Huntington's Disease

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Abstract

Changes in the level and activity of brain-derived neurotrophic factor (BDNF) have been described in a number of neurodegenerative disorders since early 1990s. However, only in Huntington disease (HD) gain- and loss-of-function experiments have mechanistically linked these abnormalities with the genetic defect.

In this chapter we will describe how huntingtin protein, whose mutation causes HD, is involved in the physiological control of BDNF synthesis and transport in neurons and how both processes are simultaneously disrupted in HD. We will describe the underlying molecular mechanisms and discuss pre-clinical data concerning the impact of the experimental manipulation of BDNF levels on HD progression. These studies have revealed that a major loss of BDNF protein in the brain of HD patients may contribute to the clinical manifestations of the disease. The experimental strategies under investigation to increase brain BDNF levels in animal models of HD will also be described, with a view to ultimately improving the clinical treatment of this condition.

Keywords

Huntingtin • BDNF • BDNF polymorphism • RE-1 silencing transcription factor/ neuron-restrictive silencer factor (REST/NRSF) • BDNF promotor • BDNF transport • Neurodegeneration • Post-mortem brain • Neurotrophin • Neurodegeneration

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G.R. Lewin and B.D. Carter (eds.), *Neurotrophic Factors*, Handbook of Experimental Pharmacology 220, DOI 10.1007/978-3-642-45106-5_14,
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Abbreviations

3NP	3-Nitropropionic acid
AAV	Adeno-associated viral vector
ALS	Amyotrophic lateral sclerosis
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BAC	Bacterial-derived artificial chromosome
ARNT2	Aryl hydrocarbon receptor nuclear translocator 2
BDNF	Brain-derived neurotrophic factor
CaM kinase II	α-Subunit of Ca^{2+}/calmodulin-dependent kinase II
cAMP	Cyclic adenosyne 3′ 5′ monophosphate
CaRE1/2/3	Ca^{2+} Responsive element 1, 2 and 3
CaRF	Calcium responsive transcription factor
CBP	CREB Binding protein
C/EBP/beta	CCAAT/Enhancer binding protein beta
ChIP	Chromatin immunoprecipitation
CNS	Central Nervous System
coREST	REST Co-repressor 1
CRE	cAMP/Ca^{2+} Responsive element
CREB	CRE Binding protein
DARPP-32	Dopamine- and cyclic AMP-regulated phosphoprotein 32 kDa
DR	Dietary restriction
ES	Embryonic stem
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and drug administration
GDNF	Glial cell line-derived neurothrophic factor
Emx	Empty spiracles homolog
eGFP	Enhanced green fluorescent protein
GSK-3β	Glycogen synthase kinase 3-beta
HAP1	Huntingtin-associated protein 1
HDAC	Histone deacetylase
HD	Huntington’s disease
Hdh	Huntington disease gene homolog
hsp70	Heat shock protein cognate 70 kDa
HSJ1B	Heat shock protein DNAJ-containing protein 1b
muHTT	Mutant huntingtin
wtHTT	Wild-type huntingtin
IT15	Interesting transcript 15
LiCl	Lithium chloride
L-VDCC	L-Type voltage-dependent Ca^{2+} channel
MEF2	Myocyte enhancer factor-2
MeCP2	Methyl-CpG binding protein 2
MEKK	Mitogen-activated protein kinase kinase
MLK	Mixed lineage kinase

MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSNs	Medium sized spiny neurons
mTOR	Mammalian target of rapamycin
NGF	Nerve growth factor
NMDA	*N*-Methyl-D-aspartic acid
NPAS4	Neuronal PAS domain protein 4
$p75^{NTR}$	p75 Neurotrophin receptor
PCR	Polymerase chain reaction
$p150^{Glued}$	150 kDa Dynein-associated polypeptide
PasRE	Basic helix-loop-helix (bHLH)-PAS transcription factor response element
PGC-1alpha	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PKA	Protein kinase A
Pro-BDNF	BDNF Precursor
RE1/NRSE	Repressor element 1/neuron-restrictive silencer element
REST/NRSF	RE-1 Silencing transcription factor/neuron-restrictive silencer factor
RILP	REST/NRSF-Interacting LIM domain protein
Sin3a	Switch independent homologue 3a
SOX 11	SRY (sex determining region Y)-box 11
Sp1	Specificity protein 1
SSRIs	Selective serotonin reuptake inhibitors
SVZ	Subventricular zone
TAFII-130	TATA box binding protein (TBP)-associated factor 130 kDa
TGases	Transglutaminases
TrkB	Tyrosine receptor kinase B
USF	Upstream stimulatory factor
Val66Met	Valine-to-methionine substitution at position 66
YAC	Yeast-derived artificial chromosome

1 Introduction

Huntington's disease (HD) is a dominant inherited neurodegenerative disorder that is caused by an unstable expansion of a CAG repeat within the coding region of the *interesting transcript 15* (*IT15*) gene (HDCRG 1993). The gene encodes for a protein called huntingtin whose mutation results in an elongated stretch of glutamine in the N-terminal of the protein (HDCRG 1993). Prevalence of the mutation is about 7–8 cases per 100,000 in populations of Western European descent, with many more at risk of having inherited the mutant gene. Neuropathological and neuroimaging studies revealed that the consequence of carrying the HD mutation is a widespread brain neurodegeneration characterised by the prevalent loss of efferent medium spiny neurons (MSNs) in caudate nucleus and putamen of the basal

ganglia (Reiner et al. 1988; Rosas et al. 2008). The typical HD symptoms include personality changes, cognitive declines and generalised motor dysfunction. The disease is with no effective therapies and progresses inexorably for 10–15 years from the onset.

The expansion of the CAG tract in huntingtin is the triggering event that endows the protein with new toxic functions deleterious for brain cells. Since the discovery of the HD gene in 1993, most of the research has focussed on elucidating the toxic activities of mutant huntingtin (Zuccato et al. 2010). In addition, we now know that the HD mutation also impairs the ability of normal huntingtin to exert activities that are fundamental for the survival and functioning of neurons (Cattaneo et al. 2001, 2005). As we proposed in 2001 (Cattaneo et al. 2001), this *loss of function hypothesis* in HD originates from the evidence that an expanded polyQ tract is present also in other proteins that cause at least eight different neurodegenerative diseases characterised by the loss of different types of neurons. Accordingly, we put forward the idea that "*whereas the CAG domain always evokes cell death, the different proteins in whose backbone the CAG is expressed identify the neurons that will die. If such proteins have crucial functions for the neurons that die in the disease, the resulting selective neuronal death might be directly attributable to the loss of those functions*" (Cattaneo et al. 2001). A number of findings now indicate that the ubiquitously expressed huntingtin protein has physiological function(s) that are particularly important for the brain, both during development and in adulthood (Zuccato et al. 2010; Cattaneo et al. 2005). *It is in the context of these studies that brain-derived neurotrophic factor (BDNF) has been mechanistically linked, through gain and loss of function experiments, to normal and mutant huntingtin for the first time.*

Most of the striatal BDNF is produced in the cerebral cortex and anterogradely delivered via the cortico-striatal afferents to the *corpus striatum* where it controls the activity of the cortico-striatal synapse while promoting the survival and maturation of the medium spiny neurons that are affected in HD (Altar et al. 1997; Baquet et al. 2004; Rauskolb et al. 2010). A 50 % reduction in BDNF levels at this synaptic site may thus contribute to striatal and cortical vulnerability. The hypothesis of a link between huntingtin and BDNF is supported also by the fact that they are co-localised in 99 % of the pyramidal neurons of motor cortex (Fusco et al. 1999, 2003)

The first proof in favour of this hypothesis was obtained in 2001. We reported that a crucial function of wild-type huntingtin is to contribute to the pool of BDNF protein produced in the cerebral cortex and that a loss or reduction in wild-type huntingtin as well as the presence of the CAG expansion in huntingtin diminishes BDNF cortical production and its striatal level (Zuccato et al. 2001). We also showed that huntingtin's ability to control cortical BDNF production occurs at a transcriptional level. Two years later huntingtin's target on the BDNF promoter was identified (Zuccato et al. 2003). In 2004, a new piece of data was added by the group led by Frederic Saudou in Paris who showed that wild-type huntingtin, in addition to controlling BDNF production, also controls its transport, at least in cells in vitro. Huntingtin is part of the molecular machinery that drives BDNF vesicles along

microtubules, and reduced BDNF transport was found in cultured HD cells (Gauthier et al. 2004). In light of the evidence indicating reduced levels of BDNF in HD, a number of studies involving HD mice have tested the impact of reducing or augmenting the level of this neurotrophin on disease onset and progression. The general conclusion is that "*the BDNF loss*" contributes to clinical manifestations in mice. This has generated considerable excitement about the idea of establishing a "*BDNF therapy*" for HD.

In this chapter we will describe the relevant data indicating that the production and transport of BDNF are under the stimulatory control of wild-type huntingtin, and that the mutation in the huntingtin gene as it occurs in HD causes the loss of this stimulatory activity, leading to a reduced BDNF protein level in cortex and striatum. We will emphasise the experiments performed on HD animal models and on tissue from patients with HD, as these have revealed defects in BDNF transcription, intracellular transport and postsynaptic targeting, as well as alterations in downstream signalling pathways. We will also present the available evidence highlighting the effect of reduced BDNF in HD, along with data showing that increased levels of BDNF are neuroprotective in the HD brain. Finally, we will describe the current experimental strategies under investigation that are aimed at increasing brain BDNF levels in animal models of HD, with a view to ultimately improving the clinical treatment of this condition.

2 Wild-Type Huntingtin and BDNF Gene Transcription

In this section we describe the evidence linking BDNF gene transcription to wild-type huntingtin as well as the data demonstrating that a well-known DNA regulatory sequence located within the BDNF promoter represents the first identified downstream molecular target of wild-type huntingtin's activity on the BDNF gene. We also discuss the mechanism by which wild-type huntingtin facilitates BDNF gene transcription and summarise the evidence showing that the same mechanism underlies the control of wild-type huntingtin over the transcription of other important neuronal genes.

2.1 In Vitro and In Vivo Evidence of a Link Between Wild-Type Huntingtin and BDNF

It was 2001 when huntingtin's ability to stimulate BDNF production was reported by means of a cell model of HD represented by immortalised ST14A cells stably transfected with human full-length wild-type or mutant huntingtin (Zuccato et al. 2001; Rigamonti et al. 2000). Enzyme-linked immunosorbent assays (ELISAs) of the different stable ST14A transfectants showed increased BDNF protein production in the cells overexpressing wild-type huntingtin in comparison with the mutant clones, which had a lower BDNF content than the mock-transfected ST14A cells. RNase protection assays further indicated that wild-type, but not

mutant huntingtin, facilitates BDNF production by acting at the level of BDNF gene transcription (Zuccato et al. 2001; Zuccato and Cattaneo 2007, 2009).

A second series of experiments showed that the pro-stimulatory effect of wild-type huntingtin on BDNF gene transcription depends on the activation of one specific BDNF promoter. At the beginning of 2000s the only data available about the structure and regulation of the BDNF gene were from work by Tonis Timmusk and colleagues at that time at Karolinska Institute in Stockholm, which identified four 5′ untranslated exons linked to separate promoters and one 3′ exon that encodes the BDNF protein (Timmusk et al. 1993). They also found that these promoters were alternatively used, generating a tissue-specific and stimulus-induced pattern of BDNF expression in the brain (Timmusk et al. 1993, 1995). It was later found that these different transcripts may also have different subcellular localisation and targets (Pattabiraman et al. 2005). Further studies from the same group published in 2007 clarified that the rodent BDNF gene contains a total of nine exons (I, II, III, IV, V, VI, VII, VIII and IX). The functional BDNF protein is produced following splicing at the 3′ end of exon IX, which contains the coding region (Aid et al. 2007) (Fig. 1). To evaluate whether the modulatory effect of huntingtin on BDNF gene transcription results from the preferential activation of one or more of these promoter regions, promoter reporter assays and polymerase chain reaction (PCR) for the specific mRNAs were performed (Zuccato et al. 2001). These experiments demonstrated that enhanced transcription from BDNF promoter II accounts for the increased BDNF level found in the presence of wild-type huntingtin, whereas transcription from BDNF promoter I, III and IV [the two last now renamed IV and VI, according to the new description of the gene by (Aid et al. 2007)] was unaffected (Zuccato et al. 2001). See Fig. 1.

This was further verified in vivo, in yeast-derived artificial chromosome (YAC) mice produced by Michael Hayden's group at the University of British Columbia and expressing increased full-length wild-type huntingtin with 18 glutamines (YAC18) (Hodgson et al. 1999). We have found that these mice carry higher BDNF protein levels as a consequence of the positive regulation on BDNF gene transcription by wild-type huntingtin. In particular, lysates from the cerebral cortex of these mice contained 47 $\pm$ 12 % more BDNF protein than that of their littermates and, consistently, there was 50 % increase in BDNF protein levels in the striatum (Zuccato et al. 2001). Increased transcription from BDNF promoter II accounted for the increased amount of BDNF protein in the cerebral cortex of YAC18 mice, whereas the transcriptional activity of other BDNF promoters was unchanged (Zuccato et al. 2001).

