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Huntington's disease: novel therapeutic perspectives hanging in the balance

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Abstract

Introduction: Huntington's disease (HD), an autosomal dominant neurodegenerative disorder caused by an expansion of CAG repeats in the huntingtin gene, has long been characterized by the presence of motor symptoms due to the loss of striatal projection neurons. Cognitive dysfunction and neuropsychiatric symptoms are also present and they occur in the absence of cell death in most mouse models, pointing to neuronal dysfunction and abnormal synaptic plasticity as causative mechanisms.

Areas covered: Here, we focus on those common mechanisms altered by the presence of mutant huntingtin affecting corticostriatal and hippocampal function as therapeutic targets that could prove beneficial to ameliorate both cognitive and motor function in HD. Specifically, we discuss the importance of reestablishing the balance in (1) synaptic/extrasynaptic *N*-methyl-D-aspartate receptor

signaling, (2) mitochondrial dynamics/trafficking; (3) TrkB/p75^{NTR} signaling and (4) transcriptional activity.

Expert opinion: Mutant huntingtin has a broad impact on multiple cellular processes, which makes it very challenging to design a curative therapeutic strategy. As we point out here, novel therapeutic interventions should look for multi-purpose drugs targeting common and early affected processes leading to corticostriatal and hippocampal dysfunction that additionally operate in a feedforward vicious cycle downstream the activation of extrasynaptic *N*-methyl-D-aspartate receptor.

Keywords: BDNF, CREB, extrasynaptic NMDAR, HDAC, mitochondria, TrkB/p75^{NTR} balance

Abbreviations: BDNF, brain-derived neurotrophic factor; CBP, CREB binding protein; CREB, cAMP response element-binding protein; Drp-1, dynamin related protein-1; ERK1/2, extracellular signal-regulated kinase 1/2; HAT, histone acetyltransferase; HDAC, histone deacetylase; Htt, huntingtin; HD, Huntington's disease; LTD, long-term depression; LTP, long-term potentiation; mHtt, mutant huntingtin; MSNs, medium-sized spiny neurons; NMDAR, *N*-methyl-D-aspartate receptor; PDE, phosphodiesterase; PGC-1 α , peroxisome proliferator-activated receptor γ coactivator 1 α ; STEP, STriatal-Enriched protein tyrosine Phosphatase.

1. Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an expansion of CAG repeats in the huntingtin gene [1]. The mutant huntingtin protein (mHtt) has a polyglutamine expansion in the N-terminal region that causes its misfolding and aggregation. The clinical course of the disease includes motor symptoms that progress inexorably towards serious disability and death. Currently, there is no effective therapy for HD, and treatment of HD patients is addressed at its symptoms [2]. The classical pathological hallmark of HD is the preferential degeneration and death of GABAergic medium sized spiny neurons (MSNs) in the putamen and caudate nucleus (globally termed striatum). The enkephalin expressing neurons that project from the striatum to the external *globus pallidum* and are part of the indirect pathway are the ones that degenerate first. This neuropathology correlates directly with the conspicuous motor symptoms in HD patients, and many studies have been focused on discovering the pathophysiology of striatal neurodegenerative mechanisms in an attempt to spare the dramatic striatal cell death in HD. Lately, it has been recognized that neuronal dysfunction, cognitive impairment and neuropsychiatric symptoms precede neuronal death in HD patients, and they occur long before, or in the absence, of cell death in HD mouse models. This indicates that the early disease processes, rather than cell loss, involve cellular dysfunction and abnormal synaptic plasticity that are responsible for cognitive decline and motor deficits [3], and offers an opportunity for therapeutic interventions. Strategies aimed at preventing or ameliorating cellular dysfunction are expected to delay the onset or block the progression of HD better than those targeting cell death alone, which is a late event and occurs once neuronal function is already severely compromised.

mHtt alters numerous cellular and molecular mechanisms including protein trafficking and aggregation, protein-protein interaction, calcium signaling, mitochondrial function, gene transcription, neurotransmitter release and receptor activity, and neurotrophic support [4, 5]. Potentially, all these alterations can impact on neuronal functioning, synaptic plasticity, cognitive and motor function and, ultimately, on cell survival. Therefore, a variety of potential targets associated with the aforementioned cellular processes can be considered for therapeutic intervention. On the other hand, despite occurring early in the disease and being functionally disabling for HD patients [6], cognitive impairment remains to be successfully addressed as there are no effective drugs to improve cognitive function in HD patients (reviewed in [2]). However, studies in mice are increasingly paying attention to cognitive dysfunction in HD, and searching for the molecular mechanisms underlying synaptic plasticity deficits and potential therapeutic options to prevent or delay them (reviewed in [7–9]).

The discovery of the HD causative mutation in 1993 produced a vast body of knowledge into HD pathophysiology and raised hopes for an eventual cure. It is indeed straightforward to first pursue therapies targeting mHtt transcription and translation [10], and in fact two clinical trials are currently in phase I/II using antisense oligonucleotides (clinicaltrials.gov: NCT02519036; NCT03225846; NCT03225833). Nevertheless, the continuous research of new potential targets to address neurodegeneration, especially in early stages, independently of correcting mHtt levels, is warranted for several reasons. First, despite recent advances, the practical considerations of the timeline, cost, feasibility and safety still may pose obstacles for human genetic therapy. Second, it is unknown if the simultaneous lowering of Htt levels has deleterious effects, or if the complete elimination of mHtt can stop the pathological processes already ongoing in the body of HD patients. Hence, targets that have promise in ameliorating neurodegeneration might have clinical value even after the causative mutation has been corrected. Lastly, as evidenced in several studies, many pathological mechanisms such as increased extrasynaptic N-methyl-D-aspartate receptor (NMDAR)

signaling, mitochondrial dysfunction or reduced brain-derived neurotrophic factor (BDNF) levels are common to many diseases [11, 12], extending the clinical interest of HD-centered studies into diseases with higher incidence such as Alzheimer's (AD) and Parkinson's (PD) diseases. Supporting the latter, cognitive deficits in HD are becoming prominent because of their early onset and the burden they represent for HD patients. This links HD pathology more closely to AD and PD, and opens the possibility of finding common therapies for these neurodegenerative diseases. Thus, we have centered this review in therapeutic targets more readily available for modulation through pharmacological interventions, and aimed at broadening the classically striatal-centered conceptualization of HD. Here, we will focus on those therapeutic strategies that could prove beneficial to ameliorate both cognitive and motor function in HD, including targeting the imbalances of synaptic/extrasynaptic NMDAR signaling, mitochondrial fusion/fission dynamics, TrkB/p75^{NTR} signaling and transcriptional dysregulation (Figure 1). Many of these operate in a feedforward deleterious loop (Figure 1; see section 2 for details) and can be implicated in cognitive, motor and even psychiatric HD symptoms [13–17]. Thus, their simultaneous therapeutic targeting using pleiotropic drugs, or drug combinations, should help to stop or at least slow down neuronal dysfunction to provide symptoms' relieve.

2. Targeting NMDARs

NMDARs play central roles in CNS function as mediators of brain plasticity by converting different patterns of neuronal activity into long-term changes in the structure and function of synapses, which are believed to underlie higher cognitive functions. They are also important players in cell survival/death signaling [12, 18–20].

With some exceptions, the localization hypothesis of NMDAR function has been supported by several studies (reviewed in [12]; see also [21]). It proposes that synaptic NMDAR stimulation

promotes extracellular signal-regulated kinase 1/2 (ERK1/2) activation, cAMP response element-binding protein (CREB) phosphorylation and BDNF expression, enhances antioxidant defense, and provides neuroprotection. Conversely, extrasynaptic NMDAR stimulation does the opposite thereby promoting cell death signaling [20, 22]. Moreover, nuclear calcium, an important regulator of gene expression that plays a key role in the prosurvival effects of synaptic stimulation, is disrupted by extrasynaptic NMDAR activity (reviewed in [23]).

In human HD brains there is evidence of impaired glutamate uptake [24, 25], and initial data from different HD mouse models (e.g. [26–28]) also supported this notion. This was challenged by recent evidence showing, by quantification of real-time glutamate dynamics, that glutamate clearance in HD striatum is normal or even accelerated [29]. However, NMDAR hyper-function can be detected in HD MSNs at early stages [13, 30], well before synapse and spine loss, behavioral deficits and neuronal death, pointing to signaling through these receptors as a key player in the pathogenesis of the disease [3, 31]. Several mechanisms and pathways are related to enhanced NMDAR signaling in HD and they have been recently reviewed [12]. Importantly, redistribution of NMDAR from synaptic to extrasynaptic locations is well documented in HD, with extrasynaptic NMDAR signaling playing an important role in HD pathogenesis [13, 32]. Namely, it has been demonstrated that stimulation of extrasynaptic NMDARs increases the vulnerability of mHtt-containing neurons to cell death by impairing the neuroprotective CREB-peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α) cascade, and increasing the level of the small guanine nucleotide-binding protein Rhes [32], a striatal enriched protein that participates in HD pathogenesis by inducing sumoylation and disaggregation of mHtt [33]. Moreover, increased extrasynaptic NMDAR expression and current were reported in the striatum of YAC128 mice, accompanied by reductions in CREB activation [13]. The mechanisms underlying NMDAR mislocalization in HD likely involve alterations in posttranslational modifications including phosphorylation, palmitoylation, and proteolytic cleavage [34].

NMDAR trafficking at the plasma membrane is regulated by dephosphorylation of the NMDAR subunit GluN2B at tyrosine 1472 (Tyr1472) by Striatal-Enriched protein tyrosine Phosphatase (STEP) [35]. Interestingly, activation of synaptic NMDARs promotes STEP degradation through the ubiquitin-proteasome system and parallel ERK1/2 activation, whereas extrasynaptic NMDARs promote STEP cleavage and increased p38 cell death signaling [36]. Remarkably, synaptic STEP activity is significantly higher in the YAC128 striatum, correlating with decreased GluN2B^{Tyr1472} phosphorylation, which reduces synaptic NMDAR retention and facilitates movement to extrasynaptic sites. A substrate trapping STEP protein (TAT-STEP C-S) significantly increased VGLUT1-GluN2B colocalization, synaptic GluN2B expression and phosphorylation at Tyr1472 [37]. Therefore, targeting STEP at early disease stages may prove to be beneficial to promote synaptic NMDAR signaling in HD striatum and prevent loss of corticostriatal connectivity. In this line, it has been demonstrated that STEP negatively regulates BDNF expression [38].

A specific role for NMDAR subtypes containing the GluN3A subunit in NMDAR hyperfunction in HD was recently identified. It was found that mHtt binds to and sequesters PACSIN1, responsible for the endocytic removal of GluN3A-containing NMDARs [39], causing an accumulation of GluN3A-containing NMDARs in striatal membrane fractions from different HD mouse models [40]. Given that GluN3A expression has a negative effect on synapse formation/stabilization [41], it can be speculated that the increased levels found in HD striatum might impact deleteriously on synaptic prosurvival signaling through NMDARs (see above). Indeed, GluN3A overexpression promoted reduced synaptic connectivity, whereas lack of GluN3A corrected the early enhancement of NMDAR currents and prevented both early and progressive dendritic spine pathology in MSNs from YAC128 mice. In addition, genetic deletion of GluN3A in the YAC128 HD mouse model prevented striatum-dependent motor and cognitive decline and cell death [40]. Therefore, GluN3A might be a good target for therapeutic interventions in HD.

It has been proposed that activation of extrasynaptic NMDARs causes neurodegeneration and cell death through the induction of a pathological triangle including 1) mitochondrial dysfunction, 2) loss of integrity of neuronal structures and connectivity, and 3) dysregulation of transcription caused by disruption of excitation-transcription coupling due to CREB shut-off and nuclear accumulation of class IIa histone deacetylases (HDACs). Reciprocal feedback within this pathological triad promotes synaptic dysfunction that culminates in failure of mitochondrial energy production and cell death [42].

mHtt expression affects a wide variety of cellular and synaptic processes in addition to promoting an increase in extrasynaptic NMDAR expression/signaling. However, it is noteworthy that many signaling pathways implicated in HD pathogenesis, namely mitochondrial impairment, spine loss and synaptic dysfunction, as well as transcriptional dysregulation, can be associated with extrasynaptic NMDARs signaling. Memantine is a noncompetitive, moderate affinity NMDAR antagonist that has been shown to block preferentially extrasynaptic NMDARs [43]. Remarkably, the beneficial effects of memantine in different HD models include amelioration of neuropathological signs, improvement of motor performance and reduction of cell death [13, 32, 44–46] (Figure 2). This is accompanied by restoration of pro-death p38 activity back to wild-type levels in YAC128 striatum [47]. Moreover, signaling through extrasynaptic NMDAR lead to CREB shut-off to a greater extent in cultured neurons from HD mice [48], but treatment with memantine restored CREB phosphorylation (pCREB) levels in HD mouse striatum [13, 47, 48]. Interestingly, blockade of NMDAR with memantine increased BDNF expression and reduced glial fibrillary acidic protein (GFAP) immunoreactivity in the 3-nitropropionic acid (3-NP) model of HD [45] (Figure 2). A recent study showed that memantine also increased the threshold for NMDA spikes and proposed that it has other functional targets in addition to extrasynaptic NMDARs that could participate in its therapeutic effects [21]. Interestingly, a small study suggested a potential neuroprotective effect in HD patients following long-term treatment with memantine [49], and memantine treatment prevented progression

of cognitive impairment in a HD patient [50]. Therefore, the results of the ongoing clinical trial using memantine [51] will be informative in determining whether indeed it can be useful to treat HD patients.

