore, these drugs cannot stop or slow down disease progression and are therefore categorized as 'symptomatic' rather than 'disease-modifying' treatments.

The second aspect to the modulation of synapse function involves facilitation of synaptic plasticity. Synaptic plasticity, by strict definition, is a change in synaptic strength in response to a brief increase in neuronal activity. It is different from simple enhancement of synaptic transmission induced by a chemical agent or drug as highlighted above. Numerous studies over the past two decades have demonstrated that synaptic plasticity mediates diverse brain functions, ranging from memory and emotion to fine motor control and executive function. Long-term potentiation (LTP), the most extensively studied form of synaptic plasticity, is thought to be the cellular mechanism underlying memory. LTP deficits are consistently reported in transgenic animal models of Alzheimer's disease[23]. Facilitation of LTP in animal models is often considered to be an important criterion in the selection of candidate drugs for neurodegenerative diseases. The key to synaptic plasticity is its activity-dependence. Thus, an attractive feature of plasticity-modulating agents is that they may preferentially alter synapses that are actively engaged in brain functions (for example, episodic memory) relevant to the disease.

The third aspect to the modulation of synapse function involves stimulation of synaptic growth (also termed synaptogenesis). Synaptic growth is a highly dynamic process that persists throughout adulthood. Unlike neuronal loss, which is irreversible, disease-associated loss of synaptic connectivity could be rescued through the growth of new terminals and/or dendritic spines. In addition, degenerating synapses could be 'stabilized' through strengthening of the existing pre- and postsynaptic structures and increasing the levels of synaptic proteins. A unique feature of the synaptogenesis-promoting agents, such as brain-derived neurotrophic factor, is that they may elicit long-lasting effects even after their withdrawal.

application of BDNF after E-LTP-inducing, weak theta-burst stimulation resulted in sustained L-LTP in hippocampal slices[79]. Similarly, in VP16-cyclic AMP-responsive element-binding protein (CREB) mice (in which BDNF levels are raised) the E-LTP-inducing, weak tetanus is able to induce L-LTP, which could be reversed by the BDNF scavenger TRKB-specific immunoglobulin G[80]. Furthermore, when all protein synthesis is blocked, application of BDNF after theta-burst stimulation is sufficient to maintain L-LTP[79]. Fourth, a critical step in L-LTP is the extracellular conversion of proBDNF to mature BDNF through a tissue plasminogen activator (tPA)–plasmin-dependent mechanism. The L-LTP impairment observed in mice lacking tPA or plasminogen could be completely rescued by mature BDNF perfusion but not by proBDNF perfusion[79]. Fifth, recent evidence suggests that TRKB at a particular synapse may act as a tag to capture BDNF[81]. This synaptic tagging mechanism is thought to ensure synapse-specific expression of L-LTP.

BDNF effects on synaptic growth. BDNF also promotes synapse formation[82] by regulating axonal branching[83], dendritic growth[84] and activity-dependent synapse refinement[85]. Here, we focus on BDNF regulation of synaptic growth, which is defined as an increase in the number and/or the size of synapses. For example, treatment of postnatal hippocampal slice cultures with BDNF for 2–3 days increased the spine density in CA1 pyramidal neurons[86] and enhanced the expression of synaptic proteins[87]. In general, chronic exposure to BDNF increases spine motility[88], which in turn increases the potential to form new synapses. Interestingly, fast delivery of BDNF enlarged the size of mushroom spines, whereas slow perfusion of BDNF induced more thin spines. This is suggestive of the consolidation of existing synapses and the formation of new synapses, respectively[70].

Heterozygous BDNF knockout mice showed reduced hippocampal expression of synaptic proteins such as synaptobrevin and a reduction in the number of synaptic vesicles docked at the active zone[89]. Analysis of TRKB knockout mice revealed that, in addition to the changes in vesicle docking and synaptic protein expression, there was a substantial reduction in synaptic density (17–39% reduction) in mossy fibre terminals in the dentate gyrus[90]. Transgenic mice overexpressing BDNF had an increased number of synapses (63%) and increased synaptic vesicle docking in area CA1 (REF. 91). A study in which BDNF was re-expressed in neurons derived from BDNF knockout mice showed that BDNF increased the number of synapses within 16 hours[92].

Although functional changes at synapses generally precede structural alterations, the two might be intricately linked through activity-dependent BDNF secretion[46]. Repetitive pairing of synaptic stimulation (through glutamate photo-uncaging) and postsynaptic spiking induced both LTP and a gradual enlargement of spine heads. Blockade of BDNF–TRKB signalling prevented the spine head enlargement, whereas synaptic stimulation plus the addition of exogenous BDNF induced spine enlargement in the absence of postsynaptic spikes[46]. These results suggest that activity-dependent BDNF secretion mediates both LTP and synaptic growth, but at different timescales.

BDNF effects on learning and memory. BDNF regulation of synaptic plasticity and synaptic growth suggests that it has a crucial role in cognitive functions. Indeed, a reduction of hippocampal BDNF levels through either genetic or pharmacological means not only impaired LTP and reduced the number of synapses but also caused deficits in the formation and consolidation of hippocampus-dependent memory[93–95]. Similar effects were observed when the level of TRKB or its activity was manipulated[68,96,97]. Furthermore, infusion of a BDNF antisense oligonucleotide into the hippocampus in rats several hours after learning impaired memory retention[98]. Conversely, overexpression of TRKB improved memory and occluded LTP[99]. Behavioural experiments have also shown a role for BDNF in episodic memory, fear memory extinction[100], motor learning[51] and mood