While extra copies of wild-type (but not mutant) huntingtin increase BDNF production in vitro and in vivo, one should expect that cells or brain tissues depleted of endogenous huntingtin are characterised by reduced BDNF levels. In 2003, we reported that BDNF mRNA levels were lower in the cerebral cortex of constitutive heterozygous huntingtin knockout mice (Zuccato et al. 2003). Similarly, the neuronal inactivation of huntingtin in conditional homozygous knockout mice (Dragatsis et al. 2000) led to a statistically significant reduction in BDNF mRNA levels in the cerebral cortex (Zuccato et al. 2007). Moreover, BDNF mRNA was progressively

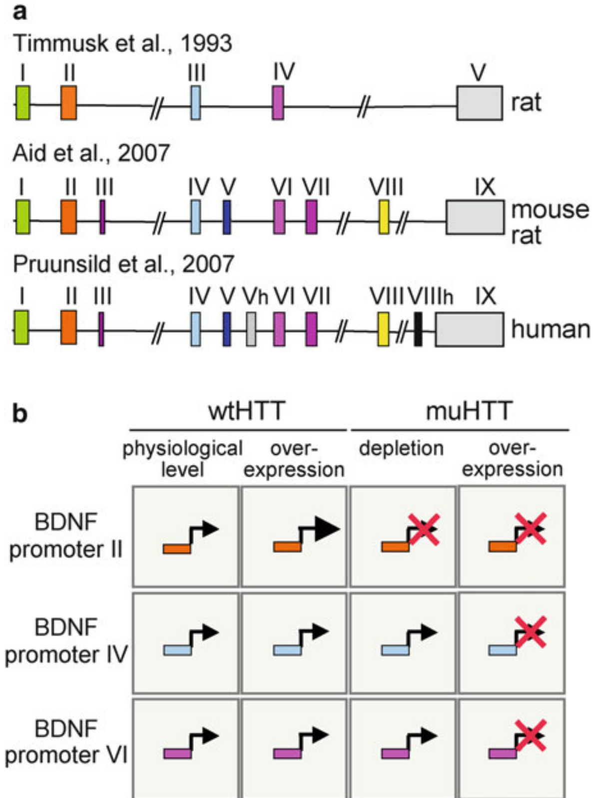

Fig. 1 (**a**) BDNF gene structure in humans and rodents proposed by different studies. Exons are shown as *boxes* and introns as *lines*. The BDNF coding region is indicated in *grey*. Homologous exons are highlighted with the same colour. (**b**) Transcription from BDNF promoter II is enhanced by wild-type huntingtin overexpression, whereas BDNF promoter IV and VI transcriptional activity is unaffected. Reduced wild-type huntingtin levels causes reduction of BDNF gene transcription from promoter II, while transcriptional activity of promoter IV and VI is unaffected. Mutant huntingtin overexpression reduces BDNF gene transcription from promoter II, IV and VI

reduced in mouse embryonic stem (ES) cells in which one or two alleles of the *Huntington disease gene homolog* (*Hdh)* have been inactivated via removal of exon 4 and 5 (Zuccato et al. 2007). This reduction in BDNF mRNA was attributable to a specific loss of BDNF mRNA II. These studies confirmed that loss of wild-type huntingtin specifically affects transcription from BDNF exon II promoter (Zuccato et al. 2003, 2007).

More recently, the group of David Rubinsztein at the University of Cambridge has used zebrafish to study wild-type huntingtin function. They demonstrated that loss of BDNF function is a major contributor to many of the developmental defects seen when huntingtin levels are knocked down in the embryo. BDNF mRNA levels were reduced in the huntingtin knockdown zebrafish, and these fishes also showed phenotypes that were very similar to those observed in the BDNF knockdown. Furthermore, the effects of huntingtin loss, which include brain atrophy, were attenuated by supplementation of the fish growth medium with recombinant BDNF protein (Diekmann et al. 2009; Henshall et al. 2009).

The data described above show that the ability of huntingtin to regulate BDNF expression is a component of its normal function which contributes to maintain the BDNF pool in the brain through a stimulatory action on BDNF promoter II.

2.2 The Involvement of REST/NRSF in Huntingtin's Activity in the CNS

The investigation of the mechanism by which wild-type huntingtin stimulates BDNF gene transcription has concentrated on BDNF promoter II. In 1998 a study by Tonis Timmusk highlighted that the BDNF promoter II contains a 21- to 23-bp DNA responsive element named repressor element 1/neuron-restrictive silencer element (RE1/NRSE), whose activity depends on its cognate transcription factor RE1 silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) (Timmusk et al. 1999). REST/NRSF is a master regulator of neuronal genes that is highly expressed in immature Central Nervous System (CNS) cells and at a much lower level in mature neurons, while remaining abundant in peripheral cells. Its role is to repress a large cohort of neuron-specific genes, through specific recruitment of a multi-subunit repressor complex to the RE1/NRSE (Ooi and Wood 2007).

In 2003, REST/NRSF was linked to HD with the discovery that wild-type but not mutant huntingtin inhibits the silencing activity of the RE1/NRSE within BDNF promoter II. In particular, wild-type huntingtin was found to retain REST/NRSF in the cytoplasm, thus reducing RE1/NRSE's activity and allowing BDNF gene transcription (Zuccato et al. 2003). Instead, mutated huntingtin causes the pathological entry of REST/NRSF into the nucleus where it can bind to the RE1/NRSE site and lead to BDNF repression (Zuccato et al. 2003).

In 2008, studies from Masahito Shimojo's laboratory at University of Kentucky College of Medicine demonstrated that huntingtin does not interact with REST/NRSF directly, but is part of a complex that contains huntingtin-associated protein 1 (HAP1) and REST-interacting LIM domain protein (RILP), a perinuclear protein that directly binds REST/NRSF and promotes its nuclear translocation. When huntingtin is mutated, REST/NRSF is released from the perinuclear protein complex and accumulates in the nucleus, where it binds to the RE1/NRSE sites within BDNF exon II and causing reduced BDNF gene transcription (Zuccato et al. 2003; Shimojo 2008) (Fig. 2).

2.3 Beyond BDNF: An Expanded Role for Wild-Type Huntingtin in Neuronal Gene Transcription

Bioinformatic studies from Noel Buckley's group at the University of Leeds indicated that the potential repertoire of REST/NRSF-regulated genes is extensive. In fact, in addition to the BDNF gene, the RE1/NRSE is found in thousands of neuronal genes including those encoding other growth factors, hormones, neuronal transcription factors, ion channels, proteins involved in axonal guidance, neurotransmitters, proteins involved in vesicle trafficking, fusion and synaptic transmission (Bruce et al. 2004). This suggested that wild-type huntingtin may play a broader role in regulating neuronal gene transcription via inhibition of the REST/NRSF–RE1/NRSE pathway.

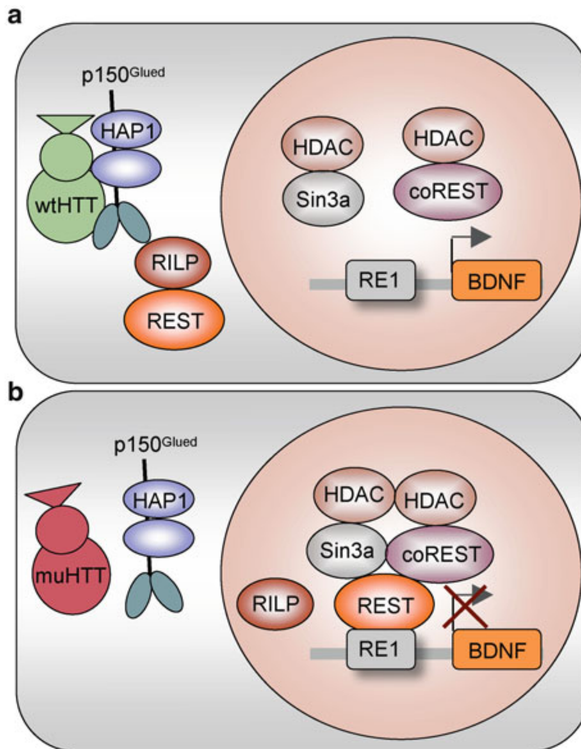

Fig. 2 (**a**) Regulation of BDNF gene transcription by huntingtin. Wild-type huntingtin (as part of a complex with HAP1, p150^{Glued} and RILP) sequesters REST/NRSF in the cytoplasm, thereby preventing the formation of a co-repressor complex (involving sin3a, coREST and HDAC) at RE1/NRSE sites and allowing the BDNF gene to be transcribed. The binding between huntingtin and REST is indirect: p150^{Glued}, the large subunit of the dynactin complex, bridges the interaction between wild-type huntingtin and RILP, with the latter directly binding REST/NRSF. (**b**) The mutant huntingtin complex in HD is less capable of retaining REST/NRSF in the cytoplasm than the wild-type complex. REST/NRSF enters the nucleus and the repressor complex is able to form, leading to reduced transcription of the BDNF gene

Several experiments confirmed the above-mentioned hypothesis. ST14A cells and YAC18 mice overexpressing wild-type huntingtin showed increased levels of the mRNAs transcribed from many other RE1/NRSE-containing neuronal genes, in addition to BDNF (Zuccato et al. 2003). In particular, the levels of synapsin-1, cholinergic receptor nicotinic beta-polypetide 2 and dynamin 1 mRNA were higher in the cerebral cortex of YAC18 mice, thus indicating that huntingtin may act as a general facilitator of neuronal gene transcription in the nervous system (Zuccato et al. 2003). Evidence in favour of a role of wild-type huntingtin in controlling RE1/NRSE-controlled neuronal gene transcription came also from chromatin immunoprecipitation (ChIP) data showing that REST occupancy is significantly lower in cells and mice expressing wild-type huntingtin than in HD models (Zuccato et al. 2007). Consistently, depletion of endogenous huntingtin in cells

and mice is associated with increased occupancy of REST/NRSF at RE1/NRSE loci and reduced transcription from the same genes (Zuccato et al. 2003, 2007).

These results identify a key role for normal huntingtin in facilitating transcription of REST/NRSF-regulated genes essential for neuronal development and maintenance. Proper control of transcription of the BDNF gene is particularly important for the activity of the cortico-striatal synapse and for the survival of striatal and cortical neurons, but reduced wild-type huntingtin function in HD may have broader consequences on neuronal gene transcription through the mechanism described herein. These findings have also potential therapeutic implications and suggest that treatment of HD may benefit from the production of drugs that mimic wild-type huntingtin physiological activity on the REST/NRSF–RE1/NRSE regulon (Zuccato et al. 2003, 2007; Rigamonti et al. 2007; Conforti et al. 2012).

3 Reduced BDNF Gene Transcription in HD

A 1997 landmark discovery by Stanley J. Wiegand and colleagues at Regeneron Pharmaceuticals, in New York, showed that most of BDNF protein found in striatum is produced in the cerebral cortex and anterogradely transported along the cortico-striatal tract to the MSNs (Altar et al. 1997). MSNs depend on cortically derived BDNF for their survival and activity (Zuccato and Cattaneo 2007, 2009). Thus, it has been proposed that reduction in BDNF level in the cerebral cortex or in its delivery may contribute to striatal (and cortical) vulnerability in HD. The finding that wild-type huntingtin stimulates BDNF gene transcription and protein production has prompted analyses of BDNF levels in the brain of transgenic mice and patients with HD.

3.1 Evidence from HD Cell and Animal Models

A first indication of a specific molecular defect in BDNF protein and mRNA levels in HD came from experiments on striatum-derived ST14A cells overexpressing full-length wild-type or mutant huntingtin. Although cells overexpressing wild-type huntingtin produce more BDNF protein, the production of both BDNF mRNA and protein in mutant huntingtin cells was less than in control cells (Zuccato et al. 2001). A similar decrease was also found in mutant huntingtin knockin cells obtained from heterozygous and homozygous huntingtin knockin mice in which a 109 CAG triplet has been inserted in exon 1 of the murine *Huntington disease gene homolog (Hdh)* gene ($Hdh^{109/7}$ and $Hdh^{109/109}$) (Zuccato et al. 2001, 2003, 2007; Soldati et al. 2011; Trettel et al. 2000). Moreover, Josep Canals and collaborators at the University of Barcelona transiently expressed exon 1 of mutant human huntingtin with 47, 72 or 103 CAG repeats in a striatum-derived cell line and showed reduced BDNF content. They also indicated that the increase in CAG size did not exacerbate the BDNF phenotype (Canals et al. 2004). More recently, reduced level of BDNF mRNA has been reported also in a novel series of mouse neural stem (NS) cells lines that carry varying number of CAG repeats (20, 50, 111)

in the mouse huntingtin gene (Conforti et al. 2013). We revealed that reduction in BDNF mRNA level during neuronal differentiation is CAG dependent up to 111 CAG (Conforti et al. 2013). According to recent in vivo studies some HD phenotypes may be more promptly revealed in the presence of shorter CAG expansion (Dragatsis et al. 2009; Morton et al. 2009; Cummings et al. 2012).