3. Targeting mitochondrial dysfunction

The participation of mitochondrial dysfunction in the pathophysiology of HD is supported by compelling evidence from both genetic and toxic animal and cellular models, as well as postmortem human tissue. It includes mitochondrial localization of mHtt, defective calcium homeostasis, impairment of mitochondrial respiration, altered mitochondrial morphology and fusion/fission dynamics, impairment in mitochondrial transport, and reduced activity of PGC-1 α , a transcriptional regulator of genes involved in mitochondrial biogenesis and oxidative phosphorylation [11, 52]. Mitochondrial dysfunction may be a contributing factor to the preferential vulnerability of MSNs in HD because their unique energy requirements to maintain a hyperpolarized state may turn them more susceptible [53]. Moreover, there is evidence that mitochondria may differ across tissues, cell types and subcellular compartments (reviewed in [54]), which could provide another explanation for this preferential vulnerability. In this line, R6/1 mice show reduced mitochondrial respiratory capacity in the striatum, but not in the cortex [55, 56].

It is noteworthy that PGC-1 α controls extrasynaptic NMDAR activity and its suppression contributed to mHtt-induced increases in extrasynaptic NMDAR activity and vulnerability to excitotoxic insults. Conversely, exogenous expression of PGC-1 α reversed mHtt-dependent increases in extrasynaptic NMDAR activity and protected neurons against excitotoxicity [57]. In this line, there is evidence that memantine treatment can improve mitochondrial function in 3-NP-injected mice ([45] and references therein), but it would be important to extend these results to genetic models of the disease. Interestingly, N-acetylcysteine (NAC) prevented mitochondrial dysfunction

and behavioral abnormalities in the 3-NP chemical model of HD [58], and a combination of NAC and dietary cysteine supplementation delayed the onset of motor abnormalities and neurodegeneration and enhanced survival in R6/2 mice [59]. Treatment with NAC also had beneficial effects on oxidative stress and mitochondrial function and delayed the onset and severity of motor symptoms in R6/1 mice [55]. Remarkably, further studies have indicated that increased levels of GluN2B subunit in the striatum of R6/1 mice could be normalized after chronic treatment with NAC indicating that aberrant striatal NMDAR signaling was ameliorated [60]. Thus, in addition to mitochondrial dysfunction, the amelioration of some aspects of glutamatergic function by NAC likely contributes to the behavioral improvement reported in earlier studies. Moreover, administration of NAC also promoted antidepressant effects, but it was not able to rescue hippocampal-dependent cognitive deficits in R6/1 mice possibly because the reductions in GluN2A or AMPAR in the hippocampus or frontal cortex of HD mice were not prevented by NAC treatment [60].

Although mitochondrial energy impairment may be particularly critical for MSNs degeneration in HD, cell death does not occur until late stages. Nevertheless, it is likely that alterations in mitochondrial dynamics and trafficking contribute to neuronal and synaptic dysfunction earlier in the disease process. Expression of mHtt alters mitochondrial function in peripheral tissues from HD patients ([61–64] and references therein), and mouse models [65, 66]. Thus, it can be anticipated that alterations in mitochondrial function in other brain areas, in addition to those well documented in the striatum, can also participate in neuronal dysfunction, synaptic plasticity deficits and loss of connectivity leading to HD symptoms. Indeed, mitochondria need to be dynamically transported along neuronal processes to provide energy to nerve terminals and maintain normal neuronal function. Actually, manipulations that reduce dendritic mitochondria content lead to loss of synapses and dendritic spines [67], and thus it is conceivable that defects in this process will lead to impairment of neural interactions/network function. In addition to impaired hippocampal, cortical and striatal synaptic plasticity, namely altered long-term potentiation (LTP) and long-term

depression (LTD), evidence for synaptic dysfunction in HD also includes spine loss (reviewed in [7, 8]). Remarkably, mHtt impairs the transport of mitochondria in neuronal processes both in cortical [68, 69], and striatal [68, 70] neurons. Altered motility was also reported in hippocampal mitochondria from BACHD mice [71]. In agreement with these trafficking defects, mHtt decreases ATP levels in striatal [72] and cortical [68] synaptosomes from *Hdh*(CAG)^{150Q} knock-in mice, without changes in total ATP levels, suggesting that mHtt is more likely to affect ATP levels in synapses than in the cell body. This has pathophysiological implications because several synaptically located processes are susceptible to impairment in conditions of low ATP levels/deficient mitochondrial transport [73]. Therefore, in contrast to the model proposed by Bading [42], we believe that there is evidence supporting the potential involvement of mitochondrial dysfunction in synaptic and cognitive impairment, but this warrants direct investigation in HD mouse models.

It was recently reported that dopamine D1 receptor signaling enhanced the sensitivity of mitochondria from striatal cells to mHtt toxicity by promoting mitochondrial fission and disrupting mitochondrial branching network. These phenomena were associated with increased dynamin related protein-1 (Drp-1) activity, and mediated by the cyclin-dependent kinase 5 (Cdk5; [74]). Cdk5 activity is increased in the striatum of *Hdh*^{Q111} mice [75], and it is an upstream regulator of mitochondrial fission during neuronal apoptosis [76]. Accordingly, pharmacological or genetic inhibition of Cdk5 ameliorated basal and dopamine-induced mitochondrial fission in *STHdh*^{Q111} striatal cells through normalization of Drp-1 activity [74]. Remarkably, genetic reduction of Cdk5 in mHtt knock-in mice attenuated both corticostriatal learning deficits and hippocampal-dependent memory decline [77], but the possible contribution of balanced fission/fusion to the behavioral improvement reported in double-mutant mice was not investigated in this study. The abnormal interaction of mHtt with the mitochondrial protein Drp-1 enhances its activity and promotes excessive mitochondrial fragmentation that leads to defective axonal transport and abnormal mitochondrial distribution, particularly in neurites and synapses [78]. Conversely, inhibiting Drp-1

GTPase activity ameliorated mitochondrial fission and transport, and improved cell survival, motor deficits, neuropathology and mortality in HD mice [14, 79]. However, the potential beneficial effect of reducing mitochondrial fission on cognitive function in HD remains to be addressed (Table I). Therefore, it would be interesting to determine whether the genetic reduction of Cdk5 impacts on Drp-1 activity and correlates with mitochondrial morphology to favor the behavioral improvements reported in mHtt knock-in:Cdk5^{+/-} double mutant mice [77].

It is noteworthy that manipulations that reduce dendritic mitochondria content lead to loss of synapses and dendritic spines, whereas increasing dendritic mitochondrial content or mitochondrial activity enhances the number and plasticity of synapses in hippocampal neurons [67]. In the context of HD what has been shown in a mHtt expressing cell line is that in conditions where mitochondrial function was enhanced there was up-regulation of synaptic genes [80, 81], suggesting that impaired mitochondrial function participates in synaptic alterations in the presence of mHtt. It would be interesting to extend these findings to HD mouse models. As the reciprocal relation also occurs, i.e., synaptic activity modulates the motility and fusion/fission balance and controls mitochondrial distribution in dendrites [67], loss of synaptic connectivity can also favor the loss of synaptic mitochondrial content, and it is likely that both process fuel each other in HD pathophysiology. Therapeutic interventions to stop this deleterious loop should provide some symptomatic relieve.

4. Targeting impaired neurotrophic signaling: reduced BDNF levels and TrkB/p75^{NTR} imbalance

BDNF is a potent neurotrophic factor for the development, neurite outgrowth, synaptic plasticity and survival of MSNs [82–84]. Globally, a reduction in BDNF levels was reported in several brain regions of HD patients and mouse models ([82], but see also [85]), with the lack of BDNF in HD being caused by a loss of function of wild-type Htt and by mHtt interfering with BDNF synthesis and

transport [86–88]. An additional contributor to reduced BDNF levels reported in HD can be the enhanced extrasynaptic NMDAR activity (see above) because BDNF is a downstream target of CREB. Accordingly, memantine treatment restores pCREB levels in the striatum of YAC128 mice [13] and increases striatal BDNF expression in the 3-NP model of HD [45].

The deficit in neurotrophic support is considered a key player in HD neuropathology. Indeed, genetic manipulation of BDNF levels has been shown to modulate the onset and severity of motor [89–91] and cognitive [89, 92] impairment in HD mouse models. BDNF also plays an important role in synaptic plasticity deficits because treatments that upregulate BDNF [93, 94], or direct exogenous BDNF application [95], restored LTP and cognitive performance in HD mice. Moreover, lack of BDNF participates in the disruption of the corticostriatal glutamatergic transmission since electrophysiological abnormalities of GABAergic function in striatal slices from HD mice were ameliorated by BDNF supply [96]. BDNF exerts its effects by binding to its receptors TrkB and p75^{NTR}. However, reduced TrkB levels have been found in HD patients, and in cellular and animal models, and imbalanced TrkB and p75^{NTR} expression/signaling has also been documented in HD (reviewed in [97]). The considerable overlap between the loss of signaling pathways regulated by BDNF-TrkB, the aberrant activation of p75^{NTR} signaling and those signaling networks affected by mHtt and leading to HD neuropathology has led to the proposal of neurotrophin receptor signaling modulation as a therapeutic strategy for HD (reviewed in [97]) (Figure 3).

A deficiency in BDNF-mediated intracellular signaling causes dendritic abnormalities in the striatum and cerebral cortex [98, 99], and BDNF is required for the activity-dependent maintenance of the mature spine phenotype of hippocampal neurons [100]. On the other hand, BDNF expression is regulated by neuronal activity [22, 101]. Therefore, a reduction in BDNF levels caused by HD related factors [102], together with loss of activity-induced BDNF transcription promoted by imbalanced synaptic/extrasynaptic NMDAR signaling (see above) likely create a vicious loop of gradual loss of synaptic activity and network connectivity, leading to pathology. Interestingly, it was

recently demonstrated that BDNF is a regulator of the balance between survival-promoting synaptic and death-inducing extrasynaptic [12, 20, 22] NMDARs, and that it promotes neuroprotection by reducing toxic NMDAR signaling [103]. In this study Lau and colleagues reported that BDNF afforded neuroprotection to hippocampal neurons through its ability to promote synaptic NMDAR activation, causing a nuclear calcium-regulated genomic response, including the induction of the expression of inhibin b-A. Once secreted, inhibin b-A activates its receptor leading to dephosphorylation of the NMDAR subunit GluN2B^{Tyr1472}, together with a reduction in neurotoxic extrasynaptic NMDAR-mediated calcium influx, which protects mitochondria from excitotoxicity [103]. In HD this neuroprotective process is likely impaired and thus, in addition to defective trophic support, reduced levels of BDNF can compromise the balance between synaptic and extrasynaptic NMDAR signaling. This renders neurons more vulnerable through the activation of neurotoxic extrasynaptic NMDAR signaling, and by loss of synaptic activity-dependent and calcium signal-regulated gene expression crucial for mitochondrial function and antioxidant defenses. Overall, neurons enter a state of increased vulnerability, with reduced length and complexity of dendrites, and synapse loss [20, 23, 42]. Since functional and structural loss of synaptic connectivity accelerates disease progression, it is important to attenuate/stop this pathological interrelated chain of events that promotes neuronal dysfunction and compromises cell survival. Hence, therapeutic strategies aimed at improving BDNF signaling may help to break this vicious circle.