Box 3 | **Pro-survival effects of BDNF**

In vitro, brain-derived neurotrophic factor (BDNF) prevents neuronal death induced by several different types of insults, including ischaemia due to oxygen-, glucose- or serum-deprivation[203,204], oxidative stress (50 μM H_2O_2)[205], glutamate toxicity[206] and toxic proteins such as amyloid-β[207]. The neuroprotective effects of BDNF have also been demonstrated *in vivo* in animal models of ischaemia and stroke[208,209], for oxidative stress associated with Parkinson's disease[210,211], for glutamate toxicity associated with seizures[212] and in an amyloid-β overexpression animal model of Alzheimer's disease[106,207]. The readers are referred to an excellent review for detailed accounts of the neuroprotective role of BDNF in neurological and psychiatric diseases[104]. Perhaps surprisingly, conditional deletion of either *Bdnf*[67] or the neurotrophic receptor tyrosine kinase *Trkb*[213] gene in the adult mouse brain did not lead to obvious changes in neuronal number or brain morphology, suggesting that the pro-survival functions of BDNF are manifested primarily when neurons are under stress. Thus, BDNF may serve as a homeostatic regulator, eliciting neuroprotective functions only when neurons are damaged in disease conditions. The pro-survival effects of BDNF, together with its synapse-enhancing properties, make the BDNF–TRKB pathway an attractive therapeutic target for neurodegenerative diseases. Nerve growth factor (NGF), which is another neurotrophin that is expressed at low levels in the brain, has also been shown to promote the survival of cholinergic neurons in the brain. The therapeutic potential of NGF has been reviewed elsewhere[214].

control[101–103]. Moreover, BDNF can protect synapses against various toxic insults in animal models of neurodegenerative diseases, such as AD, Huntington's disease, amyotrophic lateral sclerosis (ALS) and PD[104]. In a transgenic mouse model of AD (APP/PS1), inhibition of TRKB signalling exacerbated the spatial memory deficit, whereas overexpression of TRKB rescued spatial memory[105]. Remarkably, BDNF has been shown to protect and/or repair hippocampal neurons and synapses despite Aβ build-up and neuronal toxicity in a mouse model of AD[106] and to rescue plasticity deficits induced by synthetic Aβ oligomers in rat hippocampal slices *ex vivo*[107], suggesting its potential as a therapeutic even in the presence of pathogenic factors.

The *BDNF* Val66Met polymorphism

The study of BDNF function has greatly benefited from the identification of the single-nucleotide polymorphism (SNP) in the gene encoding BDNF in humans that converts a valine to methionine at codon 66 (Val66Met)[108]. The *BDNF* Val66Met polymorphism does not alter the expression or processing of proBDNF or the structure of mature BDNF[108]. Rather, the $BDNF^{Met}$ protein results in impairment in the dendritic trafficking and synaptic localization of the protein and, most importantly, an 18–30% reduction in activity-dependent BDNF secretion[108,109]. The *BDNF* Val66Met polymorphism is associated with alterations in brain structure, network and function in healthy humans and has been implicated in several neurological and psychiatric disorders.

Effects on hippocampal volume. A reduction in BDNF secretion may affect dendritic and axonal growth, leading to changes in volume of certain brain areas. Structural MRI revealed a small but significant bilateral reduction (~10%) in the grey matter volume of the hippocampus, amygdala and neocortex in $BDNF^{Met}$ carriers (that is, $BDNF^{Val/Met}$ individuals and $BDNF^{Met/Met}$ individuals) compared with $BDNF^{Val/Val}$ individuals[110–112]. A longitudinal study revealed a twofold higher incidence of age-related reductions in hippocampal volume in healthy subjects carrying the $BDNF^{Met}$ allele compared with healthy $BDNF^{Val/Val}$ individuals[113]. However, a meta-analysis suggested that the effect of the $BDNF^{Met}$ allele on hippocampal volume may be overestimated owing to underpowered studies[114].

A reduction in brain volume has been associated with (susceptibility to) brain illnesses. Indeed, hippocampal volume is consistently reduced in $BDNF^{Met}$ carriers compared with $BDNF^{Val/Val}$ patients with major depressive disorder, independently of age[115,116]. Perhaps surprisingly, in patients with multiple sclerosis, the $BDNF^{Met}$ genotype was associated with the preservation of grey matter volume and was inversely correlated with autoimmune-induced lesions[117], suggesting that the $BDNF^{Met}$ genotype may be protective in certain diseases. Adult healthy $BDNF^{Met}$ carriers exhibit structural phenotypes similar to those seen in AD — a reduction in the thickness of temporal lobe structures, including the entorhinal cortex, and in white matter tracts that connect temporoparietal and temporofrontal areas[118].

Effects on cognitive performance and neuronal network activity. Since the initial report[108], numerous studies have reported changes in brain activation and function associated with the $BDNF^{Val/Met}$ polymorphism. However, the findings have not been consistent, probably owing to small sample sizes or population differences in factors such as ethnicity, age and gender. For example, functional MRI experiments have revealed reduced hippocampal activation during the encoding or retrieval of episodic memory in $BDNF^{Met}$ carriers compared with $BDNF^{Val/Val}$ subjects[108,110] even when performance levels were matched[106]. By contrast, when effects on successful memory-related activation were examined, $BDNF^{Met}$ carriers showed a greater engagement of the hippocampus and other medial temporal lobe areas during encoding and retrieval, potentially suggesting that there is neural inefficiency in memory-specific networks[119]. Excess hippocampal activation may contribute to memory impairment and may be associated with widespread degenerative processes in prodromal AD[120,121]. $BDNF^{Met}$ carriers exhibit impaired performance in various memory tasks (including episodic, visuospatial and working memory)[108,122–125], which is consistent with the role of BDNF in LTP and hippocampus-dependent memories[71]. However, many studies have failed to observe robust and consistent effects of the $BDNF^{Val/Met}$ genotype on various cognitive functions[126], probably because the tasks are relatively insensitive to a small reduction in BDNF secretion.