Consistent with the in vitro data, many laboratories have found reduced BDNF levels in total brain or cortical and striatal samples from a large panel of mouse models of HD that show different degrees of similarity to the human condition. The first in vivo evaluation of BDNF levels in a mouse model of HD has been performed on YAC mice that express human full-length mutant huntingtin with 72 glutamines (YAC72) and was described to develop striatal degeneration of MSNs at 12 months of age. An approximately 30 % decrease in BDNF protein levels has been found in the cortex of 9-month-old YAC72 mice with no disease symptoms (Zuccato et al. 2001). Another study found reduced BDNF mRNA levels in YAC72 mice from the age of 3 months, thus confirming that BDNF gene transcription can be affected before the onset of disease symptoms in this animal model (Hermel et al. 2004). A 40 % reduction in BDNF content has also been detected in the hippocampus, a finding that may be consistent with observations of impaired spatial memory in HD mice, as well as reports of hippocampal cell proliferation and neurogenesis deficits (Gil et al. 2005; Grote et al. 2005; Lazic et al. 2004; Ben M'Barek et al. 2013). Although preliminary, these data may have a clinical correlate insofar as HD patients show cognitive abnormalities (Schmidtke et al. 2002). The battery of YAC mice includes also mice carrying 128 CAG repeats (Slow et al. 2003) which are especially interesting because they show an earlier disease onset with respect to YAC72 mice, with age dependent striatal and cortical degeneration, and development of well-characterised progressive motor and cognitive deficits (Zuccato et al. 2010). Recently, Baoji Xu and colleagues at Georgetown University Medical Center have reported similar BDNF mRNA levels in the cerebral cortex of symptomatic 16-month-old mice YAC128 compared to wild-type mice (Xie et al. 2010). In the same study levels of mature BDNF determined by western blot in YAC128 mice were similar in the cerebral cortex, but significantly reduced in the striatum when compared with control mice (Xie et al. 2010). It is surprising that BDNF mRNA level and protein do not change in YAC128 cortex at symptomatic stages, whereas significant BDNF reduction has been found in the cortex, striatum and hippocampus of YAC72 mice in the absence of neuropathological and behavioural phenotype (Zuccato et al. 2001; Hermel et al. 2004). Data from our group have shown that BDNF mRNA level, as determined by quantitative PCR, is approximately 30 % lower in the cortex of YAC128 mice from pre-symptomatic stages compared to controls (unpublished data). These different results may be due to the different techniques used for BDNF mRNA quantisation. Baoji Xu and colleagues used in situ hybridisation while we have used quantitative PCR.

In 2008 bacterial-derived artificial chromosome (BAC)-mediated transgenesis was used to develop mouse models of HD expressing full-length mutant huntingtin with 103 glutamine repeats (BACHD). These mice, produced by William Yang at the University of California Los Angeles, exhibit progressive motor deficits starting from 2-months of age, neuronal synaptic dysfunction and late onset selective

neuropathology, which includes significant cortical and striatal atrophy and numerous degenerating neurons in striatum (Gray et al. 2008). BACHD cortical tissues have been tested for the BDNF content and significant deficit in BDNF transcription was found at 8 and 6 months of age (Simmons et al. 2013; Gray et al. 2008). More recently, reduction in BDNF cortical mRNA has been revealed at earlier time points (2 and 4 months of age) (Conforti et al. 2012).

Other studies have shown reduced BDNF mRNA and protein levels in HD mice transgenic for the N-terminal portion of the mutant huntingtin. These mice are characterised by early onset of symptoms and a fast progression of the disease that makes them particularly useful to test BDNF levels along disease progression. The analyses usually cover an experimental window that is no longer than 24 weeks. The R6/2 line produced by Gill Bates group at King's College in London and expressing a 63 amino acid N-terminal fragment of mutant huntingtin with 150 glutamines (Mangiarini et al. 1996) has been tested independently by four groups. Zhang et al. have reported a 50 % reduction in BDNF protein in total brain from 12-week-old symptomatic (Zhang et al. 2003) while Wang et al., using animals of the same age, reported a 20 % decrease in perikarial BDNF mRNA in corticostriatal neurons located in layer V (which have projections to the striatum) (Wang et al., abstract 450.4/W11, Society for Neuroscience 36th Annual Meeting 2006). In line with the rapid disease progression—subtle motor and learning deficits appear after approximately 4–5 weeks and the animals usually die after 13–14 weeks—we found reduced BDNF mRNA levels in the cerebral cortex from early pre-symptomatic stages (Zuccato et al. 2005). Luthi-Carter et al. have shown that the same mice exhibit reduced BDNF gene transcription in the cerebellum from 8 weeks of age, possibly leading to cerebellar dysfunction and altered motor coordination (Luthi-Carter et al. 2002). In the last years, the reduction of BDNF in the brain of R6/2 mice has been confirmed by additional studies (Conforti et al. 2008; Apostol et al. 2008; Johnson et al. 2008; Mielcarek et al. 2011; Giampà et al. 2013).

Brain BDNF protein levels have been tested, but with conflicting results, in another transgenic mouse line, R6/1, created at the same time as R6/2. R6/1 mice show slower disease progression because of the smaller amount of expressed mutant huntingtin (Mangiarini et al. 1996). Spires et al. (2004) reported that BDNF protein levels were reduced in R6/1 striatum but not in the cerebral cortex at the age of 5 months (Spires et al. 2004), whereas Canals et al. found no deficiency in striatal BDNF protein levels at the age of 6 months (Canals et al. 2004). The latter authors suggested that the unchanged BDNF levels in R6/1 mice may be due to the low transgene level, as cells expressing low levels of an exogenous mutant huntingtin tract do not show a reduction in BDNF protein content (Canals et al. 2004). Pang et al. have reported similar BDNF protein levels in the striatum and hippocampus of 5-month-old controls and R6/1 mice, but increased levels were found in the frontal cortex and, in the same study, reduced BDNF mRNA levels in the striatum, anterior cortex and hippocampus was detected (Pang et al. 2006). Reduced BDNF mRNA level in the R6/1 hippocampus has been confirmed by a study from Zajac and colleagues (2010). These conflicting findings may be explained by the different methods used for BDNF protein quantification. Spires et al. (2004) used western blot, which differentiates mature BDNF (which is found

decreased) from the immature form (which remained unmodified), whereas Canals and Pang used ELISA, which is more quantitative but does not distinguish mature and immature BDNF. It is possible that the striatal level of mature BDNF protein is significantly decreased but levels of immature BDNF remain largely unchanged (Pang et al. 2006). Moreover, the reduced levels of BDNF mRNA in striatal neurons (which transcribe little or no BDNF) probably also affect the still uncertain BDNF levels in R6/1 mice, and so further investigations are necessary in this mouse model.

BDNF levels have also been tested in N171-82Q mice produced by David Borchelt laboratory at Johns Hopkins University and expressing a 517 amino acid N-terminal portion of huntingtin with 82 glutamine repeats driven by a mouse prion protein promoter (Duan et al. 2003; Schilling et al. 1999). Compared with the R6 mice, the N171-82Q model has fewer polyglutamine repeats resulting in a later onset of symptoms. ELISA assays showed that BDNF protein levels were significantly decreased by 70–80 % in the striatum and cortex of symptomatic 3-month-old N171-82Q mice (Duan et al. 2003). Quantitative PCR analyses have recently shown that BDNF mRNA is reduced in the cortex of N171-82Q mice at 4 months of age (Conforti et al. 2012). The above data indicate that R6/2 and N171-82Q mice are attractive tools for the study of pre-symptomatic therapies aimed at isolating drugs that increase BDNF levels.

BDNF levels have also been analysed in knockin mice that carry the HD mutation in the appropriate genomic context and express huntingtin protein at a physiological concentration, thus more reliably replicating the pathogenesis of HD. BDNF protein levels were first evaluated in a knockin mouse model produced by Marcy MacDonald at Massachusetts General Hospital in Boston and in which mouse exon 1 has been replaced with the human exon 1 carrying 111 CAG repeats (Wheeler et al. 1999). Immunoblots showed a less intense BDNF band in striatal and cortical extracts from homozygous mutant huntingtin knockin mice ($Hdh^{111/111}$) aged 5 months (Gines et al. 2003). Data from Borrell-Pages et al. indicating a small reduction in BDNF protein levels in total brain samples taken from 3-month-old homozygous knockin mice further support the notion of a BDNF deficit in this mouse model (Borrell-Pages et al. 2006). Support for an early BDNF reduction in the brain of mutant huntingtin knockin mice came also from a study by Simmons et al. who found that BDNF protein was reduced by 40–45 % in the hippocampus, cortex and striatum of 2-month-old $Hdh^{111/111}$ mice and from a work by our group highlighting reduced BDNF mRNA in cortex at 1 month of age (Lynch et al. 2007; Zuccato et al. 2007).

With a few exceptions that require further investigation, this evidence together speaks in favour of reduced BDNF level in HD cells and animal models and opens up the possibility that a similar dysfunction may be present in the human disease.

3.2 Reduced BDNF Promoter II Activity in HD

As previously described, wild-type—but not mutant—huntingtin stimulates BDNF gene transcription by acting at the level of BDNF promoter II. Several evidences indicate that the presence of a pathological CAG expansion in huntingtin abolishes the ability to sustain BDNF gene transcription from BDNF promoter II. Reduced

BDNF mRNA II levels are found in ST14A cells overexpressing full-length mutant huntingtin (Zuccato et al. 2001), as well as in heterozygous and homozygous mutant huntingtin knockin cells (*Hdh*$^{109/7}$ and *Hdh*$^{109/109}$) (Zuccato et al. 2003). Furthermore, reporter gene assays confirm that BDNF exon II promoter is 60 % less active in cells overexpressing mutant huntingtin than in parental cells (Zuccato et al. 2001). Earlier in vivo data support these observations and indicate that BDNF mRNA II levels are much reduced in the cerebral cortex and hippocampus of pre-symptomatic YAC72 mice expressing human full-length mutant huntingtin (Zuccato et al. 2001), and similar findings were reported in an independent study of the same YAC mice at 3 months of age (Hermel et al. 2004). Reduced BDNF mRNA II levels have been recently described also in cortical tissues from BAC-HD and in N171-82Q mice (Conforti et al. 2012). Cortical BDNF mRNA II levels are 25 % less in 8-week-old R6/2 mice than in controls and 60 % less in 12-week-old symptomatic R6/2 mice (Zuccato et al. 2005). Similar analyses by other authors have shown a significant depletion of wild-type huntingtin in 7-week-old R6/2 mice that parallels the timing of the reduced BDNF mRNA II level, thus suggesting that the decreased transcription from BDNF II promoter in this model may be due to the reduced level of endogenous huntingtin (Zhang et al. 2003).

The mechanism by which BDNF exon II promoter activity is reduced in HD has been described previously. As indicated, the RE1/NRSE silencer is the target of wild-type huntingtin on BDNF promoter II, and the wild-type protein inhibits its silencing activity by retaining the REST/NRSF transcription factor (which binds and activates the silencer) in the cell cytoplasm (Zuccato et al. 2003). ChIP assays have highlighted increased REST/NRSF binding at the RE1/NRSE of BDNF exon II in mutant huntingtin homozygous HD cells, in animal models (BAC-HD mice, R6/2 mice and homozygous mutant huntingtin knockin mice) as well as in the cerebral cortex of HD subjects, and this leads to increased activity of the silencer and to reduced BDNF mRNA II levels (Zuccato et al. 2007; Conforti et al. 2012) (Fig. 2).

Increased binding of REST/NRSF in the presence of mutant huntingtin is not confined to the RE1/NRSE of the BDNF gene. Increased REST/NRSF occupancy is evident in a cohort of RE1/NRSE-regulated genes in different cellular and animal HD models (Zuccato et al. 2007; Soldati et al. 2011; Johnson et al. 2008; Conforti et al. 2012; Soldati et al. 2013), resulting in repression of gene transcription. Furthermore, bioinformatic analyses of published microarray data of HD brain have shown that RE1/NRSE genes are preferentially repressed in HD patients (Johnson and Buckley 2009). This suggests that increased REST/NRSF repression can explain a significant fraction of gene dysregulation in the HD brain.

3.3 A Gained Toxic Activity of Mutant Huntingtin on BDNF Promoter IV and VI

In addition to reduced activity of BDNF promoter II, transcriptional activities of BDNF mRNA IV and VI are affected in HD cells and mice and contribute to reduction of the BDNF pool in HD brain. Short regions flanking promoters IV and

IV have been thoroughly characterised in terms of their regulatory elements of gene transcription. In HD cells, mouse and human tissue transcription from other BDNF promoters (BDNF promoter IV and VI) is also affected, suggesting that, in addition to reduced activity of BDNF promoter II (caused by loss of wild-type huntingtin activity), other mechanisms are in operation that lead to reduced BDNF gene transcription that are more specifically linked to mutant huntingtin's gain of toxic function (Zuccato et al. 2001, 2007; Zuccato and Cattaneo 2009) (Fig. 1). Information on these promoter exons is given below, followed by a summary of experiments indicating the deleterious effect of mutant huntingtin and speculation about the underlying mechanisms.

Early studies indicated that BDNF promoter I is physiologically activated at low levels and stimulated by the administration of kainic acid, which evokes calcium signals through different subtypes of glutamate receptors (Metsis et al. 1993; Zafra et al. 1990). For this reason BDNF exon I was originally defined as the inducible brain-specific promoter (Timmusk et al. 1993; Metsis et al. 1993). Recent studies by Liu et al. (2006) and by Aid et al. (2007) have shown that BDNF promoter I is subject to physiological activation as the mRNA transcribed from it can be detected in the cerebral cortex, cerebellum, hippocampus, thalamus and brain stem (Aid et al. 2007; Liu et al. 2006), but little is known about the mechanisms regulating the transcriptional activation of BDNF promoter exon I. It is known that BDNF promoter exon I has distal and proximal cyclic adenosine 3′, 5′ monophosphate (cAMP)/Ca^{2+} responsive elements (CRE), and a proximal CRE is overlapped by an upstream stimulatory factor (USF) binding element (Tabuchi et al. 2002). We also know that the proximal element is bound by CRE binding protein (CREB) and upstream stimulatory factor 1 and 2 (USF1/USF2) and responds to Ca^{2+} signals evoked via L-type voltage-dependent Ca^{2+} channels (L-VDCC) and *N*-methyl-D-aspartic acid (NMDA) (Tabuchi et al. 2000, 2002). A study from Hara and colleagues suggest that Ca^{2+} signal-induced transcription of BDNF promoter I is mediated by REST/NRSF (Hara et al. 2009). More recently, the group of Tonis Timmusk has identified a asymmetric E-box-like element named PasRE [basic helix-loop-helix (bHLH)-PAS transcription factor response element] in human BDNF promoter I and demonstrated that binding of this element by bHLH-PAS transcription factors ARNT2 (aryl hydrocarbon receptor nuclear translocator 2) and NPAS4 (neuronal PAS domain protein 4) is crucial for neuronal activity-dependent transcription from promoter I (Pruunsild et al. 2011).