Since BDNF protein-based therapies have several limitations, an alternative would be to use small agonists of its high affinity receptor. Actually, the TrkB agonist LM22A-4 reduced motor impairment, inflammation and mHtt aggregates and prevented spine loss in R6/2 and BACHD mouse models of HD [104]. Treatment with 7,8-dihydroxyflavone (7,8-DHF), originally described as a potent and selective TrkB agonist [105], also promoted motor and pathological improvements and extended survival in N171-82Q mice [106]. Another recent study demonstrates that chronic administration of 7,8-DHF delayed motor deficits and prevented deficits in object recognition

memory in R6/1 mice. This behavioral amelioration correlated with improved striatal levels of enkephalin, prevention of striatal volume loss and reduced mHtt aggregates, together with normalized striatal levels of induced and neuronal nitric oxide synthase (NOS) [107].

As mentioned above, imbalanced TrkB and p75^{NTR} expression/signaling have been demonstrated in HD. mHtt negatively affects signaling downstream the activation of TrkB while potentiating p75^{NTR}-related signaling (reviewed in [97]). Independently of whether, in a brain region- and/or disease stage-dependent manner, the imbalance simultaneously occurs in the same cell or in distinct cell types, it has pathophysiological implications. Signaling downstream TrkB and p75^{NTR} has opposing effects on synaptic plasticity, and BDNF binding to TrkB receptor is associated with neuronal survival, whereas signaling through p75^{NTR} can activate cell death cascades (reviewed in [97]). Importantly, genetic normalization of p75^{NTR} in Hdh^{Q7/Q111} mice rescued hippocampal synaptic plasticity and memory function, and prevented hippocampal dendritic spine alterations, likely by normalization of RhoA GTPase activity [108]. Moreover, overexpression of p75^{NTR} in the hippocampus of wild-type animals reproduced those memory deficits observed in HD mice, while specific hippocampal p75^{NTR} knockdown prevented the manifestation of cognitive impairment [108]. It is noteworthy that chronic administration of fingolimod (FTY720), used in the treatment of multiple sclerosis [109], ameliorated long-term memory deficits and dendritic spine loss in CA1 hippocampal neurons from R6/1 mice, and these effects were accompanied by normalization of p75^{NTR} levels and reduced astrogliosis in the hippocampus [110]. Nevertheless, motor learning deficits or corticostriatal LTP abnormalities were unaffected by p75^{NTR} deletion in Hdh^{Q7/Q111} mice [108]. In BACHD mice, despite unaltered levels of p75^{NTR}, impairment of LTP at corticostriatal synapses of the indirect pathway could be rescued by specifically knocking down striatal p75^{NTR} or inhibiting its downstream targets, indicating that enhanced signaling through p75^{NTR} antagonizes TrkB function and corticostriatal LTP [85]. It will be important to determine if this improvement in corticostriatal connectivity can be reproduced in other HD models, and reflected into an amelioration

of corticostriatal-dependent learning and/or motor symptoms. Moreover, reduction of p75^{NTR} levels in corticostriatal slices of Hdh^{Q111/Q111} mice increased cell survival and prevented BDNF-induced cell death [111]. In contrast, another report showed unaltered TrkB and p75^{NTR} levels in the striatum of presymptomatic Q175 mice, together with increased survival signaling that was lost upon p75^{NTR} deletion [112]. Interestingly, treatment with fingolimod, which promoted the normalization of p75^{NTR} levels in the hippocampus of R6/1 mice [110], improved motor function, increased survival and reduced brain atrophy in R6/2 mice, in parallel with increased BDNF levels, strengthening of neuronal activity and connectivity, reduction of mHtt aggregates and increased phosphorylation of mHtt in residues predicted to attenuate its toxicity [113]. However, the possible contribution of striatal p75^{NTR} to this beneficial outcome remains to be determined. Also treatment with the TrkB agonist 7,8-DHF reduced striatal p75^{NTR} levels in mutant mice, thus favoring the balance in p75^{NTR}/TrkB levels, which might also have contributed to the behavioral improvement reported in 7,8-DHF-treated R6/1 mice [107]. Interestingly, LM11A-31, a small molecule p75^{NTR} ligand, has been shown to restore striatal prosurvival signaling, while inhibiting degenerative signaling in R6/2 mice. Normalization of p75^{NTR} signaling upon treatment with LM11A-31 was accompanied by reduced Htt aggregates and extended survival in R6/2 mice. Importantly, there was also decreased inflammation, increased striatal and hippocampal dendritic spine density, and improved motor and cognitive function in R6/2 and BACHD mice receiving LM11A-31 [15].

Overall, this evidence suggests that p75^{NTR}/TrkB imbalance plays a role in the synaptic and learning and memory deficits observed in HD mice, and that the use of TrkB agonists or p75^{NTR} ligands could represent an excellent approach to restore TrkB-mediated synaptic plasticity while reducing aberrant p75^{NTR} signaling in HD. This is expected to improve corticostriatal connectivity thereby promoting motor improvement, ameliorate hippocampal synaptic dysfunction and memory deficits and, ultimately, increase cell survival. Additionally, although the main focus of research in HD has been neurodegeneration, glia can both induce and rescue aspects of the HD phenotype *in*

vitro [114], and *in vivo* [115]. Thus, as reviewed above and evidenced by the reduction of neuroinflammation, therapeutic strategies based on the modulation of neurotrophin receptor signaling are likely benefiting from both a neuronal and a glial effect (Table II).

5. Targeting transcriptional dysregulation

Transcriptional dysregulation has been shown to occur in HD human brain, and in mouse and cellular models of the disease. mHtt can cause transcriptional dysregulation through several mechanisms including sequestration of positive transcriptional regulators, loss of interaction with negative transcriptional regulators, increased ubiquitination and histone methylation, and reduced histone acetylation [116]. Since transcriptional dysregulation is an early and progressive event in HD [116], it emerges as a relevant therapeutic target.

5.1 Targeting cAMP/cGMP signaling to improve CREB-dependent transcription

The transcription factor CREB mediates stimulus-dependent changes in the expression of genes critical for neuronal survival, plasticity and growth, and its activity is regulated by phosphorylation and by association with CREB co-activators [117]. Increases in cyclic nucleotide levels are expected to activate CREB-dependent signaling pathways, known to be dysregulated in HD models and proposed as a contributing factor to HD pathology ([118] and references therein). Thus, phosphodiesterases (PDEs), the enzymes responsible for cAMP/cGMP degradation, represent a target to restore cyclic nucleotide levels and their downstream signaling cascades in HD.

The most interesting PDE in HD is the cAMP/cGMP dual-substrate PDE10A due to its striatal-enriched distribution [119]. Even though the levels of PDE10A in the striatum are reduced well before the onset of motor symptoms in HD patients [120], PDE10A inhibitors have attracted interest as potential novel pharmacotherapies for HD [121, 122]. The beneficial effects of PDE10A

inhibition in HD mouse models include reduced striatal and cortical pathology, amelioration of motor function, improved corticostriatal input, increased pCREB/BDNF levels and partial reversal of striatal transcriptional dysregulation [123–126]. Moreover, chronic treatment with the PDE10A inhibitor papaverine improved spatial and object recognition memory in R6/1 mice. This memory-enhancing effect likely involved a partial, but significant, recovery of GluA1 phosphorylation levels together with increased CREB phosphorylation in the hippocampus of R6/1 mice [127]. TAK-063, a novel PDE10A inhibitor, improved deficits in procedural, but not in contextual, memory in R6/2 mice [126]. Unfortunately, despite the promising results obtained with PDE10A inhibitors in preclinical models, the clinical trials from Pfizer using the PDE10 inhibitor PF-02545920 have led to disappointing results in HD patients [51].

The nitric oxide/soluble guanylyl cyclase/cGMP/cGMP-dependent protein kinase signaling pathway is implicated in synaptic plasticity, and in learning and memory (reviewed in [128]). Hippocampal cGMP levels were found to be reduced in HD mice, and targeting the cGMP-specific PDE5 with sildenafil increased cGMP levels and improved object recognition memory and passive avoidance learning in R6/1 mice, leading to the proposal that normalization of cGMP levels could counteract deficits in hippocampal cognitive function in HD [129]. Moreover, sildenafil treatment improved memory performance in the Morris water maze in the rat 3-NP model [130]. Importantly, cGMP levels were also reduced in the hippocampus of HD patients compared with control individuals [129]. Thus, PDE5 inhibition may show beneficial effects in hippocampal-dependent cognitive function in HD. In addition, biochemical and behavioral abnormalities in the 3-NP toxic model of HD were improved after treatment with PDE5 inhibitors [130, 131], accompanied by increased levels of striatal pCREB and BDNF [131]. As recently reviewed, the neuronal NOS (nNOS) pathway is highly affected also in HD striatum and cortex [8], but the effect of sildenafil or other PDE5 inhibitors on corticostriatal connectivity, corticostriatal-dependent learning and/or motor dysfunction in genetic models of the disease has not been investigated so far.

5.2 Targeting histone deacetylation

CREB binding protein (CBP) is a key regulator of CREB-mediated transcription by acting as a CREB transcriptional co-activator and as a histone acetyltransferase (HAT) to disrupt repressive chromatin structure and allow gene transcription [132]. Importantly, CBP was found in mHtt aggregates in HD cell cultures, transgenic mice, and human postmortem brain, thereby suppressing CREB/CBP-mediated transcription [133, 134] and CBP HAT activity [135, 136]. Thus, abnormal histone acetylation and chromatin remodeling might be crucial processes leading to transcriptional dysregulation, and histone acetylation became a potential therapeutic target in HD. In this line, different HDAC inhibitors (HDACis) are being tested, and the details of various preclinical studies using HDACis in HD models have been recently reviewed [16]. The benefits of HDAC inhibition in HD mice include increased survival, prevention of body weight loss, reduced brain atrophy, reduced aggregate formation, correction of gene expression abnormalities, increased BDNF levels and improved cognitive and motor function (reviewed in [16]).

Concerning the impact of transcriptional dysregulation on cognitive dysfunction in HD, a recent study tested the effect of RGFP966, an inhibitor of HDAC3. Chronic treatment with RGFP966 starting at early stages of the disease prevented deficits in hippocampal-dependent spatial and recognition memories, and normalized the expression of some specific memory-related genes in the hippocampus of Hdh^{Q7/Q111} knock-in mice. Moreover, RGFP966 treatment also prevented corticostriatal-dependent motor learning deficits in HD mice [137]. In addition, Hdh^{Q7/Q111} knock-in mice chronically treated with RGFP966 showed substantially reduced striatal CAG repeat expansions and average change in repeat length, and partial prevention of striatal protein marker expression loss, including that of DARPP-32, PDE10A and adenosine receptor A2A. The

accumulation of mHtt oligomeric forms was also reduced in the striatum of RGFP966-treated mice [137]. Complementary findings were reported in N171-82Q mice since treatment with RGFP966 improved motor deficits on the rotarod and open field, accompanied by neuroprotective effects on striatal volume and decreased GFAP immunoreactivity in the striatum [138]. Interestingly, valproate was shown to ameliorate the depressive behavior in N171-82Q and YAC128 mice, accompanied by increased striatal and cortical BDNF levels [139]. The beneficial effects, extending to improvement of spontaneous locomotor activity, motor skill learning and survival in N171-82Q mice, were significantly enhanced by combined treatment with the glycogen synthase kinase 3 β (GSK3 β) inhibitor, lithium chloride [139]. Nevertheless, there are conflicting results regarding whether GSK3 activity is elevated or decreased in HD. For instance, lipid rafts extracted from knock-in HD mouse brains had significantly more GSK3 β than lipid rafts from control mice, and treating HD primary cortical neurons with GSK3 β inhibitors reduced neuronal death [140]. Also, upregulation of the active isoform pGSK3 β ^{Tyr216} levels in the hippocampus was associated with the increased vulnerability of R6/2 mice hippocampal neurons *in vitro* [141]. On the other hand, the inhibitory phosphorylation of GSK3 β was increased in the cortex and striatum of R6/1 mice [142, 143], and the levels of cortical pGSK3 inversely correlated with cognitive function in R6/1 mice [142]. In this line, the genetic correction of the GSK3 levels/activity deficit found in R6/1 mice resulted in amelioration of their brain atrophy, motor function and hippocampal learning deficits [144].