Brain stimulation studies using transcranial magnetic stimulation, transcranial direct current stimulation or paired associative stimulation have revealed impairments in cortical excitability or plasticity in $BDNF^{Met}$ carriers[50,127], although other studies could not replicate these findings[128–131]. These inconsistencies may be due to methodological or inter-subject differences in response to brain stimulation. Moreover, *in vivo* electrophysiology, which is perhaps the best measure of synaptic and circuit function in the brain, was influenced by the $BDNF^{Val/Met}$ genotype.

Paired associative stimulation
A neurophysiological paradigm that involves stimulation of the median peripheral nerve followed by transcranial magnetic stimulation of the contralateral motor cortex. This paradigm has been successfully used to induce plasticity changes in the human motor cortex.

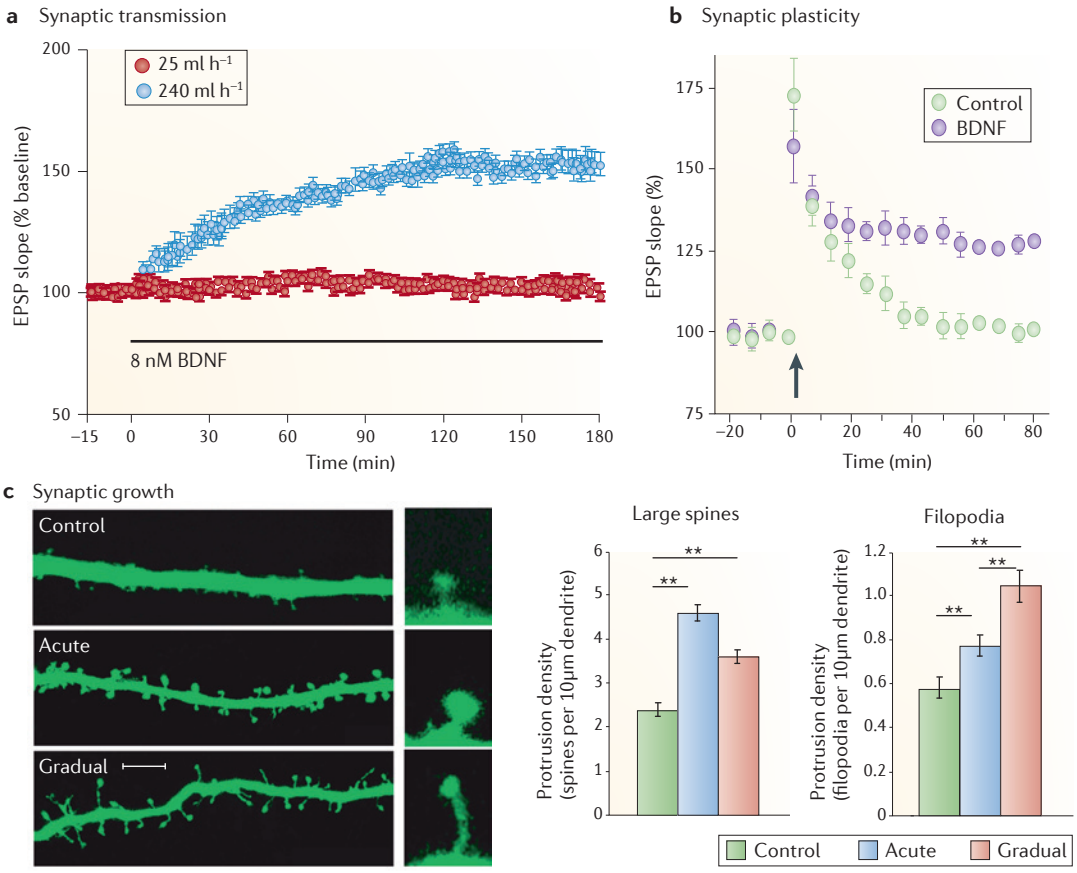

Figure 3 | **BDNF regulates synaptic transmission, synaptic plasticity and synaptic growth.** Preclinical studies in the past two decades have demonstrated the role of brain-derived neurotrophic factor (BDNF) in enhancing synaptic transmission[57,70], modulating synaptic plasticity[59] and also in promoting synaptic growth[220]. **a** | Effects of BDNF on basal synaptic transmission. Acute application of recombinant BDNF (8 nM) to hippocampal slices from 8-week-old mice at a fast (blue, 240 ml h^{-1}) but not slow (red, 25 ml h^{-1}) perfusion rate rapidly enhances synaptic transmission at CA1–Schaffer collateral synapses. **b** | Effects of BDNF on hippocampal long-term potentiation (LTP). Hippocampal slices from 2-week-old rats were incubated in BDNF (2 nM) for 2.5–4 hours before electrophysiological recordings were made. Theta-burst stimulation (indicated by the arrow) applied to CA1–Schaffer-collateral synapses induced LTP in BDNF-treated slices but not in control slices. **c** | Effects of BDNF on synaptic growth. Rat hippocampal neurons transfected with green fluorescent protein in cultures examined 20 days after a 1-day treatment with BDNF (1 nM) showed increases in spine density (mushroom and filopodia) in general (left panel). Acute application of BDNF preferentially increases the number of large spines (middle panel), whereas a gradual increase of BDNF stimulates spine motility and preferentially increases the number of filopodia (right panel). The asterisks indicate statistically significant differences. EPSP, excitatory postsynaptic potential. Parts **a** and **c** are modified, with permission, from REF. 70 © (2010) Macmillan Publishers Ltd. All rights reserved. Part **b** is modified, with permission, from REF. 59 © (1996) Macmillan Publishers Ltd. All rights reserved.