More robust attempts have been made to elucidate the structure and activity of BDNF exon IV promoter [BDNF exon III, according to the nomenclature described in (Timmusk et al. 1993)], which is characterised by the three Ca^{2+} responsive elements CaRE1, CaRE2 and CaRE3/CRE. These regulatory elements are stimulated by Ca^{2+} signals evoked by *N*-methyl-D-aspartic acid (NMDA) glutamate receptor and involve CREB together with CaM kinase IV (Shieh et al. 1998; Tao et al. 1998). Moreover, CaRE1 and CaRE3/cAMP responsive element are bound by the neuronal calcium responsive transcription factor (CaRF), whereas CaRE2 activity depends on the binding of transcription factor USF1/USF2 (Tabuchi et al. 2002; Chen et al. 2003a). Two studies by Chen et al. and Martinowich

et al. have shown that methyl-CpG binding protein 2 (MeCP2), which binds methylated CpGs island on DNA and is involved in the long-term silencing of gene transcription, can selectively bind BDNF promoter exon IV and repress BDNF gene transcription (Chen et al. 2003b; Martinowich et al. 2003). Membrane depolarisation triggers the calcium-dependent phosphorylation and release of MeCP2 from BDNF promoter IV, thus facilitating transcription. Recently, it has been shown that for a full induction of human BDNF exon IV mRNA transcription, ARNT2 and NPAS4 binding to a PasRE sequence in promoter IV is needed (Pruunsild et al. 2011).

Unlike the other BDNF promoters analysed above, BDNF promoter VI [indicated as IV by (Timmusk et al. 1993)] contains glucocorticoid-responsive elements, and its activity is influenced by thyroid hormone (Koibuchi et al. 1999) and corticosterone (Hansson et al. 2006). Additional findings indicate that CaM kinase II mediates the activation of BDNF promoter VI by Ca^{2+} influx. Transient transfection and overexpression experiments have shown that two nuclear isoforms of CaM kinase II (delta 3 and alpha B) specifically activate only promoter VI (Takeuchi et al. 2000). Takeuchi et al. has shown that mitogen-activated protein kinase kinase (MEKK) and protein kinase A (PKA) can also upregulate the activity of BDNF promoter exon VI; in particular, CaM Kinase II and MEKK, respectively, activate the promoter linked to BDNF exon VI via CCAAT/enhancer binding protein beta (c/EBP/beta) and specificity protein 1 (Sp1) transcription factors (Takeuchi et al. 2002). More recent findings indicate that MEF2 and Sox11 are also implicated in the regulation of BDNF promoter IV (Lyons et al. 2012; Salerno et al. 2012).

The first indication about a possible effect of huntingtin on BDNF promoter I, IV and VI was reported in 2001 by our group. We found that ST14A neural cells overexpressing the mutant protein do not express BDNF mRNA I (Metsis et al. 1993), but we did find that transcription from BDNF promoter IV and VI, which are physiologically subject to activation in the CNS, was significantly reduced in the presence of the mutant protein. Consequently, BDNF mRNA VI and VI are also reduced in ST14A cells expressing mutant huntingtin, and their levels were also lower in heterozygous and homozygous mutant huntingtin knockin cells (Zuccato et al. 2001). Similar results have been obtained in mouse models of HD. YAC72 mice show a reduction in BDNF mRNA IV and VI levels starting at pre-symptomatic stages (Zuccato et al. 2001; Hermel et al. 2004). A similar pattern has been found in R6/2 mice, which express mutant huntingtin exon 1. In particular, BDNF exon VI mRNA level was the first to be affected (at 6 weeks of age), while defects in transcription from promoter IV occurred only at very late stages (12 weeks of age) (Zuccato et al. 2005). Transcription from BDNF promoter IV and VI was significantly reduced also in the brain of N171-82Q and in BAC-HD mice (Conforti et al. 2012).

The mechanism leading to the reduced expression of BDNF mRNA IV and VI in HD is still unknown. However, an impaired CRE pathway has been observed (Sugars et al. 2004; Sugars and Rubinsztein 2003) and, as BDNF promoter IV has a CRE element, it is possible that a dysfunction in CRE activity may account for its

reduced transcription. Various evidences indicate that crucial proteins in this event are CREB (which directly binds to the CRE element after phosphorylation by PKA at Ser133) and the CREB binding protein (CBP), which acts as a bridge between CREB and the transcriptional machinery. A finding by Joan Steffan and Leslie Thompson at the University of California Irvine indicates that mutant huntingtin can interact with both the glutamine-rich activation domain and the acetyl transferase domain of CBP (Steffan et al. 2001). They also found that a reduction in the acetyltransferase activity of CBP causes a reduction in histone acetylation (Steffan et al. 2001), thus leading to a more compact chromatin structure that is less accessible to transcription factors and potentially explaining the decrease in CRE-dependent transcription and reduction in BDNF mRNA IV levels. Although early findings suggested that CBP can be sequestered into mutant huntingtin aggregates (McCampbell et al. 2000; Nucifora et al. 2001), a study by Yu et al. showed that altered CRE-dependent gene expression may be due to the interactions of soluble mutant huntingtin with nuclear CBP, rather than to the depletion of this transcription factor by nuclear inclusions (Yu et al. 2002). CBP is therefore subtracted from the transcriptional machinery regulating the CRE element in BDNF promoter IV. Reduced CREB phosphorylation (Gines et al. 2003; Giampa et al. 2006) and reduced cAMP levels (Gines et al. 2003) may also contribute to reduced transcription from BDNF exon IV promoter in an HD background. Moreover, CRE-mediated transcription is also activated by TATA box binding protein (TBP)-associated factor, 130 kDa (TAFII130), and evidence from Dimitri Krainc originally at Massachusetts General Hospital indicates that TAFII130 interacts with mutant huntingtin, thus further impairing the transcriptional machinery at the CRE loci (Dunah et al. 2002). The reduced transcription from BDNF promoter linked to exon VI in HD (Zuccato et al. 2001, 2005) may be also explained on the basis of evidence showing that Sp1 participates in its activation (Takeuchi et al. 2002), whereas mutant huntingtin sequesters Sp1, thus blocking its physiological interaction with TAFII130 and causing reduced transcriptional activity (Dunah et al. 2002; Li et al. 2002).

In conclusion, reduced normal huntingtin activity is responsible for decreased transcription from promoter II, whereas reduced transcriptional activity at promoters IV and VI reflects mutant huntingtin-induced toxicity. The above has potential therapeutic implications insofar as it suggests the usefulness of restoring BDNF levels in HD. The BDNF promoters can be used as reporter assays of huntingtin activity in order to identify the contribution of the activity of the mutant protein versus the loss of normal huntingtin function during HD progression. In particular, they can be used to develop reporter assays for the isolation of molecules that mimic wild-type huntingtin on BDNF exon II promoter. Such an assay would have the advantage of reflecting the activity of a much larger number of promoters located in neuronal genes and containing the RE1/NRSE element, thus anticipating the possibility that active compounds would restore transcription from a large number of RE1/NRSE controlled neuronal genes. In parallel, BDNF exon IV and VI promoters can be used in reporter assays to identify drugs capable of reducing or blocking the ability of mutant huntingtin to inactivate BDNF gene transcription from the same promoters.

4 Huntingtin and BDNF Vesicles Transport

In 2004, the French group led by Frederic Saudou at the Centre Universitaire Orsay in Paris showed that full-length wild-type huntingtin stimulates BDNF vesicular trafficking in neuronal cells and that its transport can be attenuated by reducing the levels of wild-type huntingtin using RNA interference (Gauthier et al. 2004). Huntingtin is found predominantly in the cytoplasm of neurons, and it is enriched in compartments containing vesicle-associated proteins (DiFiglia et al. 1995); it is antero- and retrogradely transported in rat sciatic nerve axons, where it associates with vesicles and microtubules (Block-Galarza et al. 1997). It is also involved in fast axonal trafficking (Gunawardena et al. 2003) and in the transport of mitochondria (Trushina et al. 2004). Wild-type huntingtin regulates axonal transport by interacting with the scaffolding proteins of the motor complex on microtubules thereby enabling retrograde transport and perhaps anterograde transport (Block-Galarza et al. 1997; Gunawardena and Goldstein 2005).

In this section we describe the studies showing that huntingtin has a role in the control of BDNF vesicle transport and the underlying mechanisms while presenting the evidence indicating that BDNF vesicle transport is reduced in HD.

4.1 Huntingtin as a Scaffolding Protein That Drives BDNF Vesicles Transport

Saudou and colleagues tested the relationship between huntingtin and BDNF vesicle transport by a series of in vitro experiments that included cells overexpressing wild-type huntingtin and cells in which endogenous huntingtin has been reduced by means of RNA interference. The distribution and dynamics of BDNF vesicles were evaluated in real time by means of ultra-fast 3D videomicroscopy after the transfection of recombinant BDNF tagged with enhanced green fluorescent protein (eGFP), followed by deconvolution microscopy and the measurement of parameters such as the percentage of static vesicles, mean velocity and the pausing time of vesicles (Gauthier et al. 2004). These analyses revealed that BDNF vesicles move faster in the presence of exogenous wild-type huntingtin while their speed is lower when the level of huntingtin is reduced. This study revealed also that BDNF vesicle transport is mediated by microtubules and requires molecular motors, such as kinesin and dynein, i.e. proteins that move vital cargoes on microtubule tracks. Within axons, vesicles from the cell body are transported anterogradely by kinesin motors to nerve terminals and synapses, whereas dynein and some kinesin motors intervene to transport organelles in the retrograde direction. Wild-type huntingtin enhances BDNF transport to both the tips of the neurite and the cell body, suggesting a possible role for huntingtin in both the anterograde and retrograde transport of BDNF (Gauthier et al. 2004).

Biofractionation studies and immunoprecipitation experiments indicated that wild-type huntingtin is part of the motor complex that drives vesicles transport along microtubules. In particular, huntingtin was found to interact with 150 kDa dynein-associated polypeptide (p150^{Glued}) subunit of dynactin via HAP1, thereby stimulating

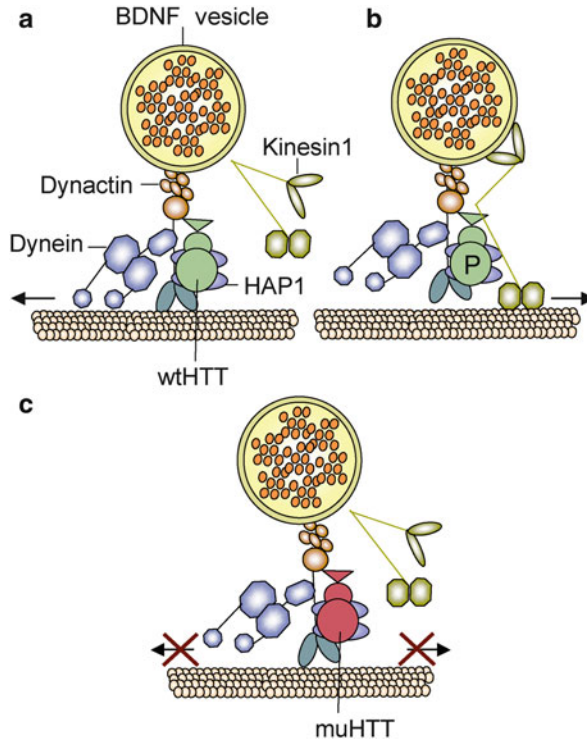

Fig. 3 The role of huntingtin in the intracellular transport of BDNF vesicles. Wild-type huntingtin forms part of a motor complex that controls BDNF vesicle intracellular transport along microtubules. *Arrows* indicate direction of transport (retrograde to the *left*, anterograde to the *right*). (**a**) when wild-type huntingtin is unphosphorylated, kinesin 1 molecules detach from the microtubules and vesicles undergo retrograde transport, mediated by dynein and dynactin. (**b**) when wild-type huntingtin is phosphorylated, kinesin 1 binds to the motor complex and microtubules, inducing a switch to anterograde transport. (**c**) mutant huntingtin is less readily phosphorylated than wild-type huntingtin and also binds more tightly to HAP1, reducing both anterograde and retrograde transport of the BDNF vesicles

BDNF transport. BDNF vesicle velocity decreased when HAP1 protein levels were reduced by RNA interference, whereas its overexpression caused the formation of BDNF vesicle clusters in which wild-type huntingtin and the p150^{Glued} subunit of dynactin are recruited to activate BDNF vesicle transport (Gauthier et al. 2004) (Fig. 3).