A general correlation between epigenetic and transcriptional dysregulation in HD has been questioned [116, 145] and histone hypoacetylation may not be a general rule in HD [146, 147]. Instead, histone deacetylation events are limited to specific loci associated with genes relevant to neuronal functions and the pathology. Moreover, histone H3 deacetylation is not necessarily linked to altered gene expression but, in some cases, it may indicate susceptibility to transcriptional change if other factors converge [147]. However, as discussed above, the beneficial effects of HDACis in HD mice are multiple and they can include the indirect activation of compensatory mechanisms, as

in the case of Sirt2 inhibition in cellular and invertebrate models of HD, which did not globally correct transcriptional dysregulation, but conferred neuroprotection by modulating cholesterol homeostasis [148]. Similarly, genetic inhibition of HDAC4 significantly ameliorated neurological features in HD mouse models by reducing cytoplasmic aggregate formation, and rescued MSNs electrophysiological properties and corticostriatal synaptic function, without having an effect on global transcriptional dysfunction or nuclear mHtt aggregation [149]. It is noteworthy that the non-histone targets of HDACs can include several proteins, namely Htt itself [145]. Several posttranslational modifications play a crucial role in Htt protein-protein interactions and are significantly altered by the presence of the HD mutation, including acetylation [150]. It was shown that increased acetylation at K444 facilitated trafficking of mHtt into autophagosomes, significantly improved its clearance by autophagy, leading to neuroprotection in primary neurons and a transgenic *C. elegans* model of HD. Conversely, an acetylation-resistant form of mHtt dramatically accumulated and lead to neurodegeneration in cultured neurons and in mouse brain [151]. Altogether, these studies show that targeting HDAC with selective inhibitors holds the promise to be a good approach for therapeutic interventions with multiple beneficial effects in HD due to their combined effects on histone and non-histone substrates.

6. Conclusions

HD is a complex neurodegenerative disorder affecting several brain functions such as motor control and cognition, accompanied by the presence of psychiatric symptoms. This multiple affectation makes difficult to design a curative therapy. It is now widely accepted that neuronal dysfunction precedes cell death and therefore therapeutic targets aimed to restore neuronal function may prove to be neuroprotective. Interestingly, mHtt seems to operate through similar mechanisms to induce neuronal dysfunction in different brain regions, providing the opportunity to target both motor and

cognitive dysfunction by using essentially the same drugs. Moreover, some of the mechanisms leading to neuronal dysfunction are interrelated, so one can intervene simultaneously at several points of the toxic cascade. Therefore, evidence discussed here points to the use of drugs with pleiotropic effects on several pathological processes as a promising therapeutic approach in HD. It can be speculated that the unintended adverse effects of the simultaneous, but individual, targeting of multiple pathological mechanisms with several drugs can be reduced by the use of single or fewer drugs with pleiotropic effects. Indeed, there is evidence that drug combinations may have additive or synergistic therapeutic effects in HD models [17, 152–157]. It is true that polypharmacy, which can be defined based on distinct criteria [158–160], raises concerns and it might have a negative connotation because it is also associated with an increased risk of adverse drug interactions and sometimes serious, or even lethal, side-effects, especially in frail older people. Recently, the term ‘appropriate polypharmacy’ was proposed. This is an attempt to recognize that patients can benefit from several medications in terms of clinical outcomes, quality of life and life expectancy, provided that prescribing is evidence-based, reflects patients’ clinical conditions and considers potential drug interactions [161]. Remarkably, several approaches are now available to assist in the process of selecting suitable drug cocktails to test [162–167]. Hopefully, their application to preclinical models will lead to important advances in treatment outcomes in HD.

7. Expert opinion

The discovery of the mutation responsible for HD 25 years ago enabled the development of genetic animal models that have provided a huge body of knowledge into HD pathophysiology and candidate therapeutic targets/drugs. Whereas the definitive cure will likely arise from therapies targeting transcription and translation of the mutant protein, several issues may still constitute hurdles for human genetic therapy for some more years. Meanwhile, pharmacologically targeting pathological

mechanisms in HD that are common to other diseases, such as increased extrasynaptic NMDAR signaling, mitochondrial dysfunction or reduced BDNF levels [11, 12], raises the possibility of finding common therapies for several neurodegenerative diseases.

Studies in R6 mice have demonstrated reduced hippocampal LTP, and abnormal ability to express LTD, and these were dependent on NMDAR signaling [168, 169]. Interestingly, activation of extrasynaptic NMDAR induces LTD in rat hippocampal CA1 neurons [170]. Therefore, it is relevant to extend the study of NMDAR localization and signaling from HD striatum to the cortex and hippocampus. Additionally, it will be important to address whether an imbalance in synaptic and extrasynaptic NMDAR signaling participates in synaptic abnormalities leading to cognitive deficits in full-length and exon-1 HD mouse models. Remarkably, memantine, which targets extrasynaptic NMDAR, is well-tolerated and FDA approved, and currently being tested in HD patients [51]. Several new classes of positive and negative allosteric modulators of NMDARs have been identified with distinct patterns of subunit selectivity and better pharmacology than previously available drugs [171]. Therefore, if memantine fails in clinical HD trials in spite the promising preclinical data reviewed here, some of these new compounds might be valuable tools to test in HD models. On the other hand, given the ability of BDNF to regulate the balance between synaptic and extrasynaptic NMDAR signaling [103], it will be important to investigate whether strategies aimed at improving BDNF signaling are able to reduce deleterious extrasynaptic NMDAR signaling in HD. In addition, targeting STEP at early disease stages may be beneficial to promote synaptic NMDAR signaling in HD striatum and prevent loss of corticostriatal connectivity. Moreover, STEP inhibition can also be useful to promote hippocampal-dependent cognitive function in HD. Indeed, it would be interesting to address these issues using TC-2153, a recently described pharmacological inhibitor of STEP that has proven to be a valuable therapeutic tool in AD [172] and fragile X syndrome [173] mouse models. Importantly, STEP opposes pERK1/2 and pCREB-mediated BDNF expression [38], while BDNF promotes the prompt degradation of STEP through the proteasome [174].

It was speculated that targeting the nNOS/cGMP pathway might also ameliorate corticostriatal dysfunction in HD and, importantly, PDE5 inhibitors also target neuroinflammation and neurodegeneration, and cGMP can promote mitochondrial biogenesis and ATP synthesis [8]. Remarkably, sildenafil has antidepressant-like effects in rodents ([175, 176] and references therein), and this is paralleled by increased cGMP/pCREB/BDNF signaling in the hippocampus and prefrontal cortex of mice subjected to chronic unpredictable mild stress [176]. Depression is a common psychiatric symptom in HD patients, and thus PDE5 inhibitors may prove to be valuable multi-purpose drugs for HD. NAC also emerges as an interesting therapeutic tool since it targets mitochondrial dysfunction, it improves some glutamate receptor imbalances and it has antidepressant effects in HD mice (Table I). Unfortunately, evidence so far is not strong to support its potential to improve cognitive deficits in HD. However, its combination with other drug/s with complementary therapeutic effects could be an interesting approach. We believe that future studies in genetic HD models should be undertaken to address these issues.

Several studies show that targeting HDACs promotes the alleviation of multiple pathological processes, and significantly prevents or delays corticostriatal- and hippocampal-dependent cognitive deficits, neuropathology, somatic CAG repeat expansions and motor dysfunction in HD mice. Moreover, by rendering chromatin more permissive for initiation of transcription, HDACs may help to counteract the impact of the nuclear import of class IIa HDACs under conditions of extrasynaptic NMDAR stimulation [177], and restore activity-dependent gene expression. In addition, HDACs may also constitute viable targets for the modulation of mitochondrial biogenesis, fission-fusion, transport and mitophagy [178, 179]. On the other hand, given the structural and functional interplay between dendritic mitochondria and spines/synapses [67], we consider that future studies exploring the therapeutic potential of drugs expected to improve mitochondrial function/dynamics/transport should go further and analyze also structural and functional synaptic plasticity, as well as cognitive function.

Finally, it is necessary to take into account the effect of any potential HD therapy on brain non-neuronal cells. Astrocytes and neurons function synergistically and mHtt is expressed in both cell types. The interplay between neuronal and glial processes is complex and therapeutic approaches focused exclusively on neuroinflammation are not likely to produce robust effects in HD [180, 181]. By unravelling astrocyte dysfunction, astrocytes could be therapeutically exploited in HD. On the other hand, targeting key pathological mechanisms in neurons and astrocytes is expected to improve astrocyte-mediated homeostasis and neural synergistic interactions with therapeutic implications [182]. Therefore, we consider that the identification of novel drugs like fingolimod, 7,8-DHF or PDE5 inhibitors, with pleiotropic effects targeting also neuroinflammation, should be increasingly pursued.

Despite the promising preclinical results with, for example creatine (reviewed in [11]) and PDE10A inhibitors (see above), the data from these clinical trials in HD have been disappointing [51, 183]. Indeed, in HD there is a low success rate at earlier phases of drug-development and a very low trial success rate at later phases [184], reflecting a gap between animal models and clinical outcomes. Therefore, in our opinion and as reviewed here, novel therapeutic interventions should look for multi-purpose/pleiotropic drugs, if possible, amenable for both cognitive and motor improvement. Moreover, targeting (individually, but preferentially in combination) those pathological mechanisms that clearly promote early neuronal dysfunction and, additionally, operate in a feedforward vicious cycle will hopefully halt or reduce disease progression. It can be speculated that the unintended adverse effects of simultaneously targeting multiple pathological mechanisms with individual drugs can be reduced by the use of single or fewer drugs with pleiotropic effects. For instance, bioinformatic tools to predict effective drug combinations can be used to test *in silico* the combination of two approved drugs like memantine and fingolimod, which target a wide range of pathophysiological mechanisms with promising results in preclinical models of HD. Information obtained from these bioinformatic analyses will likely produce results more easily translatable to

clinical-ready therapies. Undoubtedly, the incorporation of this paradigm shift in upcoming studies can, in the near future, provide a valuable amount of information relevant for HD management.

Article highlights

- Cognitive and neuropsychiatric symptoms are present early in HD pathophysiology, accompanied by synaptic pathology.
- mHtt expression creates several imbalances in neural function.
- Enhanced extrasynaptic NMDAR signaling in HD engages a feed forward pathological cycle that can be prevented by memantine.
- Deficient BDNF trophic support also arises from imbalances in receptor signaling, with therapeutic implications.
- The existence of this feed forward pathological cycle beyond the HD striatum is therapeutically relevant and deserves a better characterization.
- Deleterious feed forward mechanisms likely underlie synaptic dysfunction leading to non-motor and motor symptoms, suggesting that simultaneous targeting of the affected pathways with appropriate polypharmacy or pleiotropic drugs might prove to be a good therapeutic approach.

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Declaration of interest

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Huntington's Disease Collaborative Research. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 1993;72:971–983
2. Mason SL, Barker RA. Advancing pharmacotherapy for treating Huntington's disease: a review of the existing literature. *Expert Opin Pharmacother* 2016;17:41–52
3. Milnerwood AJ, Raymond LA. Early synaptic pathophysiology in neurodegeneration: Insights from Huntington's disease. *Trends Neurosci* 2010;33:513–523
4. Labbadia J, Morimoto RI. Huntington's disease: underlying molecular mechanisms and emerging concepts. *Trends Biochem Sci* 2013;38:378–385
5. Zuccato C, Valenza M, Cattaneo E. Molecular mechanisms and potential therapeutical targets in Huntington's disease. *Physiol Rev* 2010;90:905–981
6. Beglinger LJ, O'Rourke JJF, Wang C, Langbehn DR, Duff K, Paulsen JS. Earliest functional

declines in Huntington disease. *Psychiatry Res* 2010;178:414–418

7. Tyebji S, Hannan AJ. Synaptopathic mechanisms of neurodegeneration and dementia: Insights from Huntington's disease. *Prog Neurobiol* 2017;153:18–45
8. Puigdemívol M, Saavedra A, Pérez-Navarro E. Cognitive Dysfunction in Huntington's Disease: Mechanisms and Therapeutic Strategies Beyond BDNF. *Brain Pathol* 2016;1:1–59
9. Giralt A, Saavedra A, Alberch J, Pérez-Navarro E. Cognitive Dysfunction in Huntington's Disease: Humans, Mouse Models and Molecular Mechanisms. *J Huntingtons Dis* 2012;1:155–73
10. Wild EJ, Tabrizi SJ. Therapies targeting DNA and RNA in Huntington's disease. *Lancet Neurol* 2017;16:837–847
11. Chaturvedi RK, Beal MF. Mitochondria targeted therapeutic approaches in Parkinson's and Huntington's diseases. *Mol Cell Neurosci* 2013;55:101–114
12. Parsons MP, Raymond LA. Extrasynaptic NMDA receptor involvement in central nervous system disorders. *Neuron* 2014;82:279–293
13. Milnerwood AJ, Gladding CM, Pouladi MA, et al. Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron* 2010;65:178–190
- **This paper showed that increases in extrasynaptic NMDAR activity contribute to phenotype onset, and demonstrated the beneficial effects of memantine treatment in HD mice.**
14. Guo X, Disatnik M, Monbureau M, Shamloo M, Mochly-rosen D, Qi X. Inhibition of mitochondrial fragmentation diminishes Huntington's disease – associated neurodegeneration.