Specifically, studies using resting electroencephalography (EEG) revealed a general increase in slow-wave activity (theta and delta power) but a decrease in fast-wave activity (alpha power) in $BDNF^{Met}$ subjects compared with $BDNF^{Val}$ subjects, suggesting that there is an increase in inhibitory and/or a decrease in excitatory synaptic activity in the cortex[132]. Studies using event-related potentials have also shown that $BDNF^{Met}$ carriers exhibit impairments in synchronization processes that underlie error processing during an error-related negativity task[133] and in attention (that is, P300 latency increase and amplitude reduction)[125].

Overall, the *BDNF* Val66Met polymorphism may serve as a useful tool to elucidate genotype–phenotype relationships in humans. Given the variability and inconsistency in past studies, future investigations need to focus on a subgroup of carefully phenotyped subjects (BOX 4).

The* BDNF *Val66Met polymorphism in neurodegenerative diseases. Attempts to establish a direct association of the *BDNF* Val66Met polymorphism with the risk of AD, age of disease onset or disease progression have been inconclusive. For instance, $BDNF^{Met}$ carriers may have an increased risk of early-onset AD, whereas $BDNF^{Val}$ carriers may have an increased risk of late-onset AD[118]. $BDNF^{Val}$ seems to delay the age of onset of AD and reduce the risk of AD in female apolipoprotein E ε4 (*APOE* ε4) carriers of Han Chinese origin[134] but not

Box 4 | **Outstanding questions about the *BDNF* Val66Met polymorphism**

The extensive characterization of the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism offers a good foundation to address a number of key questions in future studies. First, the vast majority of studies published so far compared $BDNF^{Val/Val}$ carriers with $BDNF^{Met}$ carriers, owing to the rarity of $BDNF^{Met/Met}$ homozygotes in Caucasian individuals[108,215]. Gene dosage studies, by comparing all three genotypes ($BDNF^{Val/Val}$, $BDNF^{Val/Met}$ and $BDNF^{Met/Met}$), would reveal whether the phenotypic effects are truly associated with the BDNF polymorphism. The high occurrence of $BDNF^{Met/Met}$ in Asian populations[134,216] may offer an opportunity for such studies. Second, it is imperative to quantitatively measure the effect of the *BDNF* genotype on several endophenotypes: for example, hippocampal volume or cognition in the same subjects. Assuming that an alteration in BDNF trafficking or secretion correlates with changes in synaptic function, such a study will provide the most sensitive and reliable measure for synaptic changes to be used in clinical trials. Third, studying possible epistasis between the $BDNF^{Val/Met}$ genotype and other common polymorphisms may offer insights into disease risk and progression that may not be revealed by assessing the effect of one single-nucleotide polymorphism alone. This could be powerful when the effects of interactions with a disease-risk gene (for example, the gene encoding apolipoprotein E4) on endophenotypes relevant to a disease (for example, episodic memory or hippocampal volume in Alzheimer's disease) are examined. Fourth, most studies published so far have revealed negative effects of the $BDNF^{Met}$ allele on brain functions. Given that the *BDNF* Val66Met polymorphism has been selected during evolution[215], it may have some beneficial effects. For example, the Met allele appears to be protective against grey matter damage in multiple sclerosis[217], childhood-onset bipolar disorder[152] and obsessive compulsive disorder[218,219]. Last, differences in cognitive functions associated with the *BDNF* Val66Met polymorphism in adults may result from cumulative changes during decades of brain development or result from functional changes in synapses and neuronal circuitry. It is widely believed that developmental alterations of neuronal networks may be difficult to restore by pharmacological intervention; however, when the neuronal circuits formed during development remain unaltered, functional modulation of synapses can be used to restore network function. Pharmacological interventions that increase BDNF expression or secretion will not only help to distinguish these possibilities but also offer hope for therapies for diseases that result from synaptic dysfunction.

Gene dosage
A linear relationship between the number of genes (or alleles), the gene (or allele) product and the resulting effect (the phenotype).

Epistatic
The effect of one gene or gene product influencing the effect of other genes or gene products.

Endophenotypes
Intrinsic phenotypes that are relevant to a disease but not evident without a test. A good endophenotype must be tightly associated with the disease and display familial association even in non-diseased relatives with a higher odds ratio than in the general population.

Phase I
Phase I trials are typically conducted in healthy volunteers or in patients in a closely monitored clinic to evaluate safety, tolerability and pharmacokinetics of a new investigational drug.

in other ethnic populations. In addition, $BDNF^{Met/Met}$ and $BDNF^{Val/Met}$ subjects with AD showed a higher risk for depression (with odds ratios threefold and twofold, respectively) compared with $BDNF^{Val/Val}$ subjects[135].

Any effects of the polymorphism on cognitive performance and disease should become evident in longitudinal studies. Indeed, a recent study demonstrated a faster and more robust decline in episodic memory and hippocampal volume over a 36-month period in healthy elderly individuals with both the $BDNF^{Met}$ genotype and high amyloid load (but not in those with the $BDNF^{Met}$ genotype or a high Aβ load alone)[136]. More importantly, patients with MCI (who have a high Aβ load) with the $BDNF^{Met}$ genotype also exhibit a faster and more robust decline in these two measures compared with $BDNF^{Val/Val}$ patients with MCI[136]. Thus, the $BDNF^{Met}$ genotype may accelerate the progression of AD. Consistent with this finding, inhibition of TRKB signalling exacerbated spatial memory impairment in APP/PS1 mice (a model of AD) but had no effect in wild-type mice[105]. If these findings in humans are validated in independent cohorts, they should help to develop a strategy for patient stratification so that clinical studies require fewer patients and can be of shorter duration. Future studies should also examine whether the faster decline in episodic memory and hippocampal volume in the $BDNF^{Met}$ MCI subpopulation can be reversed by pharmacological interventions.