Further elucidation of the molecular mechanisms that link wild-type huntingtin to BDNF vesicle transport came from the group of Sandrine Humbert at the Institute Curie in Paris and involves one of huntingtin post-translational modifications [reviewed in Zuccato et al. (2010)]. Humbert's group found that phosphorylation at Ser 421 by Akt kinase is crucial to control the direction of BDNF vesicles (Colin et al. 2008). When huntingtin is phosphorylated, BDNF anterograde transport is favoured, whereas when the phosphorylated status is reduced, BDNF vesicles undergo retrograde transport (Colin et al. 2008). Reduced phosphorylation of huntingtin at Ser 421 is observed in cellular and animal models

of HD and in post-mortem human tissue, and this is likely to impair the transport of BDNF vesicles although in vivo proofs are still missing (Colin et al. 2008; Warby et al. 2005) (Fig. 3). A recent finding from Humbert's group has shown that BDNF vesicle transport can also be regulated by phoshorylation of huntingtin at Ser 1181 and 1201 (Ben M'Barek et al. 2013). Particularly, it was found that unphosphorylated forms of the two residues cause increased anterograde and retrograde BDNF transport (Ben M'Barek et al. 2013).

4.2 The Impact of the HD Mutation on BDNF Vesicles Transport

Since 2004, different groups have tried to understand whether a pathological polyQ expansion affects BDNF vesicles transport in HD. The Saudou's group found that BDNF vesicle velocity is reduced in heterozygous and homozygous mutant huntingtin knockin cells and that proteins involved in other neurodegenerative diseases do not affect BDNF transport, indicating the selectivity of huntingtin involvement in the transport of BDNF vesicles (Gauthier et al. 2004). To test in vivo the possible alteration of BDNF transport in the brain, Saudou and colleagues analysed the composition of the microtubule transport machinery in brain homogenates from mutant huntingtin knockin mice ($Hdh^{109/109}$ mice) and human post-mortem brain tissue. As previously mentioned, huntingtin is involved in the motor complex that includes HAP1 and the $p150^{Glued}$ subunit of dynactin (Gauthier et al. 2004; Block-Galarza et al. 1997; Engelender et al. 1997; Li et al. 1995, 1998). The results of experiments using HD mice, as well as human post-mortem brain tissues, suggest that this motor complex is altered in HD. In particular, increased binding of mutant huntingtin to HAP1 reduced the association between $HAP1/p150^{Glued}$ dynactin and microtubules in heterozygous mutant huntingtin knockin mice (Gauthier et al. 2004). This suggests that the mechanism controlling retrograde transport is altered in the presence of the polyglutamine expansion in huntingtin. As most striatal BDNF comes from anterograde (and not retrograde) transport from the cerebral cortex, it was also investigated whether the association between kinesin and microtubules is also reduced and found this to be the case in in vitro experiments using homozygous mutant huntingtin knockin cells. On the basis of the consideration that, in yeast two hybrid experiments, HAP1 may be pulled down with a human kinesin-like protein, it was also suggested that the complex consisting of huntingtin/HAP1 and kinesin may be affected by the polyglutamine expansion, leading to impaired anterograde transport (Gauthier et al. 2004; McGuire et al. 2006).

The second study was from Her and Goldstein at the University of California San Diego. By using a knockin mouse model of HD, which carries a 150 CAG triplet repeat expansion in the huntingtin gene, (*Hdh*(CAG)150) this group reported impaired movement of BDNF vesicles along microtubules in striatal and hippocampal primary neurons, but not in cortical neurons, the main source of striatal BDNF (Her and Goldstein 2008). Contrary to previous findings of Saudou and colleagues, this study shows that the observed alteration of BDNF vesicles transport in HD is not attributable to a disruption of motor protein complexes in *Hdh*(CAG)150 knockin mice (Her and Goldstein 2008). To test whether this discrepancy could be caused by differences in the

HD mouse models used (*Hdh*(CAG)150 vs *Hdh*$^{109/109}$ used in the Gauthier's study), differences of age or methods, Her and Goldstein performed sucrose gradient fractionation of brain extracts of 14-months-old *Hdh*$^{109/109}$ using a 7.5–25 % sucrose gradient as previously described by the Saudou group (Gauthier et al. 2004). No gross change in the pattern of the dynein and dynactin complexes and of kinesin or HAP1 between mutant and control mice were found. This study indicates that different mechanisms may contribute to alter BDNF vesicle transport. Mutant huntingtin may form aggregates that impair cargo movement or physically block movement in axons (Chang et al. 2006; Orr et al. 2008). However, this is unlikely to occur in the Her and Goldstein experiments because no aggregates were observed in the presymptomatic primary neurons employed (Her and Goldstein 2008). More recently, a study from Wu and colleagues suggests a new mechanism involving huntingtin, HAP1 and its direct interaction with pro-BDNF. BDNF is synthesised as a precursor (pro-BDNF), sorted into the secretory pathway, transported along dendrites and axons and released in an activity-dependent manner. Wu et al. have shown that HAP1 may participate in axonal transport and activity-dependent release of pro-BDNF by directly interacting with pro-BDNF (Wu et al. 2010). Mutant huntingtin reduces the association of HAP1 with pro-BDNF, thus leading to decreased transport and release of BDNF in HD (Wu et al. 2010).

In 2006, Sandrine Humbert linked BDNF vesicle transport to heat shock protein DNAJ-containing protein 1b (HSJ1B). HSJB is an inhibitor of heat shock protein cognate 70 kDa (hsp70), which removes clathrin from clathrin-coated vesicles (Cheetham et al. 1996). Clathrin is the main component of the protein coats decorating the cytoplasmic face of vesicles budding from the plasma membrane, the trans-Golgi network and endosomes, and is important for regulating vesicle secretion and endocytosis (Gleeson et al. 2004). This study revealed that BDNF, HSJ1B and clathrin co-localise at the cis-Golgi. The overexpression of HSJ1B positively regulates the sorting of BDNF-containing vesicles from the Golgi/trans-Golgi network, thus increasing BDNF release. Increasing levels of HSJ1B enhance the co-localisation of BDNF and clathrin, whereas reducing HSJ1B by RNA interference dramatically decreases it (Borrell-Pages et al. 2006). Reduced HSJ1B levels have been found in HD patients. This suggests that formation of the clathrin coats on BDNF vesicles can be altered, leading to impairment in BDNF processing at the Golgi and reduced BDNF vesicle transport (Borrell-Pages et al. 2006).

The finding of an altered BDNF vesicle transport in HD needs more studies. However, the evidence available suggests that increasing endogenous BDNF levels may be of therapeutic interest. On these bases, several attempts have been made to understand if BDNF levels are consistently affected in the brain of HD patients.

5 BDNF in HD Patients

In this section we describe the studies aimed at investigating BDNF levels in HD patients. We present the available data about BDNF mRNA and protein levels in autoptic brain tissues and the studies that have tested the BDNF gene

polymorphisms as potential modifiers of age at HD onset. Finally, we will review the conflicting evidence related to BDNF measurement in human blood.

5.1 Studies on Post-mortem Tissues

In a preliminary study conducted in 2000 by Ferrer and colleagues at the University of Barcelona, a small selection of post-mortem HD brain samples was evaluated. Decreased BDNF levels were found in striatum but not in the cerebral cortex. In particular, the parietal cortex, temporal cortex, hippocampus, caudate and putamen of 4 grade III HD subjects were analysed and compared with samples from 6 - age-matched controls. Western blots indicated a decreased ranging of mature BDNF protein (14 kDa) from 53 to 82 % in the caudate and putamen of HD patients when compared with age-matched controls. BDNF levels were preserved in the cerebral (parietal and temporal) cortex and the hippocampus. Immunohistochemical studies of the same tissue samples confirmed the reduced BDNF immunoreactivity in HD striatum (Ferrer et al. 2000). Although the BDNF signal was decreased in striatal neurons, BDNF labelling was maintained in scattered fibres. The authors suggested that the reduced BDNF protein levels in HD striatum could be due to a selective reduction in striatal neurons rather than reduced BDNF input from the cerebral cortex (Ferrer et al. 2000). However, most of the BDNF found in striatum is notoriously derived from cerebral cortex.

The findings of a second study by the Saudou's group published in 2004 also showed that BDNF protein levels evaluated by western blot in the cerebral cortex of ten HD patients and seven controls were reduced to about 50 % in striatum, but not in the cerebral cortex, thus suggesting a defect in cortical BDNF transport to striatum in HD; the negligible patient-to-patient variations indicated the highly homogenous nature of the patient cohort (Gauthier et al. 2004). On the contrary, the third study (published by our group in 2001) found that the levels of BDNF protein (assessed by ELISA) and BDNF mRNA in cortex were consistent with those observed in the various transgenic mouse models of HD: there was a ~50 % decrease in BDNF levels in the frontoparietal cortex of two HD subjects (grade II) in comparison with 2 age-matched controls (Zuccato et al. 2001). It is highly likely that the differences in the results of these three studies were due to their different methods and the diversity of the analysed samples (including our own limited number of samples initially analysed), and would be eliminated by analysing a larger number of samples. To this end, in 2007 we have extended the study to a larger cohort of HD and control subjects and have provided new evidence indicating a significant reduction in BDNF mRNA and protein in the cortex of 20 HD subjects in comparison with 17 controls. Analyses of the BDNF isoforms showed also that transcription from BDNF promoter II and IV is downregulated in human HD cortex (Zuccato et al. 2008; Pruunsild et al. 2007).

This study supports the notion of impaired BDNF production in human HD cortex as a consequence of an expanded CAG tract in the HD gene and suggests that increasing BDNF level or its signalling may be beneficial.

5.2 BDNF Polymorphisms in HD

Given the extensive evidence linking BDNF to HD, the BDNF gene has been tested as a potential modifier of age at HD onset caused by the presence of the BDNF polymorphisms. One known polymorphism of the human BDNF gene is a valine-to-methionine substitution at position 66 (Val66Met BDNF) that is located in the 5′ pro-BDNF sequence encoding the precursor peptide (pro-BDNF), which is proteolytically cleaved to form the mature protein. This BDNF polymorphism does not affect mature BDNF protein function nor its rate of transcription, but it has been shown to dramatically alter the intracellular trafficking and packaging of pro-BDNF, and consequently the regulated secretion of the mature peptide (Chen et al. 2004; Egan et al. 2003). The BDNF Val66Met polymorphism is highly conserved across species and relatively common in the human population with a prevalence for heterozygotes of 20–30 % and a prevalence for the homozygote of ~4 % (Egan et al. 2003; Hariri et al. 2003; Neves-Pereira et al. 2002; Sen et al. 2003). Several genetic linkage and behavioural studies have shown that this polymorphism is associated with neuropsychiatric disorders, including Alzheimer's disease, Parkinson's disease, bipolar disorders, depression, obsessive compulsive disorder and schizophrenia, as well as with normal personality traits (Neves-Pereira et al. 2002, 2005; Momose et al. 2002; Sklar et al. 2002; Ventriglia et al. 2002).

In the case of HD, it was found that mutant huntingtin does not affect the transport of Val66Val BDNF nor of Val66Met BDNF from the endoplasmic reticulum to the Golgi apparatus. Instead, it specifically alters the post-Golgi trafficking of BDNF vesicles. In particular, the post-Golgi trafficking of Val66Val BDNF was significantly blocked in mutant huntingtin cells, whereas the transport of Val66Met BDNF was not affected. These data clearly indicate that the mutant protein affects solely the trafficking of Val66Val BDNF form, without causing a major retention of Val66Met BDNF in the Golgi apparatus (del Toro et al. 2006). However, this study does not exclude the possibility that patients withVal66Met BDNF polymorphisms manifest the disease earlier.

A first linkage studies from Jordi Alberch at the University of Barcelona reported a later age of onset in HD patients who were heterozygous for the Val66Met polymorphism compared to individuals who were homozygous for valine or methionine at this position, although this association was restricted to the group of patients with huntingtin CAG repeats between 42 and 49 (Alberch et al. 2005). However, four subsequent independent studies did not confirm an effect of Val66Met and other BDNF polymorphisms, representing the entire variability of the BDNF gene, on the age of onset of HD (Di Maria et al. 2006; Kishikawa et al. 2006; Mai et al. 2006; Metzger et al. 2006).

Collectively, these studies conclude that there is no convincing genetic link between BDNF polymorphisms and HD. As the Val66Met polymorphism influences BDNF transport from the Golgi region to the appropriate secretory granules, without affecting the transcriptional or biological activities of this molecule, we proposed that the lack of an association might indicate that the defect in BDNF transport has no impact on the age of disease onset, although it may still

affect disease progression. However, this evidence does not exclude the possibility that a defect in BDNF transcriptional activity may affect age of onset and/or disease progression (Zuccato and Cattaneo 2007).

5.3 BDNF in Blood: An Unsolved Issue

BDNF is highly concentrated in the nervous system but is also found in the blood of human and other mammals, where its function is poorly understood. The BDNF in blood derives not only from synthesis in mononuclear blood and endothelial cells but also from platelets release as well as, although to a very minor extent, from the passage through the brain blood barrier (Fujimura et al. 2002; Radka et al. 1996; Rasmussen et al. 2009; Pan et al. 1998; Pan and Kastin 1999). Although it is still unclear how BDNF expression and metabolism in human peripheral tissues are regulated, changes in serum BDNF levels in rats during development correlate to those in brain (Radka et al. 1996; Klein et al. 2011). Based on these findings and on the extensive data showing that BDNF is reduced in HD brain, it was proposed that peripheral BDNF could be used to measure the state of the disease and/or the effectiveness of a given treatment. A number of clinical studies in other pathological conditions revealed that BDNF protein can be measured in human plasma and serum. Although attempts at revealing BDNF protein levels in human HD blood have been performed (Ciammola et al. 2007) in our experience, the detection of BDNF in human blood samples remains extremely complex and variable and results can be easily affected by the experimental procedure (Marullo et al. 2010; Zuccato et al. 2011).