15. Simmons DA, Belichenko NP, Ford EC, et al. A small molecule p75NTR ligand normalizes signalling and reduces Huntington's disease phenotypes in R6/2 and BACHD mice. *Hum Mol Genet* 2016;25:4920–4938
16. Sharma S, Taliyan R. Transcriptional dysregulation in Huntington's disease: The role of histone deacetylases. *Pharmacol Res* 2015;100:157–169
17. Chiu CT, Liu G, Leeds P, Chuang DM. Combined treatment with the mood stabilizers lithium and valproate produces multiple beneficial effects in transgenic mouse models of huntington's disease. *Neuropsychopharmacology* 2011;36:2406–2421
18. Fan X, Jin WY, Wang YT. The NMDA receptor complex: a multifunctional machine at the glutamatergic synapse. *Front Cell Neurosci* 2014;8:160
19. Paoletti P, Bellone C, Zhou Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nat Rev Neurosci* 2013;14:383–400
20. Hardingham GE, Bading H. Synaptic versus extrasynaptic NMDA receptor signalling: Implications for neurodegenerative disorders. *Nat Rev Neurosci* 2010;11:682–696
21. Mahfooz K, Marco S, Martínez-Turrillas R, Raja MK, Pérez-Otaño I, Wesseling JF. GluN3A promotes NMDA spiking by enhancing synaptic transmission in Huntington's disease models. *Neurobiol Dis* 2016;93:47–56
22. Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat Neurosci* 2002;5:405–414

• **First study showing that synaptic and extrasynaptic NMDARs have antagonistic effects on the regulation of CREB-mediated transcription and cell survival/death pathways.**

23. Bading H. Nuclear calcium signalling in the regulation of brain function. *Nat Rev Neurosci* 2013;14:593–608
24. Cross AJ, Slater P, Reynolds GP. Reduced high-affinity glutamate uptake sites in the brains of patients with Huntington's disease. *Neurosci Lett* 1986;67:198–202
25. Hassel B, Tessler S, Faull RLM, Emson PC. Glutamate uptake is reduced in prefrontal cortex in Huntington's disease. *Neurochem Res* 2008;33:232–237
26. Lievens JC, Woodman B, Mahal A, et al. Impaired glutamate uptake in the R6 Huntington's disease transgenic mice. *Neurobiol Dis* 2001;8:807–821
27. Faideau M, Kim J, Cormier K, et al. In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. *Hum Mol Genet* 2010;19:3053–3067
28. Huang K, Kang MH, Askew C, et al. Palmitoylation and function of glial glutamate transporter-1 is reduced in the YAC128 mouse model of Huntington disease. *Neurobiol Dis* 2010;40:207–215
29. Parsons MP, Vanni MP, Woodard CL, Kang R, Murphy TH, Raymond LA. Real-time imaging of glutamate clearance reveals normal striatal uptake in Huntington disease mouse models. *Nat Commun* 2016;7:1–12
30. Levine MS, Klapstein GJ, Koppel A, et al. Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knockin mouse models of Huntington's disease. *J Neurosci Res* 1999;58:515–532
31. Fan MM, Raymond LA. N-methyl-D-aspartate (NMDA) receptor function and excitotoxicity in Huntington's disease. *Prog Neurobiol* 2007;81:272–293

32. Okamoto S, Pouladi MA, Talantova M, et al. Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. *Nat Med* 2009;15:1407–1413

•• This study demonstrated that the balance between synaptic and extrasynaptic NMDAR signaling modulates the formation of mHtt aggregates, neurotoxicity, and disease severity in HD models. Also showed the therapeutic value of targeting extrasynaptic NMDAR with memantine.

33. Subramaniam S, Sixt KM, Barrow R, Snyder SH. Rhes, a striatal specific protein, mediates mutant-huntingtin cytotoxicity. *Science* 2009;324:1327–1330

34. Gladding CM, Raymond LA. Mechanisms underlying NMDA receptor synaptic/extrasynaptic distribution and function. *Mol Cell Neurosci* 2011;48:308–320

35. Snyder EM, Nong Y, Almeida CG, et al. Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci* 2005;8:1051–1058

36. Xu J, Kurup P, Zhang Y, et al. Extrasynaptic NMDA receptors couple preferentially to excitotoxicity via calpain-mediated cleavage of STEP. *J Neurosci* 2009;29:9330–9343

37. Gladding CM, Sepers MD, Xu J, et al. Calpain and Striatal-Enriched protein tyrosine Phosphatase (STEP) activation contribute to extrasynaptic NMDA receptor localization in a huntington's disease mouse model. *Hum Mol Genet* 2012;21:3739–3752

38. Xu J, Kurup P, Baguley TD, et al. Inhibition of the tyrosine phosphatase STEP61 restores BDNF expression and reverses motor and cognitive deficits in phencyclidine-treated mice. *Cell Mol Life Sci* 2016;73:1503–1514

39. Perez-Otano I, Lujan R, Tavalin SJ, et al. Endocytosis and synaptic removal of NR3A-containing NMDA receptors by PACSIN1/syndapin1. *Nat Neurosci* 2006;9:611–621

40. Marco S, Giralt A, Petrovic MM, et al. Suppressing aberrant GluN3A expression rescues synaptic and behavioral impairments in Huntington's disease models. *Nat Med* 2013;19:1030–1038
41. Henson MA, Roberts AC, Perez-Otano I, Philpot BD. Influence of the NR3A subunit on NMDA receptor functions. *Prog Neurobiol* 2010;91:23–37
42. Bading H. Therapeutic targeting of the pathological triad of extrasynaptic NMDA receptor signaling in neurodegenerations. *J Exp Med* 2017;214:569–578
- **Proposed a pathological triad downstream the activation of extrasynaptic NMDAR as a common converging point in neurodegenerative conditions, and provides a conceptual framework for the development of novel, broadly applicable neuroprotective treatments.**
43. Xia P, Chen HV, Zhang D, Lipton SA. Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. *J Neurosci* 2010;30:11246–11250
44. Carter RL, Chen Y, Kunkanjanawan T, et al. Reversal of cellular phenotypes in neural cells derived from Huntington's disease monkey-induced pluripotent stem cells. *Stem Cell Reports* 2014;3:585–593
45. Ranju V, Sathiya S, Kalaivani P, Priya RJ, Saravana Babu C. Memantine exerts functional recovery by improving BDNF and GDNF expression in 3-nitropropionic acid intoxicated mice. *Neurosci Lett* 2015;586:1–7
46. Lee S-T, Chu K, Park J-E, et al. Memantine reduces striatal cell death with decreasing calpain level in 3-nitropropionic model of Huntington's disease. *Brain Res* 2006;1118:199–207
47. Dau A, Gladding CM, Sepers MD, Raymond LA. Chronic blockade of extrasynaptic NMDA receptors ameliorates synaptic dysfunction and pro-death signaling in Huntington disease transgenic mice. *Neurobiol Dis* 2014;62:533–542

48. Milnerwood AJ, Kaufman AM, Sepers MD, et al. Mitigation of augmented extrasynaptic NMDAR signaling and apoptosis in cortico-striatal co-cultures from Huntington's disease mice. *Neurobiol Dis* 2012;48:40–51
49. Beister A, Kraus P, Kuhn W, Dose M, Weindl A, Gerlach M. The N-methyl-D-aspartate antagonist memantine retards progression of Huntington's disease. *J Neural Transm Suppl* 2004;117–122
50. Cankurtaran ES, Ozalp E, Soygur H, Cakir A. Clinical experience with risperidone and memantine in the treatment of Huntington's disease. *J Natl Med Assoc* 2006;98:1353–1355
51. Rodrigues FB, Wild EJ. Clinical Trials Corner: September 2017. *J Huntingtons Dis* 2017;6:255–263
52. Guedes-Dias P, Pinho BR, Soares TR, de Proença J, Duchen MR, Oliveira JMA. Mitochondrial dynamics and quality control in Huntington's disease. *Neurobiol Dis* 2016;90:51–57
53. Morigaki R, Goto S. Striatal vulnerability in huntington's disease: Neuroprotection versus neurotoxicity. *Brain Sci* 2017;7:02–25
54. Cherubini M, Ginés S. Mitochondrial fragmentation in neuronal degeneration: Toward an understanding of HD striatal susceptibility. *Biochem Biophys Res Commun* 2017;483:1063–1068
55. Wright DJ, Renoir T, Smith ZM, et al. N-Acetylcysteine improves mitochondrial function and ameliorates behavioral deficits in the R6/1 mouse model of Huntington's disease. *Transl Psychiatry* 2015;5:e492–10
56. Herbst EAF, Holloway GP. Exercise training normalizes mitochondrial respiratory capacity

within the striatum of the R6/1 model of Huntington's disease. *Neuroscience* 2015;303:515–523

57. Puudifoot C, Martel M-A, Soriano FX, et al. PGC-1alpha negatively regulates extrasynaptic NMDAR activity and excitotoxicity. *J Neurosci* 2012;32:6995–7000
58. Sandhir R, Sood A, Mehrotra A, Kamboj SS. N-acetylcysteine reverses mitochondrial dysfunctions and behavioral abnormalities in 3-nitropropionic acid-induced Huntington's disease. *Neurodegener Dis* 2012;9:145–157
59. Paul BD, Sbodio JI, Xu R, et al. Cystathionine γ -lyase deficiency mediates neurodegeneration in Huntington's disease. *Nature* 2014;508:96–100
60. Wright DJ, Gray LJ, Finkelstein DI, et al. N-acetylcysteine modulates glutamatergic dysfunction and depressive behavior in Huntington's disease. *Hum Mol Genet* 2016;25:2923–2933
61. Ciammola A, Sassone J, Sciacco M, et al. Low anaerobic threshold and increased skeletal muscle lactate production in subjects with Huntington's disease. *Mov Disord* 2011;26:130–137
62. Naia L, Ferreira IL, Cunha-Oliveira T, et al. Activation of IGF-1 and Insulin Signaling Pathways Ameliorate Mitochondrial Function and Energy Metabolism in Huntington's Disease Human Lymphoblasts. *Mol Neurobiol* 2014;51:331–348
63. Chen CM, Wu YR, Cheng ML, et al. Increased oxidative damage and mitochondrial abnormalities in the peripheral blood of Huntington's disease patients. *Biochem Biophys Res Commun* 2007;359:335–340
64. Lodi R, Schapira AH, Manners D, et al. Abnormal in vivo skeletal muscle energy metabolism

in Huntington's disease and dentatorubropallidoluysian atrophy. *Ann Neurol* 2000;48:72–76

65. Mihm MJ, Amann DM, Schanbacher BL, Altschuld RA, Bauer JA, Hoyt KR. Cardiac dysfunction in the R6/2 mouse model of Huntington's disease. *Neurobiol Dis* 2007;25:297–308
66. Choo YS, Johnson GVW, MacDonald M, Detloff PJ, Lesort M. Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. *Hum Mol Genet* 2004;13:1407–1420
67. Li Z, Okamoto KI, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* 2004;119:873–887
- This study shows that dendritic distribution of mitochondria is essential and limiting for the support of synapses. Also that, in turn, synaptic activity modulates the motility and fusion/fission balance of mitochondria and controls mitochondrial distribution in dendrites. Thus, work provides evidence that mitochondria play a significant role in structural plasticity.**
68. Orr AL, Li S, Wang C-E, et al. N-terminal mutant huntingtin associates with mitochondria and impairs mitochondrial trafficking. *J Neurosci* 2008;28:2783–2792
69. Chang DTW, Rintoul GL, Pandipati S, Reynolds IJ. Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons. *Neurobiol Dis* 2006;22:388–400
70. Trushina E, Dyer RB, Badger JD, et al. Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. *Mol Cell Biol* 2004;24:8195–8209
71. Shirendeb UP, Calkins MJ, Manczak M, et al. Mutant Huntingtin's interaction with mitochondrial protein Drp1 impairs mitochondrial biogenesis and causes defective axonal transport and synaptic degeneration in Huntington's disease. *Hum Mol Genet* 2012;21:406–