Emerging findings also provide evidence for an association of the *BDNF* Val66Met polymorphism with PD: the *Gene*PD study[137] revealed an association of the polymorphism with the risk and age of onset of familial PD[137–139], which was especially strong in patients with PD who had cognitive impairments[140]. Epistatic interactions between the $BDNF^{Met}$ allele and the most common variant of the gene encoding leucine-rich repeat kinase 2 (the G2385R variant) increases the risk of PD in Asian populations. The overall odds ratio increased from 3.2 to 4.0 and to over 6, if the age of PD onset was >60 years[141]. These emerging results continue to substantiate the role of BDNF in neurodegenerative diseases.

$BDNF^{Val/Met}$ *knock-in mice as a tool.* To study the *BDNF* Val66Met polymorphism in well-controlled conditions and to determine whether the endophenotypes seen in $BDNF^{Met}$ carriers could be rescued genetically or pharmacologically, a knock-in mouse line was generated. The mice have a point mutation in the endogenous mouse *Bdnf*, resulting in a $Bdnf^{Met}$ genotype[109] instead of the $Bdnf^{Val}$ genotype. Neurons derived from $Bdnf^{Met/Met}$ mice showed reduced activity-dependent BDNF secretion[109]. $Bdnf^{Met/Met}$ mice had normal total brain BDNF levels, a smaller hippocampus (associated with reduced dendritic complexity) and impairments in hippocampus-dependent contextual memory[100]. In addition, hippocampal slices derived from $Bdnf^{Met/Met}$ mice exhibited a deficit in NMDA receptor (NMDAR)-dependent LTP but not in basal glutamatergic transmission[142]. NMDAR-dependent LTD was also reduced, whereas metabotropic glutamate receptor-dependent LTD remained intact. These results suggest that activity-dependent BDNF secretion may be selectively involved in NMDA-dependent forms of synaptic plasticity. Moreover, $Bdnf^{Met/Met}$ mice showed anxiety-like behaviour that was resistant to treatment with antidepressants such as fluoxetine[109] and ketamine[143]. A different mouse knock-in line in which the mouse *Bdnf* coding region was replaced by the human $BDNF^{Val}$ or $BDNF^{Met}$ sequence also exhibited synaptic deficits[144]. Although $BDNF^{Met}$ knock-in mice capture some phenotypes associated with the human *BDNF* polymorphism, it is important to assess whether the deficits in $BDNF^{Met}$ knock-in mice can be rescued by pharmacological intervention *in vivo*. If so, $BDNF^{Met}$ knock-in mice could serve as a translational preclinical model not only to study synaptic dysfunction but also to use in translational drug discovery for CNS diseases.

BDNF-based therapeutic strategy

The preclinical and human studies summarized above point to BDNF as a pro-survival molecule (BOX 3) as well as a synaptic repair molecule for neurodegenerative diseases. However, results from clinical studies using BDNF as a therapeutic agent have not been encouraging. To date, five clinical trials using BDNF have been conducted (four in ALS and one in diabetic neuropathy[145]), and the results have been inconclusive. In a Phase I/II[146] open-label trial for ALS, subcutaneously administered BDNF showed a delay in the percentage of forced vital capacity decline and an improvement in walking time, whereas

a Phase II/III trial did not replicate these benefits[143]. In another Phase I/II placebo-controlled, double-blind trial[144] and in an as yet unpublished Phase III trial[145] using intrathecal administration, BDNF showed no clinical benefits on survival or on the ALS functional rating scale (ALSFRS) score[145,147]. Given the beneficial effects of BDNF on neuronal functions observed in preclinical studies, how can we explain these clinical failures? One explanation could be that BDNF is cleared rapidly *in vivo* and does not easily penetrate into the spinal cord parenchyma. Although a dose-dependent increase in BDNF levels in cerebrospinal fluid (CSF) was reported after intrathecal delivery[148], there was no clinical evidence that BDNF had reached the target site (that is, the ventral horn of the spinal cord). In fact, none of the trial design included measurements of 'target engagement' (that is, TRKB activation or downstream signalling) or of an immediate downstream pharmacodynamic response following BDNF administration. In short, these four trials in ALS did not unequivocally test the BDNF–TRKB mechanism. Thus, it may be premature to conclude that BDNF is ineffective as a therapy for ALS [145,149,150].

BDNF as a therapeutic molecule. The problem described above — namely, the lack of evidence that BDNF has been delivered to the right tissues or activated TRKB — is not unique to BDNF-based trials. A recent analysis by Pfizer indicates that 43% of Pfizer programmes that were terminated because of a negative outcome in Phase II (testing proof-of-concept) had not adequately tested the drug's mechanism of action[151]. In other words, in these trials, it was unclear whether the pharmacological agents under investigation had reached the target tissue in the disease at therapeutic concentrations, or whether they had engaged the proposed target. A 'three pillars of survival'[151] theory that is based on this analysis proposes that three specific conditions (the 'pillars') should be met to increase the likelihood that a candidate drug 'survives' a Phase II trial. The three pillars are: first, that the drug is delivered to the target site over a desired period of time; second, that the drug binds to the target under investigation at the required level; and third, that drug-induced modulation of the target results in a pharmacodynamic effect.

Two additional problems have hampered the translation of the beneficial effects of stimulating BDNF–TRKB signalling from bench to bedside: the first is the inability to deliver BDNF across the blood–brain barrier (BBB); and the second is the poor bioavailability of BDNF owing to its physiochemical properties.