Also studies in rodents can be problematic and controversial. BDNF protein was detected in mouse and primate serum and found sensitive to pharmacological treatment with cystamine (Borrell-Pages et al. 2006). By contrast, earlier findings from Radka et al. (1996) further confirmed by Klein et al. (2011) indicated that BDNF protein is not detectable in either mouse serum or plasma with the most commonly used commercially available ELISA kit (Radka et al. 1996; Klein et al. 2011). However, BDNF mRNA can be monitored systematically by quantitative PCR in the blood of control and HD mice and correlates with disease progression (Conforti et al. 2008). Blood BDNF mRNA is also sensitive to pharmacological treatments as, for example, the acute and chronic treatment of R6/2 mice with CEP-1347, a mixed lineage kinase (MLK) inhibitor with neuroprotective and neurotrophic effects in mice, leads to increased total BDNF mRNA in blood and brain when compared to untreated R6/2 mice (Conforti et al. 2008; Apostol et al. 2008). BDNF mRNA levels in blood may represent a reliable tool to assess drug efficacy in pre-clinical trials in animals.

Table 1 Role of BDNF in HD: evidence from rodent models

Model	Observation	References
Emx1-BDNF knock-out mice	Complete inactivation of BDNF in wild-type mice forebrain leads to: – HD-like behavioral phenotype – Gene expression changes similar to the ones observed in the human HD caudate	Baquet et al. (2004), Strand et al. (2007)
BDNF$^{+/-}$ R6/1	Inactivation of one BDNF allele in HD mice leads to: – Earlier onset, worsening of the behavioural, motor phentype – Loss of striatal enkephalin-positive neurons	Canals et al. (2004)
CamKIIalpha BDNF Tg;R6/1 *CamKIIalpha BDNF Tg;YAC128*	Overexpression of BDNF in the brain of HD mice leads to: – Improvement of behavioral, motor phenotype – Improvement of neuropathology and BDNF-mediated signaling in HD mice	Gharami et al. (2008), Xie et al. (2010)

6 Experimental Manipulation of BDNF Levels and Its Impact on HD Progression

The data described above indicate a clear reduction in BDNF mRNA and protein levels in the cortex of subjects with HD, thus suggesting that the administration of BDNF may be a valid therapeutic option. In this section we present a number of studies involving genetically altered mice that have been performed to evaluating the effects of the modification of BDNF levels on disease onset and progression. These studies provided further support to the idea that cortical BDNF depletion and dysfunction are one of the critical factors in the pathology of HD and that BDNF administration could be beneficial to HD patients (Table 1).

6.1 Effect of BDNF Reduction

In a first set of experiments performed by the group of Kevin Jones at the University of Colorado, *empty spiracles homolog (Emx)*-BDNF knockout mice that are genetically engineered to be deficient in BDNF production in cortical neurons with little BDNF reduction in the thalamus, midbrain and hindbrain were produced. These mice gradually develop aspects of behavioural and anatomical abnormalities seen in mouse models of HD (Baquet et al. 2004). Cortical *Emx*-BDNF knockout mutants show significantly smaller striatal volumes due to reduced MSNs soma size, thinner dendrites and fewer dendritic spines than wild-type littermates. Similar results have been reported by Yves Barde group at the University of Basel that generated a new mouse line in which the BDNF gene has been globally inactivated in post-mitotic neurons of the CNS (Rauskolb et al. 2010). These data are in agreement with earlier studies demonstrating that BDNF stimulates the morphological differentiation of striatal neurons by increasing the length of their neurites, the number of branching

points on the neurites and the soma area (Ivkovic and Ehrlich 1999). Another study has confirmed reduced BDNF support as one major molecular pathway causing striatal dysfunctions in human HD (Strand et al. 2007). The aim of this specific work was to identify the animal model that best recapitulates the striatal gene expression profile of human HD. This study included the most widely used genetic models of HD, i.e. the R6/2 line, three mechanistically motivated HD models of mitochondrial dysfunction including 3-nitropropionic acid (3NP) treated rats, 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated mice and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-alpha) and *Emx*-BDNF knockout mice. Remarkably, the authors found the *Emx*-BDNF knockout mice exhibited striatal gene expression abnormalities that are more similar to human HD than the other profiles, including those of mouse genetic HD models (Strand et al. 2007).

In a second experimental paradigm to explore the relevance of BDNF depletion in HD pathogenesis, inactivation of one BDNF allele was achieved in a transgenic mouse line expressing human huntingtin exon 1 with an expanded CAG repeat (i.e. R6/1 mouse) (Canals et al. 2004). These mice were reported to show a worsening of the HD phenotype as shown by anticipated age of onset and exacerbated behavioural deficits (Canals et al. 2004) and more accentuated cognitive and learning impairment before symptoms onset (Giralt et al. 2009).

These observations indicate that BDNF depletion may contribute to HD aetiology. The obvious clinical implication is that augmenting BDNF levels or activating downstream signalling pathways may be of therapeutic benefit.

6.2 Effect of BDNF Augmentation

Studies aimed at testing a possible neuroprotective role of BDNF in HD started in the early 1990s, soon after the discovery of the BDNF as potent pro-survival and pro-differentiative factor for developing mature neurons.

The first experiments to assess the effect of BDNF augmentation in vivo in HD mice were performed in chemically induced models. Before the isolation of the disease gene in 1993, HD animal models were produced by injecting excitotoxins into the striatum (Zuccato et al. 2010). BDNF delivery by protein infusion, intrastriatal injection of BDNF-expressing adenovirus, or grafting of BDNF-expressing cells conferred protection to striatal neurons exposed to excitotoxins (Zuccato and Cattaneo 2007). These early findings have been recently corroborated by a study in which the BDNF gene was delivered to the striatal neurons by use of adenoviral vectors. The authors found that transfer of low concentration of BDNF gene to striatal neurons using serotype adeno-associated viral vector (AAV) increased the BDNF protein level in the striatum and conferred protection to striatal neurons against excitotoxic insult, thus attenuating motor impairment (Bemelmans et al. 1999; Kells et al. 2004, 2008).

The impact of BDNF delivery has been then evaluated in genetic models of HD, which better recapitulate the human pathology. A first experiment was performed in vitro on cultured cells transfected with mutant huntingtin and showed that BDNF

conferred protection against death of neurons caused by mutant huntingtin (Saudou et al. 1998).

Later, four independent studies tried to establish whether BDNF could be neuroprotective also in vivo in HD transgenic mice. In one study, BDNF was delivered via osmotic minipump into the striatum of mice overexpressing exon 1 of human mutant huntingtin (R6/1 mice). It was found that daily treatment of BDNF for 1 week succeeded in increasing the expression of enkephalin, as well as in augmenting the number of enkephalin-expressing striatal neurons, the most severely affected cells in HD (Canals et al. 2004). The same study showed a slight improvement of the behavioural phenotype after BDNF administration. A more recent study has shown that chronic and systemic delivery of recombinant BDNF is beneficial also to R6/2 mice (Giampà et al. 2013). It was found that BDNF-treated R6/2 mice survived longer and displayed less severe signs of neurological and neuropathological dysfunctions than the vehicle treated ones (Giampà et al. 2013). To better address the potential of BDNF increase in the brain of HD mice, in two separate studies led by Baoji Xu at Georgetown University School of Medicine, the neurotrophin was constitutively overexpressed in R6/1 mice (Gharami et al. 2008) and YAC128 mice (Xie et al. 2010) by means of the promoter of the α-subunit of Ca^{2+}/calmodulin-dependent kinase II (CaM Kinase II). Such overexpression in the striatum and cerebral cortex of R6/1 mice substantially ameliorated motor dysfunction, reversed brain weight loss, restored tyrosine receptor kinase (TrkB) signalling in the striatum and reduced the formation of mutant huntingtin aggregates in neurons (Gharami et al. 2008). Similarly, BDNF overexpression in YAC128 mice prevented loss and atrophy of striatal neurons and motor dysfunction. Decreased spine density and abnormal spine morphology in striatal neurons of YAC128 mice were also reversed by increasing BDNF levels in the striatum (Xie et al. 2010). Evidence of a neuroprotective role of BDNF in HD came also from a study by Girald and colleagues that produced R6/2 mice overexpressing BDNF in astrocytes (Giralt et al. 2011). In the R6/2:p-GFAP BDNF animals, the decrease in striatal BDNF observed in R6/2 mice was prevented and mice showed an improvement in several motor coodination task and in synaptic plasticity (Giralt et al. 2011).

In 2007 the group of Steven Goldman at University of Rochester Medical Center used a different approach to increase BDNF level in R6/2 mice. BDNF was delivered to striatum by means of adenoviral vectors in combination with Noggin, a molecule that promotes neurogenesis and regulates striatal neuronal regeneration. The authors observed delayed motor impairment in the BDNF/Noggin treated R6/2 transgenic mice (Cho et al. 2007). In particular, these mice exhibited a significant slowing in latency to fall and in rotarod impairment relative to untreated R6/2 mice. Moreover, the BDNF/Noggin-treated mice survived an average of 16.8 % longer than the respective controls (Cho et al. 2007). These results suggest that the neurotrophic action of BDNF in combination with molecules that stimulate neurogenesis might confer considerable therapeutic potential for mitigating both neuropathological and motor function deficits in the brain of patients with HD (Cho et al. 2007; Benraiss et al. 2013).

7 Strategies to Increase BDNF Level In Vivo

The findings described above have generated considerable excitement about the possibility of establishing a "*BDNF therapy*" for neurodegenerative diseases. When designing therapeutic strategies based on BDNF administration, one important consideration is the level of BDNF receptor expression in the neurodegenerating brain. A study by Jordi Alberch group at the University of Barcelona has described a marked reduction in the number and activity of TrkB receptors levels in the striatum in mouse models of HD (Gines et al. 2006). Subsequent studies by our group have shown that TrkB mRNA levels are reduced in caudate tissue but not in the cortex, whereas the mRNA levels of T-Shc (a truncated TrkB isoform) and p75 neurotrophin receptor ($p75^{NTR}$) are increased in the caudate. More recently, it was also found that huntingtin can regulate TrkB transport and that the transport of TrkB is reduced in HD neurons (Liot et al. 2013). This indicates that, in addition to the reduction in BDNF mRNA and transport, there is also unbalanced neurotrophic receptor trafficking and signalling in HD (Zuccato et al. 2008; Liot et al. 2013). Overall, it remains likely that residual TrkB molecules in individuals with HD are still capable of efficiently transducing BDNF-dependent cell signalling (Canals et al. 2004), thereby justifying the effort to develop strategies aimed at increasing BDNF levels in the brain.

The first clinical trial that explicitly investigated the role of BDNF in neurodegenerative diseases was performed in patients with amyotrophic lateral sclerosis (ALS) (Bradley et al. 1995; Ochs et al. 2000; The BSG 1999). Methionyl human BDNF was infused subcutaneously or intrathecally and was well tolerated but failed to demonstrate a statistically significant effect of BDNF on the survival of patients with ALS (Ochs et al. 2000; The BSG 1999). It is possible that the promising results seen in animal models of disease have not translated well into clinical trials owing to the poor pharmacokinetics associated with the intact protein. In particular, BDNF has a short in vivo half-life, has a low blood–brain barrier penetrability and undergoes only limited diffusion in the brain parenchyma. However, there is a serious drawback associated with this intrathecal administration of BDNF. A steep concentration gradient is generated, originating from the point of infusion, which could lead to alteration of the infused tissue and the development of adverse effects such as edema (Gill et al. 2003). Moreover, the intrathecal delivery systems of recombinant BDNF need to be refilled repeatedly over time. The aforementioned problems and the limited neuroprotective effects observed led to the cessation of trials with BDNF.

For these reasons other approaches to efficiently deliver optimum doses of BDNF to the brain have been considered. Non-invasive approaches such as nanoparticle-, Trojan horse- and nose-to brain-mediated delivery of BDNF into the brain are being explored. Trojan horse technology involves conjugating BDNF to molecules that can readily cross the blood-brain barrier. 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 trancytosis or to antibodies directed against these receptors

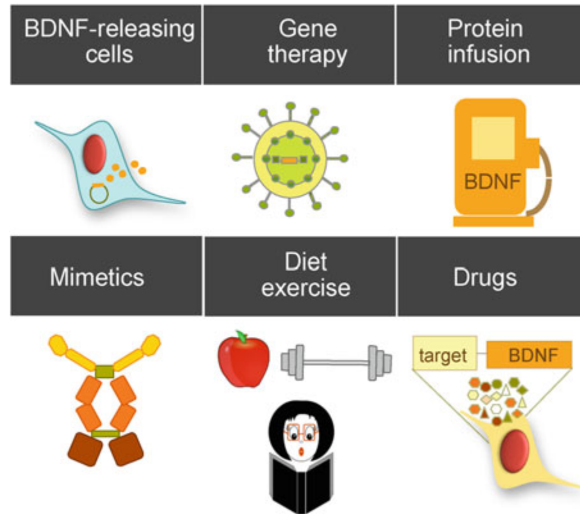

Fig. 4 Experimental therapeutic strategies for restoring BDNF function in HD

(Gabathuler 2010; Géral et al. 2013). Intranasal administration of BDNF protein is an alternative way to deliver BDNF into the CNS, and preliminary data in rodents indicate that the neurotrophin reaches the brain parenchyma (Alcala-Barraza et al. 2010; Jiang et al. 2011). The intranasal delivery method has great clinical potential due to simplicity of administration, noninvasive drug administration, relatively rapid CNS delivery, ability to repeat dosing easily, no requirement for drug modification and minimal systemic exposure. Additional approaches are represented by BDNF in vivo and ex vivo gene transfer while other strategies are aimed at stimulating the synthesis of endogenous BDNF (Zuccato and Cattaneo 2007, 2009). A number of drugs that enhance BDNF production in the brain are being studied, as well as the production of BDNF mimetics. Moreover, interesting new perspectives have arisen from the observation that physical exercise and diet markedly increase endogenous BDNF levels in the hippocampus and cerebral cortex (Zuccato and Cattaneo 2007, 2009). In this section we describe the current strategies that are under development to increase BDNF levels in the HD brain (Fig. 4).