72. Wang J, Wang CE, Orr A, Tydlacka S, Li SH, Li XJ. Impaired ubiquitin-proteasome system activity in the synapses of Huntington's disease mice. *J Cell Biol* 2008;180:1177–1189
73. Li X-J, Orr AL, Li S. Impaired mitochondrial trafficking in Huntington's disease. *Biochim Biophys Acta* 2010;1802:62–65
74. Cherubini M, Puigdellívol M, Alberch J, Ginés S. Cdk5-mediated mitochondrial fission: A key player in dopaminergic toxicity in Huntington's disease. *Biochim Biophys Acta - Mol Basis Dis* 2015;1852:2145–2160
75. Paoletti P, Vila I, Rife M, Lizcano JM, Alberch J, Gines S. Dopaminergic and glutamatergic signaling crosstalk in Huntington's disease neurodegeneration: the role of p25/cyclin-dependent kinase 5. *J Neurosci* 2008;28:10090–10101
76. Meuer K, Suppanz IE, Lingor P, et al. Cyclin-dependent kinase 5 is an upstream regulator of mitochondrial fission during neuronal apoptosis. *Cell Death Differ* 2007;14:651–661
77. Alvarez-Periel E, Puigdellívol M, Brito V, et al. Cdk5 Contributes to Huntington's Disease Learning and Memory Deficits via Modulation of Brain Region-Specific Substrates. *Mol Neurobiol* 2017;1:1–19
78. Reddy PH, Shirendeb UP. Mutant huntingtin, abnormal mitochondrial dynamics, defective axonal transport of mitochondria, and selective synaptic degeneration in Huntington's disease. *Biochim Biophys Acta* 2012;1822:101–110
79. Song W, Chen J, Petrilli A, et al. Mutant huntingtin binds the mitochondrial fission GTPase dynamin-related protein-1 and increases its enzymatic activity. *Nat Med* 2011;17:377–382
80. Yin X, Manczak M, Reddy PH. Mitochondria-targeted molecules MitoQ and SS31 reduce

mutant huntingtin-induced mitochondrial toxicity and synaptic damage in Huntington's disease. *Hum Mol Genet* 2016;25:1739–1753

81. Manczak M, Reddy PH. Mitochondrial division inhibitor 1 protects against mutant huntingtin-induced abnormal mitochondrial dynamics and neuronal damage in Huntington's disease. *Hum Mol Genet* 2015;24:7308–7325
82. Zuccato C, Cattaneo E. Role of brain-derived neurotrophic factor in Huntington's disease. *Prog Neurobiol* 2007;81:294–330
83. Rauskolb S, Zagrebelsky M, Dreznjak A, et al. Global deprivation of brain-derived neurotrophic factor in the CNS reveals an area-specific requirement for dendritic growth. *J Neurosci* 2010;30:1739–1749
84. Baydyuk M, Xu B. BDNF signaling and survival of striatal neurons. *Front Cell Neurosci* 2014;8:254
85. Plotkin JL, Day M, Peterson JD, et al. Impaired TrkB receptor signaling underlies corticostriatal dysfunction in Huntington's disease. *Neuron* 2014;83:178–188

•• These authors demonstrated the role of imbalanced TrkB/p75^{NTR} signaling in corticostriatal dysfunction in HD.

86. Cattaneo E. Dysfunction of wild-type huntingtin in Huntington disease. *News Physiol Sci* 2003;18:34–37
87. Zuccato C, Ciammola A, Rigamonti D, et al. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* (80-) 2001;293:493–498
88. Gauthier LR, Charrin BC, Borrell-Pages M, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell*

2004;118:127–138

89. Xie Y, Hayden MR, Xu B. BDNF Overexpression in the Forebrain Rescues Huntington's Disease Phenotypes in YAC128 Mice. *J Neurosci* 2010;30:14708–14718
90. Gharami K, Xie Y, An JJ, Tonegawa S, Xu B. Brain-derived neurotrophic factor overexpression in the forebrain ameliorates Huntington's disease phenotypes in mice. *J Neurochem* 2008;105:369–379
91. Canals JM, Pineda JR, Torres-Peraza JF, et al. Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. *J Neurosci* 2004;24:7727–7739
92. Giralt A, Rodrigo T, Martin ED, et al. Brain-derived neurotrophic factor modulates the severity of cognitive alterations induced by mutant huntingtin: involvement of phospholipaseCgamma activity and glutamate receptor expression. *Neuroscience* 2009;158:1234–1250
93. Anglada-Huguet M, Vidal L, Giralt A, Garcia-Diaz Barriga G, Xifro X, Alberch J. Prostaglandin E2 EP2 activation reduces memory decline in R6/1 mouse model of Huntington's disease by the induction of BDNF-dependent synaptic plasticity. *Neurobiol Dis* 2015;95:22–34
94. Simmons DA, Rex CS, Palmer L, et al. Up-regulating BDNF with an ampakine rescues synaptic plasticity and memory in Huntington's disease knockin mice. *Proc Natl Acad Sci USA* 2009;106:4906–4911
95. Lynch G, Kramar EA, Rex CS, et al. Brain-derived neurotrophic factor restores synaptic plasticity in a knock-in mouse model of Huntington's disease. *J Neurosci* 2007;27:4424–4434

96. Cepeda C, Starling AJ, Wu N, et al. Increased GABAergic function in mouse models of Huntington's disease: reversal by BDNF. *J Neurosci Res* 2004;78:855–867
97. Simmons DA. Modulating Neurotrophin Receptor Signaling as a Therapeutic Strategy for Huntington's Disease. *J Huntington's Dis* 2017;6:303–325
- This review describes in detail the imbalance of neurotrophic signaling in the presence of mHtt, and the last therapeutic interventions targeting BDNF-TrkB-p75^{NTR} in HD**
98. Baquet ZC, Gorski JA, Jones KR. Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. *J Neurosci* 2004;24:4250–4258
99. Gorski JA, Zeiler SR, Tamowski S, Jones KR. Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites. *J Neurosci* 2003;23:6856–6865
100. Kellner Y, Godecke N, Dierkes T, Thieme N, Zagrebelsky M, Korte M. The BDNF effects on dendritic spines of mature hippocampal neurons depend on neuronal activity. *Front Synaptic Neurosci* 2014;6:1–17
101. Ghosh A, Carnahan J, Greenberg ME. Requirement for BDNF in activity-dependent survival of cortical neurons. *Science* 1994;263:1618–1623
102. Zuccato C, Cattaneo E. Brain-derived neurotrophic factor in neurodegenerative diseases. *Nat Rev Neurol* 2009;5:311–322
103. Lau D, Bengtson CP, Buchthal B, Bading H. BDNF Reduces Toxic Extrasynaptic NMDA Receptor Signaling via Synaptic NMDA Receptors and Nuclear-Calcium-Induced Transcription of inhba/Activin A. *Cell Rep* 2015;12:1353–1366

104. Simmons DA, Belichenko NP, Yang T, et al. A Small Molecule TrkB Ligand Reduces Motor Impairment and Neuropathology in R6/2 and BACHD Mouse Models of Huntington's Disease. *J Neurosci* 2013;33:18712–18727
105. Jang S-W, Liu X, Yepes M, et al. A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. *Proc Natl Acad Sci U S A* 2010;107:2687–2692
106. Jiang M, Peng Q, Liu X, et al. Small-molecule TrkB receptor agonists improve motor function and extend survival in a mouse model of Huntington's disease. *Hum Mol Genet* 2013;22:2462–2470
107. Garcia-Diaz Barriga G, Giralt A, Anglada-Huguet M, et al. 7,8-dihydroxyflavone ameliorates cognitive and motor deficits in a Huntington's disease mouse model through specific activation of the PLC γ 1 pathway. *Hum Mol Genet* 2017;26:3144–3160
108. Brito V, Giralt A, Enriquez-Barreto L, et al. Neurotrophin receptor p75(NTR) mediates Huntington's disease-associated synaptic and memory dysfunction. *J Clin Invest* 2014;124:4411–4428
- **In this study, Brito and colleagues showed the contribution of aberrant p75^{NTR} signaling to hippocampal synaptic and cognitive dysfunction in HD.**
109. Chiba K, Adachi K. Discovery of fingolimod, the sphingosine 1-phosphate receptor modulator and its application for the therapy of multiple sclerosis. *Future Med Chem* 2012;4:771–781
110. Miguez A, Garcia-Diaz Barriga G, Brito V, et al. Fingolimod (FTY720) enhances hippocampal synaptic plasticity and memory in Huntington's disease by preventing p75NTR up-regulation and astrocyte-mediated inflammation. *Hum Mol Genet* 2015;24:4958–4970

• **This study demonstrated that the immunomodulator fingolimod improved**

hippocampal synaptic plasticity and memory by restoring the balance between TrkB and p75^{NTR}, promoting CREB phosphorylation and preventing astrogliosis in R6/1 mice. Together with reference 113 (Di Pardo et al., 2014) showing improved motor function in fingolimod-treated R6/2 mice, this evidence supports the use of multi-purpose drugs in HD.

111. Brito V, Puigdemivol M, Giralt A, Del TD, Alberch J, Gines S. Imbalance of p75(NTR)/TrkB protein expression in Huntington's disease: implication for neuroprotective therapies. *Cell Death Dis* 2013;4:e595
112. Wehner AB, Milen AM, Albin RL, Pierchala BA. The p75 neurotrophin receptor augments survival signaling in the striatum of pre-symptomatic Q175WT/HD mice. *Neuroscience* 2016;324:297–306
113. Di Pardo A, Amico E, Favellato M, et al. FTY720 (fingolimod) is a neuroprotective and disease-modifying agent in cellular and mouse models of Huntington disease. *Hum Mol Genet* 2014;23:2251–2265
114. Shin J-Y, Fang Z-H, Yu Z-X, Wang C-E, Li S-H, Li X-J. Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. *J Cell Biol* 2005;171:1001–1012
115. Benraiss A, Wang S, Herrlinger S, et al. Human glia can both induce and rescue aspects of disease phenotype in Huntington disease. *Nat Commun* 2016;7:11758
116. Valor LM. Transcription, Epigenetics and Ameliorative Strategies in Huntington's Disease: a Genome-Wide Perspective. *Mol Neurobiol* 2015;51:406–423
117. Lonze BE, Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. *Neuron* 2002;35:605–623
118. Choi Y-S, Lee B, Cho H-Y, et al. CREB is a key regulator of striatal vulnerability in chemical

and genetic models of Huntington's disease. *Neurobiol Dis* 2009;36:259–268

119. Xie Z, Adamowicz WO, Eldred WD, et al. Cellular and subcellular localization of PDE10A, a striatum-enriched phosphodiesterase. *Neuroscience* 2006;139:597–607
120. Niccolini F, Haider S, Reis Marques T, et al. Altered PDE10A expression detectable early before symptomatic onset in Huntington's disease. *Brain* 2015;138:3016–3029
121. Heckman PRA, van Duinen MA, Bollen EPP, et al. Phosphodiesterase Inhibition and Regulation of Dopaminergic Frontal and Striatal Functioning: Clinical Implications. *Int J Neuropsychopharmacol* 2016;19:1–16
122. Kleiman RJ, Kimmel LH, Bove SE, et al. Chronic suppression of phosphodiesterase 10A alters striatal expression of genes responsible for neurotransmitter synthesis, neurotransmission, and signaling pathways implicated in Huntington's disease. *J Pharmacol Exp Ther* 2011;336:64–76
123. Beaumont V, Zhong S, Lin H, et al. Phosphodiesterase 10A Inhibition Improves Cortico-Basal Ganglia Function in Huntington's Disease Models. *Neuron* 2016;92:1220–1237
124. Giampa C, Laurenti D, Anzilotti S, Bernardi G, Menniti FS, Fusco FR. Inhibition of the striatal specific phosphodiesterase PDE10A ameliorates striatal and cortical pathology in R6/2 mouse model of Huntington's disease. *PLoS One* 2010;5:e13417
125. Giampa C, Patassini S, Borreca A, et al. Phosphodiesterase 10 inhibition reduces striatal excitotoxicity in the quinolinic acid model of Huntington's disease. *Neurobiol Dis* 2009;34:450–456
126. Harada A, Suzuki K, Kimura H. TAK-063, a Novel Phosphodiesterase 10A Inhibitor, Protects from Striatal Neurodegeneration and Ameliorates Behavioral Deficits in the R6/2 Mouse