Regarding the first problem, several approaches are actively being pursued to deliver BDNF into the CNS. BDNF can be delivered to the required site of action through invasive procedures (by using a catheter or implantable pumps[152] or through implantation of biodegradable polyethylene glycol-based hydrogel device containing poly(lactic-co-glycolic acid) (PLGA) microparticles encapsulated with BDNF[153,154]). Although these approaches may be suitable for the short-term treatment of localized acute CNS injuries, they are of limited benefit in chronic neurological diseases because patients would have to undergo these invasive procedures repeatedly, which can lead to secondary complications.

Non-invasive approaches such as nanoparticle-, Trojan horse- and nose-to-brain-mediated delivery of BDNF into the brain are also being explored. Trojan horse technology involves conjugating BDNF to molecules that can readily cross the BBB. Emerging evidence suggests that preferential uptake of BDNF into the CNS can be achieved by conjugating BDNF to ligands that bind to certain receptors in endothelial cells that facilitate transcytosis or to antibodies directed against these receptors. For instance, BDNF conjugated to a monoclonal antibody against the human insulin receptor exhibited a ~tenfold increase in brain concentrations of BDNF and a 100-fold increase in the mean residence time of BDNF in the circulation without altering the blood glucose level[155]. Similar results have been obtained by conjugating BDNF to an antibody targeting the transferrin receptor[156]. A recent study revealed the significance of the antibody binding kinetics in Trojan horse-mediated CNS delivery[157]. Other Trojan horse carriers or ligands include low-density lipoprotein receptor-related protein 1 (LRP1)[158], diphtheria toxin receptor[159] or single-chain domain antibodies isolated from llama[160]. Current Trojan horse approaches lack tissue specificity and suffer from potential interference with the endogenous function of the transcytosis receptors. (For comprehensive reviews on the delivery of proteins, including BDNF, into the CNS, see REFS 161,162).

The nose-to-brain (or intranasal) route is an alternative way to deliver macromolecules into the brain parenchyma. The advantages of the nose-to-brain route include simple and non-invasive administration, rapid delivery to the brain, minimal systemic exposure to the drug and the option for repeated dosing. Nose-to-brain delivery of BDNF not only resulted in an increase in brain parenchymal concentrations within ~30 minutes but also induced activation of the TRKB receptor and its downstream phosphoinositide 3-kinase–AKT pathway[163]. In a rat middle cerebral artery occlusion model, nose-to-brain-administered BDNF 2 hours after the ischaemic insult was neuroprotective[164]. Further characterizations will be required to overcome the challenges associated with regulating the delivery of the intended dose to achieve region-selective delivery.

The second hurdle is the poor bioavailability and stability of BDNF (it has a half-life of few minutes in rat plasma[165] and a few hours in sheep CSF[152]). BDNF in the circulation is primarily cleared by the liver owing to its basic isoelectric pH. PEGylation of BDNF has been shown to enhance its stability (>60%) without affecting its bioactivity or mean time in the circulation[166]. Recombinant engineering methods should be considered to generate a mutant form of BDNF with higher stability, neutral isoelectric pH and enhanced brain penetration.

Development of drugs targeting the BDNF–TRKB pathway. In addition to BDNF itself as potential medicine, several strategies could be used to manipulate BDNF–TRKB signalling, including small-molecule

Forced vital capacity
The amount of air that can be forcibly exhaled from the lungs after a deep breath, which can be measured with a spirometer.

Phase II
A trial conducted primarily to evaluate the effectiveness of a drug in people who have a certain disease or condition. Safety continues to be evaluated in the clinical setting. Initial Phase II efficacy studies are also referred to as proof-of-concept studies.

ALSFRS
Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R is the revised version) is a validated measure (scores 0–48) that aids the assessment of disability of the patients with motor neuron diseases based on a questionnaire that asks about daily activities and how much help the patients need along with disease-specific symptoms.

Nanoparticle
A microscopic particle with a diameter of less than 100 nm. Here, it refers to the liposomes or exosomes that carry drug substances into the brain.

Trojan horse
A strategy to deliver drugs to target sites that are normally inaccessible. The drug is fused to a molecule or encapsulated in a cell or nanoparticle that can readily cross the blood–brain barrier.

Nose-to-brain
Delivery or transport of drugs, cells or cargoes into the brain intranasally through the olfactory or trigeminal neuronal pathway. This delivery route limits systemic exposure and bypasses the blood–brain barrier.

Table 1 | **Summary of approaches for targeting the BDNF–TRKB pathway**

Approach	Advantages	Challenges	Examples
Small-molecule neurotrophic receptor tyrosine kinase TRKB agonist, modulator and/or transactivator	• Systemic administration and delivery across the blood–brain barrier for CNS actions • TRK selectivity (not targeting the p75NTR pathway) • Transactivation through other tyrosine kinase receptors, G protein-coupled receptors and metal ions	• Identifying small molecules that can induce TRKB dimerization • Finding a true TRKB agonist and/or modulator that directly binds and activates TRKB • Off-target effects	• 7,8 -dihydroxyflavone[167] • LM-22 series[168] • De-oxygedunin[174] • Antidepressants (fluoxetine)[184] • N-acetylserotonin[221] • Adenosine 2A receptor agonist[222,223] • Apomorphine[224] • Zinc[225]
Biopharmaceutical TRKB agonist and/or modulator	• Specificity, fewer off-target effects • Better pharmacokinetics and bioavailability (compared with brain-derived neurotrophic factor (BDNF)) • Receptor selectivity (not targeting TRKA, TRKC or the pan-neurotrophin receptor p75NTR) • Low-affinity may limit hyperactivation and desensitization of TRKB	Delivery into CNS	• Peptidomimetics[171–173,226] • Agonist antibodies[169,176,177,227] • RNA aptamer[228]
Small molecules that enhance the transcription, translation or secretion of endogenous BDNF	• Limits pharmacology to physiologically relevant BDNF-expressing cells • Regulation at subcellular location (dendritic and/or synaptic synthesis and release) • Modulates endogenous mechanisms, limiting clinical adverse events	• Achieving promoter-specific activation of transcription • Increasing translation or inhibiting degradation of BDNF without affecting other molecules • Affecting secretion of growth factors, chemokines or cytokines • Generating a sufficient increase of extracellular BDNF to induce prolonged TRKB activation with a full spectrum of downstream signalling necessary for synaptic growth	• Antidepressants[181] • Memantine[188] • Ampakines[229–232] • Rolipram[233] • Fingolimod[234] • CEP-1347 (REF. 235) • Exercise[180,236] • Acetylcholinesterase inhibitors (donepezil, galantamine and huperzine A)[237,238] • Cystamine or cysteamine[239]