7.1 Gene Therapy

Durable expression of BDNF or other neurotrophins such as glial cell line-derived neurotrophic factor (GDNF), from adenoviral, adeno-associated viral or lentiviral vectors, has been successfully tested and developed over the past decade in animal models of HD (Zuccato and Cattaneo 2007). Increasing BDNF levels through constant, local production following gene transfer has produced encouraging results in preclinical studies on mouse models of HD. Nevertheless, there are still a number of problems to be overcome if this approach is to be used in the clinic. The first

challenge is to regulate the amount of BDNF produced locally, as an excess of BDNF could have a deleterious effect on neuronal circuits, learning and memory (Croll et al. 1999). The second problem is that transduction is often associated with inflammation, which is usually accompanied by some vector toxicity, and together these effects prohibit long-term therapy on safety grounds. Another major problem is the risk of accidental insertional mutagenesis by viral vectors and subsequent tumour formation (Hacein-Bey-Abina et al. 2008). To overcome these problems, a large effort is currently underway to produce new viral vectors, which lack both pathogenicity and immunogenicity (Biffi and Naldini 2005). Methods utilising integration-deficient lentiviral vectors and nontoxic viral systems have been successfully used in other pathologies and are under scrutiny (Biffi et al. 2013; Aiuti et al. 2013; Yanez-Munoz et al. 2006). These approaches would allow the transduction of BDNF in a cell-specific and inducible manner.

7.2 Grafting of BDNF-Releasing Cells

To avoid concerns about the direct injection of a virus into the brain parenchyma, another possible strategy to increase BDNF levels in the brain is to graft cells engineered to stably express BDNF. In a first attempt, immortalised rat fibroblasts genetically engineered to secrete BDNF were implanted in the rat striatum 7 days before the striatal infusion of excitotoxic quantities of an NMDA-receptor agonist that causes widespread neuropathological deficits similar to those seen in the HD brain. Analysis of striatal damage 7 days after the lesion revealed that BDNF-secreting fibroblasts offered no protection (Frim et al. 1993). A later study showed that BDNF had only limited ability to protect the striatum from damage due to an excitotoxic lesion by transplanting putative neural stem cells that had been genetically modified to overexpress BDNF, which were injected in the same area 1 week later. One month after the lesion, striatal degeneration, lesion size and the loss of striatal dopamine- and cyclic AMP-regulated phosphoprotein 32 kDa (DARPP-32) positive neurons were only slightly improved by the BDNF-secreting cells (Martinez-Serrano and Bjorklund 1996). Subsequent attempts have been more successful probably because lower and safer BDNF doses have been released (Perez-Navarro et al. 1999, 2000; Ryu et al. 2004), including a particularly interesting study by Ryu et al. (2004). The authors investigated the ability of transplanted BDNF-overexpressing *bona fide* neural stem cells taken from human foetal brain to protect animals after 3NP administration, which causes striatal cells death similar to those seen in HD. The animals receiving the intrastriatal cell implantation 1 week before 3NP treatment showed significantly improved motor performance and less striatal neuron damage, whereas those transplanted 12 h after 3NP treatment did not show any improvement in motor performance or any protection of striatal neurons from the toxicity induced by 3NP (Ryu et al. 2004). More recently, mice grafted with primary astrocytes overexpressing BDNF have showed important and sustained behavioural improvements over time after quinolinate administration as compared with wild-type mice grafted with wild-

type astrocytes (Giralt et al. 2010). These findings suggested that astrocytes engineered to release BDNF can constitute a therapeutic approach for HD.

Since grafting of BDNF-releasing cells may still have some problems—xenogenic cells are at risk of being rejected and immortalised cells can cause tumour growth—researchers envisage encapsulating cells with new materials under development. These materials would serve as biological shields, preventing immune rejection and eliminating the need for immunosuppression (Emerich et al. 1997). There is also considerable interest in the development of stable, nontumorigenic human neural stem cell lines as well as mesenchymal stem cells that release BDNF (Conti and Cattaneo 2010; Hess and Borlongan 2008; Joyce et al. 2010; Rossi and Cattaneo 2002; Somoza et al. 2010; Olson et al. 2012).

7.3 BDNF Mimetics

As many of the issues surrounding BDNF efficacy and safety result from the need to deliver the neurotrophin close to the target site, investigators have considered the interesting possibility of using peptidomimetics, agonist antibodies and small molecules directed specifically to the BDNF receptors. These BDNF mimetics have been designed in accordance with the three-dimensional structure of BDNF, in particular, loops 1, 2 and 4, which are required for binding of BDNF to TrkB receptors. The synthetic molecules are also modified in such a way as to penetrate the blood–brain barrier more efficiently than BDNF (Longo et al. 2007; Massa et al. 2010; Pardridge 2006). Recently, Frank Longo of Stanford University and colleagues from the University of California at San Francisco screened over one million compounds and discovered four chemically distinct compounds which mimic BDNF being able to bind and activate selectively TrkB, but not the other Trks and not $p75^{NTR}$. One of the compounds was selected for further study, and it was used to treat various cell models of neurodegenerative disease, including HD, with promising results on cell survival (Massa et al. 2010; Simmons et al. 2013). BDNF mimetics applied locally or systemically may be a promising strategy to increase BDNF-mediated signalling in HD and, as a consequence, to induce neuroprotection effects, because it avoids the adverse effects associated with invasive methods of delivery or uncontrolled dosing, while improving upon the diffusion properties of BDNF.

7.4 Drug Increasing BDNF Levels and Their Effectiveness in HD

Current experiments are aimed at isolating compounds that increase endogenous BDNF level. Such a strategy would circumvent the problems related to invasive methods of BDNF delivery in humans, including achieving the correct dosage and maintaining stability of the neurotrophin. Several classes of compounds are able to increase BDNF levels in the brain of HD mice, leading to improvements of the neuropathology as well as of cognitive and behavioural deficits. Among them,

considerable attention has been received by selective serotonin reuptake inhibitors (SSRIs) and lithium. Furthermore, memantine and riluzole (a non-competitive inhibitor of ionotropic glutamate NMDA receptor), cystamine and cysteamine, ampakine (a positive modulator of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors), nicotinamide (an inhibitor of sirtuin 1/class III NAD (+)-dependent histone deacetylase) and the calcineurin inhibitor FK506 have been found to significantly restore BDNF levels in HD mice. We describe below a selection of these studies.

7.4.1 SSRIs

SSRIs facilitate the signalling of serotonin by inhibiting its reuptake. SSRIs may have protective effects on striatal and cortical neurons by activating cyclic AMP and CREB signals, which also lead to BDNF expression (Mostert et al. 2008; Tardito et al. 2006). A first attempt to test the effect of SSRIs on mouse models of HD involved the administration of paroxetine (5 mg/kg/day) to N171-82Q mice, which was found to delay the onset of behavioural symptoms and increase lifespan (Duan et al. 2004). Significant impairment of the behavioural phenotype was observed specifically at the level of motor function (Duan et al. 2004), and the weight loss previously reported in this model occurred significantly more slowly than in vehicle-treated HD mice. Histological analyses also revealed a decrease in brain atrophy. N171-82Q are normally hyperglycemic but paroxetine treatment reduced blood glucose levels, thus providing evidence that, in addition to neurodegenerative processes, it improves glucose metabolism in HD. Paroxetine also increased survival even when administered after the onset of motor dysfunction (Duan et al. 2004), thus suggesting the possibility that HD patients may benefit from SSRIs after they become symptomatic.

In 2008, Duan and colleagues confirmed the beneficial effects of SSRIs by demonstrating that sertraline prolongs survival, improves motor performance and ameliorates brain atrophy in two mouse models of HD represented by the R6/2 and N171-82Q (Duan et al. 2008; Peng et al. 2008). These beneficial effects of sertraline were associated with enhanced neurogenesis and increased BDNF levels in the brain (Duan et al. 2008; Peng et al. 2008).

These findings open the way to studies of the effects of paroxetine and sertraline in human HD patients, but previous studies have found no clinical benefit with the use of other SSRIs. There is a single case report of fluoxetine exacerbating chorea (Chari et al. 2003), and although another study found it a useful antidepressant, it failed to provide any substantial clinical benefit to non-depressed HD patients (Como et al. 1997). On the contrary successful treatment with sertraline in depressed HD patients has been reported. Moreover, sertraline is safe and well tolerated for long-term administration, including in HD patients (Ranen et al. 1996). This suggests that a clinical trial of SSRI treatment in order to retard disease progression in human HD may be warranted.

7.4.2 Lithium

Lithium induces the expression of BDNF and the subsequent activation of TrkB in cortical neurons (Fukumoto et al. 2001). Early studies by Wei et al. indicated that a subcutaneous lithium chloride (LiCl) injection for 16 days before quinolinic acid infusion considerably reduces the size of quinolinic acid-induced striatal lesions (Wei et al. 2001). It was later found that it can protect against polyglutamine toxicity in cell lines by inhibiting glycogen synthase kinase 3-beta (GSK-3beta), which is involved in apoptotic cell death, and increasing beta-catenin whose overexpression protects cells from mutant huntingtin-induced toxicity (Carmichael et al. 2002).

One year later, on the basis of lithium's reported neuroprotective and anti-depressive properties, other studies determined whether chronic LiCl treatment affects the progression of the phenotype in R6/2 mice, but found that it had variable effects on motor behaviour and did not improve survival (Wood and Morton 2003). A study by Senatorov et al. has suggested that lithium may be neuroprotective in the quinolinic acid-injection model of HD because of its ability to inhibit apoptosis and induce neuronal and astroglial progenitor proliferation or migration from the subventricular zone (SVZ) (Senatorov et al. 2004).

In 2008, David Rubinsztein at Cambridge University in the UK has shown that lithium enhances mammalian target of rapamycin (mTOR)-dependent and -independent autophagic processes in HD flies when administered in combination with mTOR inhibitor rapamycin, leading to protection against neurodegeneration (Sarkar et al. 2008). More recently, it has been reported that lithium induced brain and blood BDNF expression, improved striatal neuropathology, and behavioral abnormalities in YAC128 and N171-82Q mice (Chiu et al. 2011; Pouladi et al. 2012). Together with its favorable safety profile and pharmacokinetic properties, these findings support further development of lithium as a therapeutic agent in HD.

7.4.3 Memantine and Riluzole

Memantine is a medium-affinity non-competitive NMDA receptor antagonist that has been clinically used as a neuroprotective agent to treat Alzheimer's disease and Parkinson's disease. At clinically relevant doses, it markedly increases BDNF and TrkB mRNA levels in rat brain, and its effects on BDNF mRNA were reflected in changes in BDNF protein levels (Marvanova et al. 2001). Remarkably, two different studies demonstrated that memantine ameliorates neuropathological and behavioral phenotypes in HD mice (Okamoto et al. 2009, Milnerwood et al. 2010). These studies also suggest that the neuroprotective role of memantine depends on its ability to promote the CREB pathway which controls BDNF gene transcription (Okamoto et al. 2009; Milnerwood et al. 2010). Like memantine, riluzole (a neuroprotective drug commonly used in ALS) acts by blocking glutamatergic neurotransmission in the CNS. Interestingly, it has also been found to upregulate the levels of a number of key neurotrophic factors, including BDNF and GDNF (Katoh-Semba et al. 2002; Mizuta et al. 2001). These data suggest that the anti-excitotoxic activity of memantine and riluzole is accompanied by an increase in the endogenous BDNF production in the brain. On these bases a 2-year, multicentre

open-label study of 27 HD patients was carried out in order to investigate the effectiveness of memantine (up to 30 mg/day) in delaying disease progression. The results suggest that memantine treatment may be useful in doing so (Beister et al. 2004). Another open-label trial has found that riluzole causes transient motor improvement in human HD patients (Seppi et al. 2001; HSG 2003). These promising results have led to a 3-year, randomised controlled study conducted by the European Huntington's Disease Initiative Study Group led by Bernard Landwehrmeyer on 379 HD patients. The study, concluded in 2007, showed that, although riluzole was well tolerated, no neuroprotective or beneficial symptomatic effects were demonstrated (Landwehrmeyer et al. 2007). On the contrary, a study performed on a small number of HD patients ($n = 11$) has shown that riluzole protects HD patients from brain glucose hypometabolism and grey matter volume loss and increases production of BDNF (Squitieri et al. 2009).