Model of Huntingtons Disease. *J Pharmacol Exp Ther* 2017;360:75–83

127. Giralt A, Saavedra A, Carretón O, et al. PDE10 inhibition increases GluA1 and CREB phosphorylation and improves spatial and recognition memories in a Huntington's disease mouse model. *Hippocampus* 2013;23:684–695
128. Kleppisch T, Feil R. cGMP signalling in the mammalian brain: role in synaptic plasticity and behaviour. *Handb Exp Pharmacol* 2009;549–579
129. Saavedra A, Giralt A, Arumí H, Alberch J, Pérez-Navarro E. Regulation of Hippocampal cGMP Levels as a Candidate to Treat Cognitive Deficits in Huntington's Disease. *PLoS One* 2013;8:1–10
130. Thakur T, Sharma S, Kumar K, Deshmukh R, Sharma PL. Neuroprotective role of PDE4 and PDE5 inhibitors in 3-nitropropionic acid induced behavioral and biochemical toxicities in rats. *Eur J Pharmacol* 2013;714:515–521
131. Puerta E, Hervias I, Barros-Minones L, et al. Sildenafil protects against 3-nitropropionic acid neurotoxicity through the modulation of calpain, CREB, and BDNF. *Neurobiol Dis* 2010;38:237–245
132. McManus KJ, Hendzel MJ. CBP, a transcriptional coactivator and acetyltransferase. *Biochem Cell Biol* 2001;79:253–266
133. Nucifora FCJ, Sasaki M, Peters MF, et al. Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* 2001;291:2423–2428
134. Steffan JS, Kazantsev A, Spasic-Boskovic O, et al. The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc Natl Acad Sci USA* 2000;97:6763–6768

135. Steffan JS, Bodai L, Pallos J, et al. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. *Nature* 2001;413:739–743
136. Cong SY, Pepers BA, Evert BO, et al. Mutant huntingtin represses CBP, but not p300, by binding and protein degradation. *Mol Cell Neurosci* 2005;30:12–23
137. Suelves N, Kirkham-McCarthy L, Lahue RS, Ginés S. A selective inhibitor of histone deacetylase 3 prevents cognitive deficits and suppresses striatal CAG repeat expansions in Huntington's disease mice. *Sci Rep* 2017;7:1–15

• In this study, Suelves and colleagues demonstrated that inhibition of HDAC3 prevented hippocampal long-term memory impairments and corticostriatal-dependent motor learning deficits. Moreover, memory-related gene expression, striatal CAG repeat expansions and markers' expression and accumulation of mHtt oligomeric forms were all improved in drug-treated mutant mice. This is another example of a multiple-benefit therapy for HD, and also that pathological mechanisms operating in other brain regions are common to those in HD striatum.

138. Jia H, Wang Y, Morris CD, et al. The Effects of Pharmacological Inhibition of Histone Deacetylase 3 (HDAC3) in Huntington's Disease Mice. *PLoS One* 2016;11:e0152498
139. Chiu CT, Liu G, Leeds P, Chuang DM. Combined treatment with the mood stabilizers lithium and valproate produces multiple beneficial effects in transgenic mouse models of huntington's disease. *Neuropsychopharmacology* 2011;36:2406–2421
140. Valencia A, Reeves PB, Sapp E, et al. Mutant huntingtin and glycogen synthase kinase 3- β accumulate in neuronal lipid rafts of a presymptomatic knock-in mouse model of Huntington's disease. *J Neurosci Res* 2010;88:179–190
141. L'Episcopo F, Drouin-Ouellet J, Tirolo C, et al. GSK-3 β -induced Tau pathology drives

hippocampal neuronal cell death in Huntington's disease: Involvement of astrocyte-neuron interactions. *Cell Death Dis* 2016;7:e2206–14

142. Lim NKH, Hung LW, Pang TY, et al. Localized changes to glycogen synthase kinase-3 and collapsin response mediator protein-2 in the Huntington's disease affected brain. *Hum Mol Genet* 2014;23:4051–4063
143. Saavedra a, García-Martínez JM, Xifró X, et al. PH domain leucine-rich repeat protein phosphatase 1 contributes to maintain the activation of the PI3K/Akt pro-survival pathway in Huntington's disease striatum. *Cell Death Differ* 2010;17:324–335
144. Fernández-Nogales M, Hernández F, Miguez A, et al. Decreased glycogen synthase kinase-3 levels and activity contribute to Huntington's disease. *Hum Mol Genet* 2015;24:5040–5052
145. Valor LM, Guiretti D. What's wrong with epigenetics in Huntington's disease? *Neuropharmacology* 2014;80:103–114
146. Valor LM, Guiretti D, Lopez-Atalaya JP, Barco A. Genomic landscape of transcriptional and epigenetic dysregulation in early onset polyglutamine disease. *J Neurosci* 2013;33:10471–10482
147. Guiretti D, Sempere A, Lopez-Atalaya JP, Ferrer-Montiel A, Barco A, Valor LM. Specific promoter deacetylation of histone H3 is conserved across mouse models of Huntington's disease in the absence of bulk changes. *Neurobiol Dis* 2016;89:190–201
148. Luthi-Carter R, Taylor DM, Pallos J, et al. SIRT2 inhibition achieves neuroprotection by decreasing sterol biosynthesis. *Proc Natl Acad Sci U S A* 2010;107:7927–7932
149. Mielcarek M, Landles C, Weiss A, et al. HDAC4 reduction: a novel therapeutic strategy to target cytoplasmic huntingtin and ameliorate neurodegeneration. *PLoS Biol*

2013;11:e1001717

150. Ehrnhoefer DE, Sutton L, Hayden MR. Small Changes, Big Impact: Posttranslational Modifications and Function of Huntingtin in Huntington Disease. *Neurosci* 2011;17:475–492
151. Jeong H, Then F, Melia Jr. TJ, et al. Acetylation targets mutant huntingtin to autophagosomes for degradation. *Cell* 2009;137:60–72
152. Agrawal N, Pallos J, Slepko N, et al. Identification of combinatorial drug regimens for treatment of Huntington's disease using *Drosophila*. *Proc Natl Acad Sci* 2005;102:3777–3781
153. Ferrante RJ, Andreassen OA, Dedeoglu A, et al. Therapeutic effects of coenzyme Q10 and remacemide in transgenic mouse models of Huntington's disease. *J Neurosci* 2002;22:1592–9
154. McLear J a, Lebrecht D, Messer a, Wolfgang WJ. Combinational approach of intrabody with enhanced Hsp70 expression addresses multiple pathologies in a fly model of Huntington's disease. *FASEB J* 2008;22:2003–2011
155. Ryu JK, Choi HB, McLarnon JG. Combined minocycline plus pyruvate treatment enhances effects of each agent to inhibit inflammation, oxidative damage, and neuronal loss in an excitotoxic animal model of Huntington's disease. *Neuroscience* 2006;141:1835–1848
156. Khan A, Jamwal S, Bijjem KR V, Prakash A, Kumar P. Neuroprotective effect of hemeoxygenase-1/glycogen synthase kinase-3beta modulators in 3-nitropropionic acid-induced neurotoxicity in rats. *Neuroscience* 2015;287:66–77
157. Yang CR, Yu RK. Intracerebral transplantation of neural stem cells combined with trehalose ingestion alleviates pathology in a mouse model of Huntington's disease. *J Neurosci Res* 2009;87:26–33
158. Gillette C, Prunty L, Wolcott J, Broedel-Zaugg K. A new lexicon for polypharmacy:

Implications for research, practice, and education. *Res Soc Adm Pharm* 2015;11:468–471

159. Masnoon N, Shakib S, Kalisch-Ellett L, Caughey GE. What is polypharmacy? A systematic review of definitions. *BMC Geriatr* 2017;17:1–10
160. Molokhia M, Majeed A. Current and future perspectives on the management of polypharmacy. *BMC Fam Pract* 2017;18:1–9
161. Cadogan CA, Ryan C, Hughes CM. Appropriate Polypharmacy and Medicine Safety: When Many is not Too Many. *Drug Saf* 2016;39:109–116
162. Xu K-J, Song J, Zhao X-M. The drug cocktail network. *BMC Syst Biol* 2012;6:1–12
163. Xu Q, Xiong Y, Dai H, et al. PDC-SGB: Prediction of effective drug combinations using a stochastic gradient boosting algorithm. *J Theor Biol* 2017;417:1–7
164. Wu Z, Zhao XM, Chen L. A systems biology approach to identify effective cocktail drugs. *BMC Syst Biol* 2010;4
165. Ligeti B, Pénczváltó Z, Vera R, Gyorffy B, Pongor S. A network-based target overlap score for characterizing drug combinations: High correlation with cancer clinical trial results. *PLoS One* 2015;10:1–18
166. Huang H, Zhang P, Qu XA, Sanseau P, Yang L. Systematic prediction of drug combinations based on clinical side-effects. *Sci Rep* 2014;4:1–7
167. Hsu YC, Chiu YC, Chen Y, Hsiao TH, Chuang EY. A simple gene set-based method accurately predicts the synergy of drug pairs. *BMC Syst Biol* 2016;10
168. Murphy KP, Carter RJ, Lione LA, et al. Abnormal synaptic plasticity and impaired spatial cognition in mice transgenic for exon 1 of the human Huntington's disease mutation. *J Neurosci* 2000;20:5115–5123

169. Milnerwood AJ, Cummings DM, Dallérac GM, et al. Early development of aberrant synaptic plasticity in a mouse model of Huntington's disease. *Hum Mol Genet* 2006;15:1690–1703
170. Liu D, Yang Q, Li S. Activation of extrasynaptic NMDA receptors induces LTD in rat hippocampal CA1 neurons. *Brain Res Bull* 2013;93:10–16
171. Burnell ES, Irvine MW, Fang G, Sapkota K, Jane DE, Monaghan DT. Positive and Negative Allosteric Modulators of N-Methyl-D-Aspartate (NMDA) Receptors ; Structure-Activity Relationships and Mechanisms of Action Positive and Negative Allosteric Modulators of N-Methyl-D-Aspartate (NMDA) Receptors ; Structure-Activity . 2018
172. Xu J, Chatterjee M, Baguley TD, et al. Inhibitor of the tyrosine phosphatase STEP reverses cognitive deficits in a mouse model of Alzheimer's disease. *PLoS Biol* 2014;12:1–17
173. Chatterjee M, Kurup PK, Lundbye CJ, et al. STEP inhibition reverses behavioral, electrophysiologic, and synaptic abnormalities in Fmr1 KO mice. *Neuropharmacology* 2018;128:43–53
174. Saavedra A, Puigdellívol M, Tyebji S, et al. BDNF induces striatal-enriched protein tyrosine phosphatase 61 degradation through the proteasome. *Mol Neurobiol* 2016;53:4261–4273
175. Matsushita H, Matsuzaki M, Han X-J, et al. Antidepressant-like effect of sildenafil through oxytocin-dependent cyclic AMP response element-binding protein phosphorylation. *Neuroscience* 2012;200:13–18
176. Wang C, Zhang J, Lu Y, et al. Antidepressant-like effects of the phosphodiesterase-4 inhibitor etazolate and phosphodiesterase-5 inhibitor sildenafil via cyclic AMP or cyclic GMP signaling in mice. *Metab Brain Dis* 2014;29:673–682
177. Chawla S, Vanhoutte P, Arnold FJL, Huang CL-H, Bading H. Neuronal activity-dependent

nucleocytoplasmic shuttling of HDAC4 and HDAC5. *J Neurochem* 2003;85:151–159

178. Guedes-Dias P, Oliveira JMA. Lysine deacetylases and mitochondrial dynamics in neurodegeneration. *Biochim Biophys Acta* 2013;1832:1345–1359
179. Guedes-Dias P, de Proenca J, Soares TR, et al. HDAC6 inhibition induces mitochondrial fusion, autophagic flux and reduces diffuse mutant huntingtin in striatal neurons. *Biochim Biophys Acta* 2015;1852:2484–2493
180. Norflus F, Nanje A, Gutekunst C-A, et al. Anti-inflammatory treatment with acetylsalicylate or rofecoxib is not neuroprotective in Huntington's disease transgenic mice. *Neurobiol Dis* 2004;17:319–325
181. Alto LT, Chen X, Ruhn KA, Trevino I, Tansey MG. AAV-dominant negative tumor necrosis factor (DN-TNF) gene transfer to the striatum does not rescue medium spiny neurons in the YAC128 mouse model of Huntington's disease. *PLoS One* 2014;9:e96544
182. Khakh BS, Beaumont V, Cachope R, Munoz-Sanjuan I, Goldman SA, Grantyn R. Unravelling and Exploiting Astrocyte Dysfunction in Huntington's Disease. *Trends Neurosci* 2017;40:422–437
183. Hersch SM, Schifitto G, Oakes D, et al. The CREST-E study of creatine for Huntington disease. *Neurology* 2017;89:594–601
184. Travessa AM, Rodrigues FB, Mestre TA, Ferreira JJ. Fifteen years of clinical trials in Huntington's disease: A very low clinical drug development success rate. *J Huntingtons Dis* 2017;6:157–163

Figure legends

Figure 1. Neuronal dysfunction and imbalances in HD. Neuronal dysfunction preceding cell death appears in HD and contributes to motor and cognitive symptoms. It includes: neurotrophic receptor imbalance (TrkB/p75^{NTR}), aberrant extrasynaptic NMDAR signaling, mitochondrial morphology, localization and function abnormalities, and transcriptional dysregulation of a wide array of genes necessary for neuronal function. All these mechanisms interact and potentiate dysfunction. 1) Extrasynaptic NMDAR signaling, through aberrant calcium buffering, elicits 2) mitochondrial dysfunction, and 3) dysregulation of transcription by CREB shut-off and abnormal histone deacetylases (HDACs) activity. These processes 4) affect neurotrophic support, which in turn has been shown to increase deleterious extrasynaptic NMDAR signaling closing a vicious loop.