TRKB agonists or modulators[167,168], TRKB agonistic antibodies[169,170] or peptidomimetics[171–173], or small molecules that stimulate endogenous BDNF expression or BDNF–TRKB signalling (see TABLE 1 for a summary). The discovery of a specific and selective small-molecule TRKB agonist or activator has remained a significant challenge, primarily because of a lack of distinct binding pockets for small molecules in the extracellular domain of TRKB, and the requirement of a dimeric ligand to form extensive protein–protein interactions to activate TRKB. Several recent studies[167,168,174] reported the identification of small-molecule agonists or modulators of TRKB based on the crystal structure of BDNF and even demonstrated binding of such molecules to the extracellular domain of TRKB using biochemical or biophysical methods. However, in the absence of the co-crystal structure of the proclaimed TRKB-bound agonists or activators and the difficulties in reproducing the results[175] one must be cautious about designating them as 'true' agonists or activators. Nevertheless, the *in vivo* effects of these molecules could be true due to 'indirect' activation of TRKB (for example, through transactivation) or due to 'off-target' effects.

Agonist antibodies and peptidomimetics have been shown to activate the TRKB receptor in a dose-dependent manner both *in vitro* and *in vivo*, although their binding affinities, potency and magnitude of TRKB activation are not comparable with those of BDNF[169,176,177]. The agonistic antibodies are both selective and specific to TRKB: that is, they do not bind or activate TRKA (also known as NTRK1) or TRKC (also known as NTRK3) receptors, nor the pan-neurotrophin receptor p75NTR (also known as NGFR). Obtaining the ligand–TRKB co-crystallographic structures would provide further evidence for such non-BDNF-mediated pharmacological activation of the TRKB receptor and may even uncover novel ways to activate the receptor. However, the difficulty in CNS delivery limits the use of TRKB agonistic antibodies or peptidomimetics as a therapy for neurodegenerative diseases.

Physical exercise has been shown to enhance endogenous levels of BDNF[178]. However, a systematic analysis of the effect of exercise on circulating BDNF levels in healthy subjects revealed that the BDNF increase (~10% above the baseline plasma BDNF levels) was mostly transient, with levels returning to baseline within 10–60 minutes[179]. Consistent with this, BDNF protein and mRNA levels increase in the hippocampus of young and middle-aged rats in a transient manner after voluntary exercise[180].

An even more promising strategy is to pharmacologically enhance the expression of endogenous BDNF in the brain using a small molecule approach. Indeed, chronic but not acute administration of antidepressant drugs has been shown to enhance hippocampal *BDNF* expression[181]. However, it seems that the magnitude

and duration of the increase in BDNF levels are not sufficient to ensure TRKB activation[182]. Acute and chronic antidepressant treatment can also increase TRKB signalling. This effect is transient, independent of BDNF and incomplete (that is, tyrosine phosphorylation occurs at the autophosphorylation site and phospholipase Cγ site but not the SHC binding site of TRKB), possibly through a transactivation mechanism[183,184]. Thus, it is unclear whether the classic antidepressants could have a meaningful impact on the BDNF–TRKB pathway to promote synaptic growth. The NMDAR channel blocker ketamine has been shown to produce rapid (within hours) antidepressant actions in treatment-resistant patients[185]. Intriguingly, ketamine administration in animals induced a rapid but transient increase in cortical BDNF expression[186] and an increase in synaptic protein expression by activating the mammalian target of rapamycin pathway, and these effects were associated with an increase in dendritic spines and synaptic transmission[187]. Future studies should determine whether the synaptogenic effect of ketamine is truly mediated by ketamine-induced BDNF expression.

Memantine, a drug commonly used to treat patients with moderate to severe AD, has also been shown to increase BDNF expression in the limbic cortex in preclinical models[188]. However, the increase in BDNF expression was only observed at a dose of 50 mg per kg, which is predicted to be toxic in humans, and the effect was marginal (~25%) at a non-toxic dose[188]. Donepezil and galantamine, the acetylcholinesterase inhibitors commonly used to treat cognitive deficits in MCI and early AD, have also been shown to increase serum BDNF levels[189], although it remains unclear whether they increase BDNF in the human brain. These molecules exhibit properties similar to that of memantine, activating the AKT pathway but not the mitogen-activated protein kinase (MAPK) pathway[190]. Given that activation of the MAPK pathway is essential for BDNF-mediated regulation of spine growth[191], these drugs may not promote synaptic growth.

Thus, several factors must be considered when using pharmacological agents to stimulate endogenous BDNF expression. First, the molecule must induce BDNF expression at non-toxic doses and induce sufficiently high concentrations of extracellular BDNF to activate TRKB. Second, the molecule should induce sustained levels of TRKB activation with the full spectrum of downstream signalling that is necessary for synaptic growth. Third, the molecule should have synaptogenic effects: namely, facilitating L-LTP, promoting dendritic spine growth and/or enhancing synaptic protein expression. Any existing drugs with these properties could be considered for synaptic repair therapy.