7.4.4 Cystamine and Cysteamine

Transglutaminases (TGases) play a critical role in the pathogenesis of HD because they cross-link huntingtin and catalyse the formation of aggregates. As TGases activity is increased in HD brain, they represent an attractive target for possible therapeutic intervention in HD (Gentile and Cooper 2004; Hoffner and Djian 2005). Early findings indicated that cystamine, a competitive inhibitor of TGases activity, limits the aggregation of proteins with an expanded polyglutamine tract (de Cristofaro et al. 1999; Igarashi et al. 1998) and has also been shown to decrease apoptosis in cultured cells exposed to glutamate or an N-terminal fragment of mutant huntingtin (Ientile et al. 2003; Zainelli et al. 2005). Cystamine protects against 3NP striatal lesions in mice (Fox et al. 2004) and, more importantly, improved behaviour and survival in two independent therapeutic trials in R6/2 mice (Dedeoglu et al. 2002; Karpuj et al. 2002). Other findings indicated that cystamine reduces striatal volume loss and neuronal atrophy in YAC128 mice, but does not reverse progressive motor dysfunction or the downregulation of the striatal marker DARPP-32, whose expression is significantly reduced in this model (Van Raamsdonk et al. 2005). Recent evidence suggests that the improved survival and motor function in cystamine-treated R6/2 mice may not be solely due to TGase inhibition because R6/2 mice not expressing tissue transglutaminase also benefit from cystamine administration (Bailey and Johnson 2005). Other beneficial effects of cystamine include the inhibition of caspase-3 activity, increased cell levels of the anti-oxidant glutathione and cysteine (Fox et al. 2004; Lesort et al. 2003) and an increase in the expression of heat-shock proteins (Karpuj et al. 2002).

In 2006 cystamine and cysteamine (the Food and Drug Administration (FDA)-approved reduced form of cystamine) were linked to BDNF secretion, thus opening up the possibility that the neuroprotection observed in treated animals may be due to a cystamine-mediated increase in BDNF secretion (Borrell-Pages et al. 2006). In their study, Borrell-Pages et al. found that cystamine increases the levels of HSJ1B, which are low in HD patients. HSJ1B stimulates the BDNF secretory pathway through the formation of clathrin-coated vesicles containing BDNF. Therefore, the authors suggested that cystamine is neuroprotective because it increases BDNF

secretion from the Golgi. Cystamine and cysteamine are both neuroprotective in HD mice (Borrell-Pages et al. 2006). Tolerated cysteamine doses have been evaluated in HD patients, thus strengthening the case in favour of using cystamine and cysteamine as a therapeutic approach to HD. Although cysteamine can cross the blood–brain barrier, it takes larger doses to detect a variation in cysteamine or its metabolites in the brain (Bousquet et al. 2010). In 2011, Raptor Pharmaceutical Corporation initiated a collaboration with the Centre Hospitalier Universitaire (CHU) d'Angers in France to support a phase II clinical study of a delayed release preparation of cysteamine bitartrate in HD patients. Clinical researchers at the CHU d'Angers, in collaboration with the Curie Institute, have designed a 96 HD patients trial to investigate the efficacy of this new cysteamine delivery, using BDNF as a marker of efficacy (Gibrat and Cicchetti 2011). The trial has been recently concluded and results are pending.

7.4.5 FK506

BDNF vesicle transport depends on S421 phosphorylation and constitutive phosphorylation of mutant huntingtin restores impaired BDNF vesicle transport in HD (Colin et al. 2008; Zala et al. 2008). Pineda and colleagues found that pharmacological inhibition of calcineurin, the *bona fide* huntingtin S421 phosphatase, restored the BDNF transport defects observed in HD (Pineda et al. 2009). Particularly, FK506, an FDA-approved drug capable of crossing the blood–brain barrier, restored BDNF transport in two complementary models: rat primary neuronal cultures expressing mutant huntingtin and mouse cortical neurons from mutant huntingtin knockin mice ($Hdh^{Q111/Q111}$). This effect was the result of specific calcineurin inhibition, as calcineurin silencing restored both anterograde and retrograde transport in neurons from $Hdh^{Q111/Q111}$ mice (Pineda et al. 2009). These results indicate that drugs as FK506, which target a specific mechanism responsible for altered BDNF transport, may be of interest in HD.

7.4.6 Ampakine and Nicotinamide

Ampakine is a positive modulator of AMPA-type glutamate receptors. In 2009, Gary Lynch's group at the University of California Irvine showed that ampakine upregulates endogenous hippocampal BDNF levels, rescues neuronal plasticity and reduces learning problems in mutant huntingtin knockin mice (Simmons et al. 2009). A study from the same group published 2 years later has confirmed these data and showed that long-term ampakine treatment markedly slows the progression of striatal neuropathology and locomotor dysfunction in an additional mouse model of HD represented by the R6/2 transgenic line by increasing BDNF protein levels in the neocortex (Simmons et al. 2011). Ampakines are well tolerated in clinical trials and have shown efficacy in this study after brief exposures, suggesting that they may be useful for chronic treatment of the cognitive difficulties in the early stages of HD.

Nicotinamide is an inhibitor of sirtuin 1/class III NAD (+)-dependent histone deacetylase. The group of Anne Messer at the Albany Medical College has examined the effects of nicotinamide after administration to R6/1 mice. BDNF levels

were found to be significantly increased in the brain of R6/1 mice, and motor deficits associated to HD phenotype were significantly improved (Hathorn et al. 2011).

7.4.7 Towards the Identification of RE1/NRSE Modulators

Since some of the mechanisms of reduced BDNF gene transcription and protein transport have been elucidated, a valid option could be to increase BDNF levels by targeting specific mechanisms that are responsible for the BDNF dysfunction. Strategies that act specifically on a defined molecular dysfunction could be more effective than drugs that increase BDNF levels but do not specifically target a disease mechanism.

We have previously shown that the REST/NRSF–RE1/NRSE transcriptional system, important regulator of BDNF gene transcription, is impaired in HD, thus contributing to reduced BDNF levels in the disease as well as to reduced transcription of other REST/NRSF-regulated genes (Zuccato et al. 2003, 2007; Johnson and Buckley 2009; Hodges et al. 2006). These data opened to the development of therapeutic strategies that target the REST/NRSF–RE1/NRSE silencer complex. In vitro evidences suggested that this could be a feasible strategy. Overexpression of a dominant negative protein of REST/NRSF lacking any co-repressor domain resulted in attenuation of REST/NRSF binding at its target sites and restoration of the expression level of several target genes (Zuccato et al. 2007). A new study from Noel Buckley at King's College London has further demonstrated this concept. By delivering oligonucleotide decoys targeting REST/NRSF, REST/NRSF occupancy at several RE1/NRSE loci was reduced in mutant huntingtin knockin cells, thus restoring transcription of BDNF and other neuronal genes (Soldati et al. 2011). Compounds that specifically interfere with the REST/NRSF pathway in HD may represent a valid therapeutic approach to increase the transcription of REST/NRSF-regulated genes (Rigamonti et al. 2007; Leone et al. 2008). To this purpose Cell-based reporter assays to monitor RE1/NRSE activity in cultured brain cells with the final aim to identify compounds that specifically upregulate BDNF in HD have been developed (Rigamonti et al. 2007; Charbord et al. 2013; Conforti et al. 2013). Compounds identified in high-throughput screening as blockers of the RE1/NRSE silencing activity alleviate the REST/NRSF-dependent repression and, hence, ameliorate the global transcriptional repression in the disease (Conforti et al. 2013; Charbord et al. 2013). Other human pathologies exhibit abnormal REST activity, highlighting the importance of REST/NRSF-mediated regulation to the integrity of the cell. Abnormalities in REST/NRSF transcriptional activity have been demonstrated also in cardiac hypertrophy, ischaemia and Down syndrome (Rigamonti et al. 2009). Future therapeutics pointing at targeting REST/NRSF or the RE1/NRSE site might consequently be applied to an extended set of pathologies in addition to HD.

7.5 Other Interesting Perspectives for Increasing Endogenous BDNF

Physical exercise and diet cause a marked increase in BDNF levels in rat brain, particularly the hippocampus and cerebral cortex. Early studies showed that dietary restriction (DR) and physical exercise can have profound effects on brain functions and vulnerability to injury and disease (Spires et al. 2004; Mattson et al. 2003; Zoladz and Pilc 2010).

7.5.1 Diet and Environmental Enrichment

DR promotes neuronal survival by enhancing resistance against cell stress (Guo et al. 2000; Yu and Mattson 1999), reducing oxidative damage (Dubey et al. 1996), stimulating the production of new neurons (neurogenesis) and improving synaptic plasticity (Mattson et al. 2003). Data in mouse models of neurodegenerative diseases indicate that DR can protect neurons against neurodegeneration, suggesting that dietary changes may reduce disease severity (Mattson et al. 2003). When rats were kept on a periodic fasting/dietary restriction regimen for several months before the administration of 3NP acid to induce a striatal lesion, their motor function improved and more striatal neurons survived (Bruce-Keller et al. 1999). In DR condition BDNF levels increase in several brain regions (Duan et al. 2001a; Lee et al. 2002). The fact that beneficial effect of DR are mediated by BDNF came from studies showing that the infusion of a BDNF blocking antibody into the lateral ventricle of rats and mice significantly attenuated the neuroprotective effect of DR in the kainate model of seizure-induced hippocampal damage (Duan et al. 2001a, b). Other findings indicate that DR increases BDNF protein level in the cerebral cortex and striatum of HD mice (the N171-82Q line), which results in delayed disease onset and increased survival (Duan et al. 2003). DR reduces brain atrophy and the formation of huntingtin aggregates and diminishes caspase activation in N171-82Q mice, thus apparently blocking the toxic effects elicited by mutant huntingtin (Duan et al. 2003). DR may therefore be considered a potential early strategy (before the development of symptoms) for counteracting HD phenotypes and restoring normal brain BDNF levels.

Environmental enrichment also markedly delays the onset and progression of HD in transgenic mice. It involves providing the mice with environments containing regularly changed, complex and stimulating objects. The impact of such a strategy was reported for the first time in 2000, when it was shown that R6/1 mice exposed to environmental enrichment experienced a delayed disease onset and slower rate of disease progression, and had improved behavioural performances on motor tests (van Dellen et al. 2000). Further studies have indicated that environmental enrichment also slows disease progression in the more severe R6/2 mouse model of HD (Hockly et al. 2002), as well as in N171-82Q transgenic HD mice (Schilling et al. 2004). Environmental stimulation delays the onset of cognitive deficits (van Dellen et al. 2005), and its beneficial effects have also been demonstrated by studies of HD patients (Sullivan et al. 2001). The mechanisms by which these beneficial effects are mediated are still unclear, but there are a number

of plausible possibilities. Several studies indicated that environmental enrichment upregulates neurotrophins such as BDNF and nerve growth factor (NGF) in the hippocampus and cortex (Falkenberg et al. 1992; Keyvani et al. 2004; Pham et al. 1999a, b; Young et al. 1999). There is evidence that environmental enrichment or physical exercise upregulates the transcription of genes encoding neuronal proteins that are important for neuronal plasticity, learning and memory (Rampon and Tsien 2000). Enrichment is associated with increased synaptic signalling and the stimulation of second messenger systems; it also has an effect on neuronal morphology, as it is associated with increased spine density. The stimulatory role of enrichment and BDNF on neurogenesis (Bath et al. 2012) suggests that this may be an additional avenue for the therapeutic effects of environmental stimulation. Studies of R6/1 transgenic mice have shown that environmental enrichment rescues striatal and hippocampal BDNF protein deficits, leading to improvement of the disease phenotype (Spires et al. 2004; Pang et al. 2006). These observations suggest that the beneficial effect of enrichment may be partially mediated by increased BDNF levels.

Conclusions

BDNF seems to be necessary for the phenotypic maintenance and activity of mature, fully developed neurons, so it has been suggested that changes in its level or distribution could be important in the pathogenesis of neurodegenerative conditions in humans. The best example is given by HD. BDNF is crucial for cortical and striatal neurons, the most affected neuronal populations in the HD brain. The evidence described in this chapter points to BDNF deficit as one major contributor to HD pathogenesis.

Findings of the last decade indicated that the normal huntingtin protein, whose mutation causes HD, is involved in the physiological control of BDNF synthesis and transport in the brain. Wild-type huntingtin sustains cortical BDNF gene transcription and drives BDNF vesicles sorting in neuronal cells. Multiple experiments in HD cells and animal models indicated that BDNF production and possibly also its transport are impaired in the disease since early stages. Moreover, BDNF levels are reduced in the brain of HD patients and this is due to decreased normal huntingtin activity, but also to the toxicity of mutant huntingtin. The "*BDNF defect*" in HD has been documented by roughly 20 laboratories and corroborated by the elucidation of the underlying molecular mechanisms. BDNF measures are currently used as read-outs, both to test the quality of new cellular or animal models of HD as well as the efficacy of new compounds in pre-clinical studies.

Several groups are working to establish a "*BDNF therapy*" for the treatment of HD, but numerous methodological and safety issues will need to be addressed in patients before this approach can be widely adopted. In our opinion, one promising strategy is the use of BDNF mimetics directed to the BDNF receptors or small molecules that increase endogenous BDNF levels by acting on well-characterised molecular targets generated by the knowledge of the mechanisms underlying BDNF transcription and transport. We also believe that an important

problem to solve is the reliable and robust measurement of BDNF protein and mRNA levels in human material. It is true indeed that studies on postmortem samples, although quantitatively and systematically performed, may not mimic what happens in vivo. Moreover, in humans, BDNF synthesis is subjected to a wide range of influences (dietary restriction, physical exercise, circadian rhythms, stress) affecting the level of BDNF. The imprecise evaluation of the BDNF level in the diseased brain may lead to the administration of uncorrected doses of BDNF that could be inefficacious as well as deleterious. A better understanding of the timing of BDNF loss in patients and the precise measurement of its levels are crucial before proposing BDNF treatment as a beneficial and feasible therapeutic approach in the clinic.

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