General challenges for BDNF-based synaptic repair therapies. Patient heterogeneity makes it difficult to perform a well-controlled clinical study with a small number of patients, especially in the more common neurodegenerative diseases such as AD and PD. A proper investigation of a targeted mechanism — and hence the drug efficacy — is best performed in a clinically homogeneous patient cohort. For instance, patients with AD who have the *BDNF*^{Met} genotype have more severe endophenotypes (in terms of, for example, episodic memory and hippocampal volume) or faster disease progression[136]. Such patients could therefore be selected for clinical trials to enhance the sensitivity of detecting drug efficacy or to shorten the length of the trial. Recent studies have shown an epistatic interaction between the *BDNF*^{Met} and *APOE4* alleles on disease progression in preclinical AD[192,193] as well as between the *BDNF*^{Met} allele and high Aβ amyloid levels in prodromal AD[136]. Similar genetic interaction studies may identify new traits that could predict disease risk, age at onset and/or progression of neurodegenerative diseases, and therefore be used for patient stratification.

In addition to the three pillars of survival highlighted above, we have proposed a fourth pillar: to demonstrate the efficacy of drugs early in clinical studies using sensitive and reliable biomarkers[194]. In the case of synaptic repair therapy, this would involve measuring synaptic dysfunction and repair or regeneration *in vivo*, both preclinically and clinically. Unfortunately, the commonly used preclinical measures of synaptic plasticity, such as hippocampal LTP and performance in the Morris water maze test, are not translatable in humans. Conversely, methods that indirectly measure *in vivo* synaptic function, such as functional MRI and FDG-PET, are technically challenging in animals, and their spatial and temporal resolution is too low in humans. Although cognitive measures such as episodic memory are relevant to synaptic function, they are subjective, highly variable and too insensitive to monitor drug efficacy. Finally, for a drug to achieve disease modification, it would have to demonstrate long-term effects on synaptic plasticity and synaptogenesis that persist after drug withdrawal rather than a transient effect such as an increase in synaptic transmission.

Non-invasive *in vivo* electrophysiological methods may be able to measure synaptic function in both animals and humans. Recent studies have shown that genetic perturbations of the BDNF–TRKB pathway that are known to alter hippocampal synaptic networks decrease gamma oscillatory activity in hippocampal slices, reflecting desynchronization of neuronal activity within the hippocampal synaptic circuits[195,196]. Thus, BDNF-induced changes in synaptic connectivity in the cortex could be recorded using surface EEG, which probes the spatial and temporal summation of synchronous current flow through postsynaptic dendrites of cortical pyramidal neurons. Indeed, human *BDNF*^{Met} carriers exhibit a slower EEG profile[132] and abnormal event-related potential activity and/or synchrony in cognitive tasks[125]. Interestingly, some of these EEG and event-related potential changes are associated with hippocampal and frontal activation[125], and are correlated with MCI-to-AD conversion and AD progression[197,198]. To ascertain the translational value of using EEG to measure synaptic changes, future studies should assess the EEG phenotypes of *BDNF*^{Met} knock-in mice.

Conclusions and future directions

It is generally thought that a toxin-reducing approach may have beneficial outcomes in neurodegenerative diseases if it is started early, but the lack of sensitive biomarkers for disease progression and drug efficacy makes such early intervention studies extremely challenging. Increasing evidence suggests that in the case of progressive neurodegenerative disorders such as AD and PD, it might be more effective to treat the pathophysiology that directly underlies the clinical syndromes than to target the pathogenesis. Synaptic dysfunction seems to be a key pathophysiological feature for all neurodegenerative disorders. Given that synapse loss is reversible and predictive of disease progression, targeting mechanisms that stabilize and protect, or repair and regenerate synapses would enable clinical intervention at both early and late stages of the disease. Although the success of such synaptic repair approaches can ultimately only be measured by assessing their clinical efficacy, confidence in the potential of synaptic repair therapy will be strengthened if synaptic dysfunction and repair and/or regeneration can be measured reliably in the clinic.

Our understanding of BDNF–TRKB biology and the role of BDNF in synaptic plasticity and synaptic growth should now be translated into disease-modifying therapies for neurodegenerative disorders. BDNF is by far the best known synaptogenic molecule and perhaps the only one that has been associated with synaptic regulation in humans. Unlike most of the existing drugs that target synaptic transmission or plasticity[59], BDNF also promotes synaptic growth, and this can form a basis for a disease-modifying therapy. Animal experiments have established the role of BDNF in cognitive functions. More importantly, BDNF is neuroprotective and can repair synaptic deficits, despite the build-up of toxic proteins, in animal models of neurodegeneration[106]. The discovery of the *BDNF* Val66Met polymorphism, which influences synaptic localization and activity-dependent secretion of BDNF, provides an unprecedented opportunity to assess how changes in synaptic function in humans influence endophenotypes that are relevant to neurodegeneration. A systematic comparison of imaging, electrophysiological and behavioural findings in the three $BDNF^{Val/Met}$ genotypes should be able to identify suitable (that is, reliable and sensitive) measures of BDNF-induced synaptogenic effects in carefully phenotyped or at-risk human subjects. A better understanding of epistatic interactions between the *BDNF* Val66Met polymorphism and 'disease genes' in the endophenotypes of PD or AD may aid the development of strategies for patient stratification. Taken together, a combination of activating the BDNF pathway and a more reliable and sensitive method to measure the resulting synaptic changes could pave the way for the development of disease-modifying therapies.

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