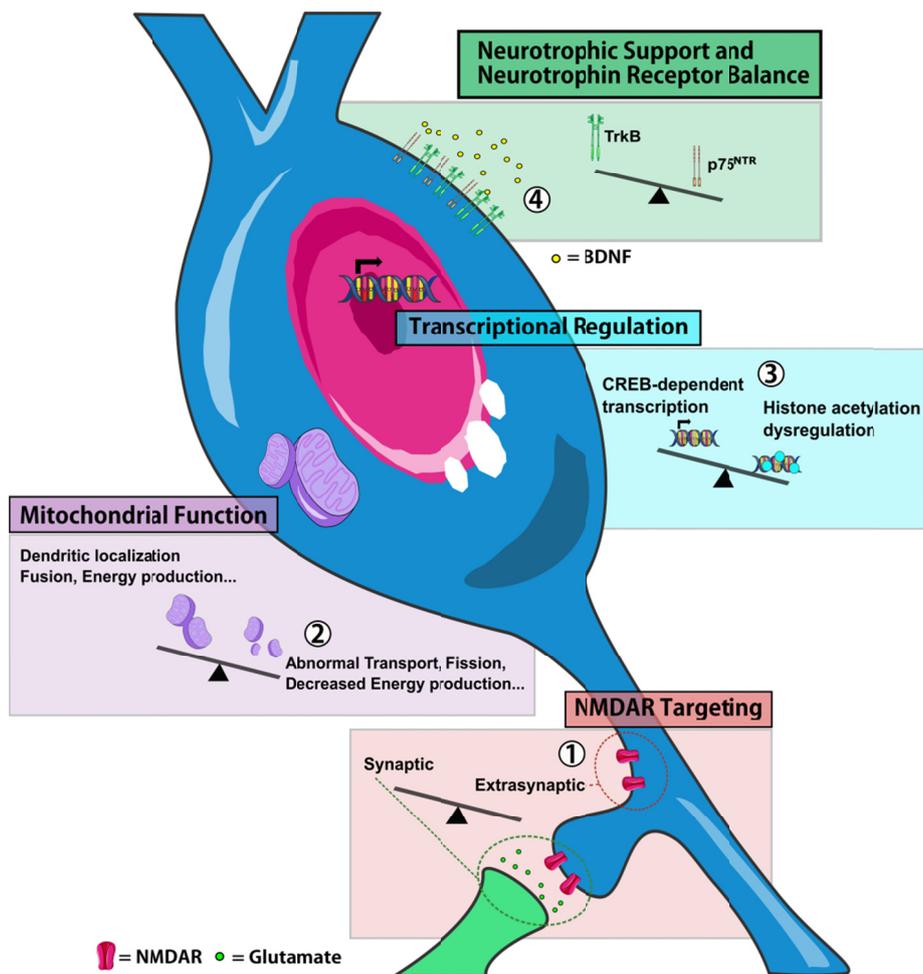
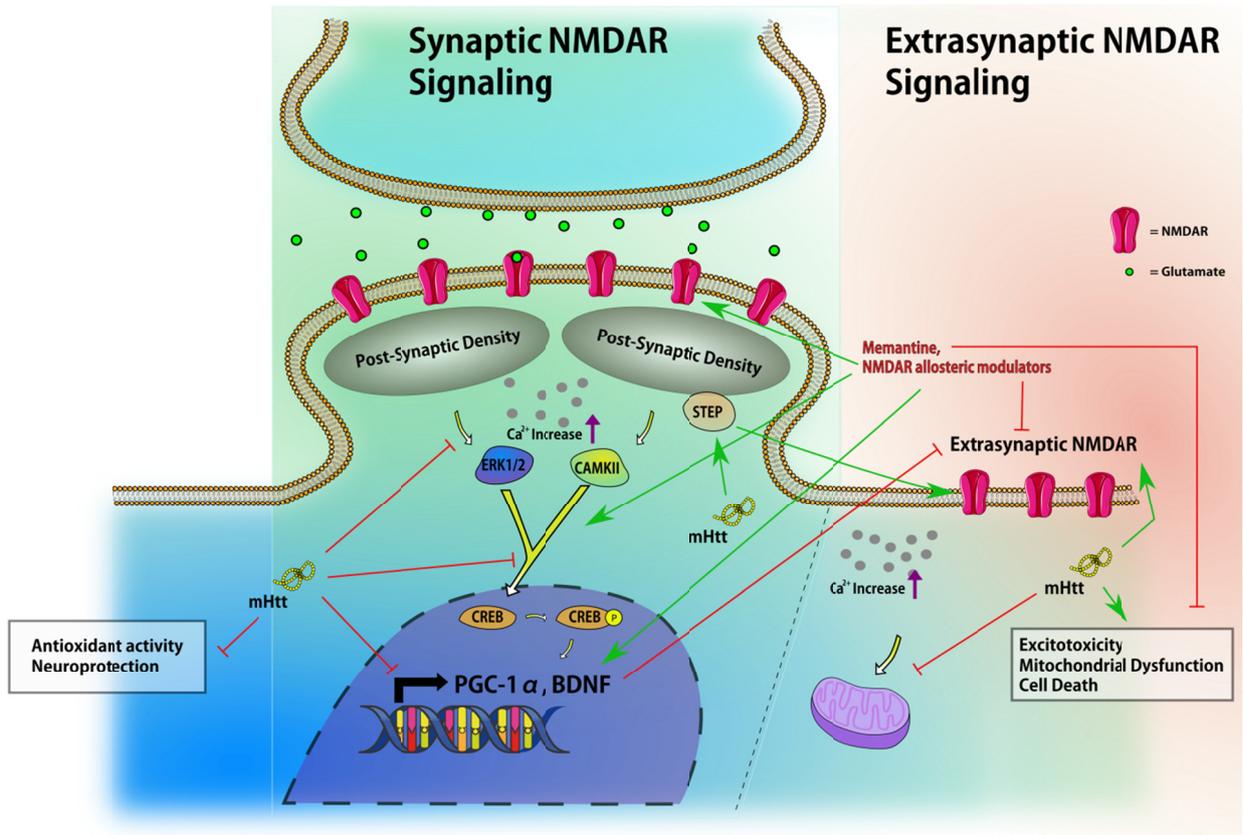


Figure 2. Synaptic/extrasynaptic NMDAR signaling in HD. Restoring the balance between synaptic and extrasynaptic NMDAR activity targets pathways deleteriously affected by mHtt. Memantine, as an antagonist of extrasynaptic NMDAR, holds the potential of ameliorating excitotoxicity, mitochondrial dysfunction and cell death elicited by mHtt. Additionally, memantine has been shown to improve synaptic activity which, through mechanisms as early gene response and neurotrophic-producing loops, can provide additional neuroprotection. Positive modulators of synaptic NMDAR and negative modulators of extrasynaptic NMDAR are also candidates to reestablish NMDAR signaling in the presence of mHtt.



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Figure 3. Neurotrophin signaling imbalance in HD. An imbalance between TrkB and p75^{NTR} has been recently established in HD patients and models. TrkB signaling through Erk/Akt and PLC γ 1 pathways promotes synaptic plasticity and neuroprotection that are impaired by interaction with mHtt. At the same time, mHtt affects p75^{NTR} signaling, increasing noxious outcomes of downstream effectors such as JNK and NF- κ B pathways. TrkB agonists or drugs that increase BDNF expression (7,8-DHF, LM22A-4 and fingolimod) favor TrkB downstream signaling, while 7,8-DHF, LM11A-31 and fingolimod reduce p75^{NTR} levels and/or signaling.

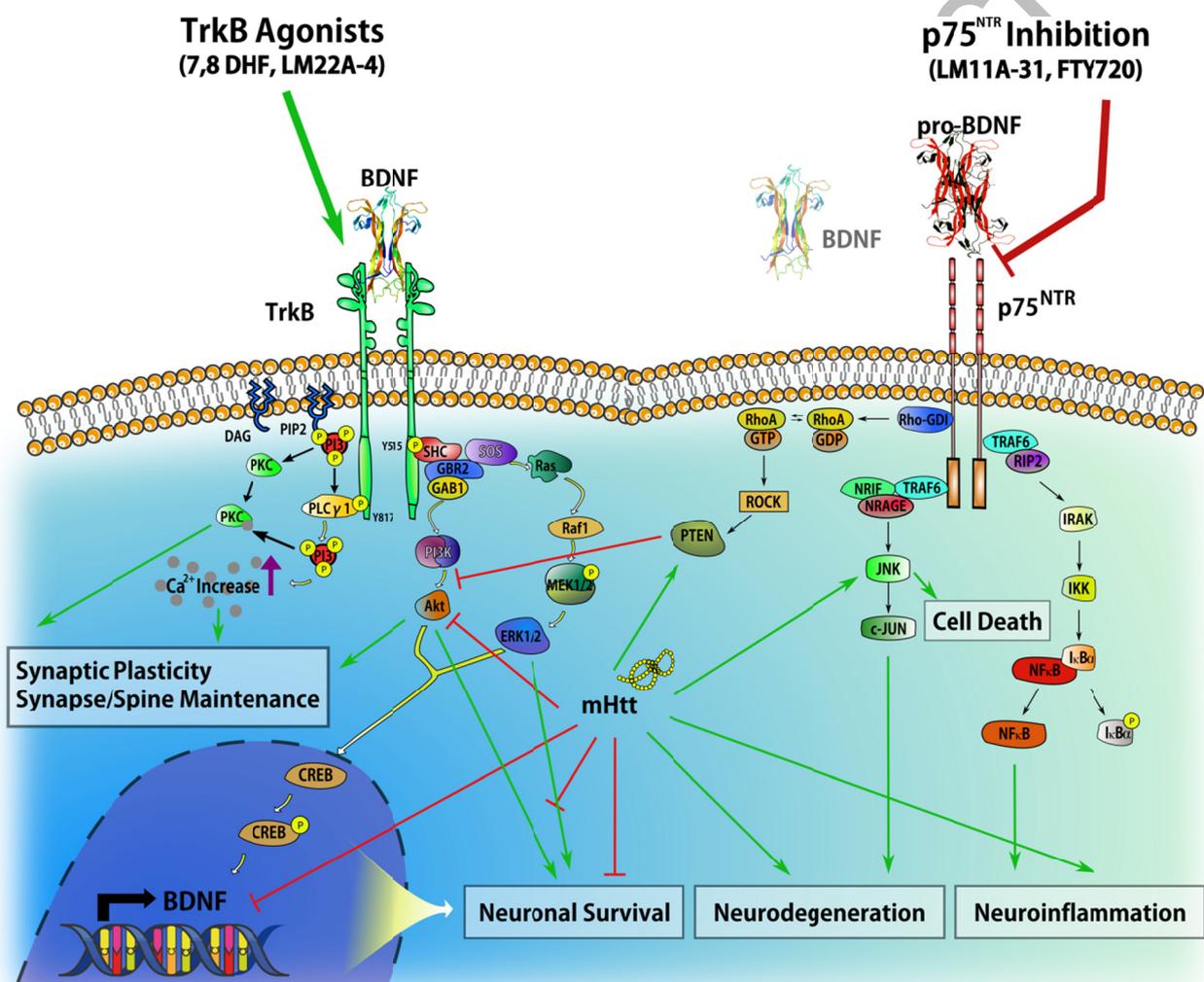


Table I. Therapeutic strategies based on improving mitochondrial function

Intervention	Mitochondrial improvement	Improvement			
		Motor	Cognitive	Psychiatric	Neuropathology
N-Acetylcysteine	No swelling Complexes activity ROS levels Membrane permeability Mitochondrial function	[55, 58] [59]*	[58]	[55, 60]	[58, 60] [59]*
Cdk-5 inhibition	Morphology and mitochondrial network		[77]**		[74]***
Drp1 inhibition	Fragmentation Transport Membrane potential ROS levels	[14]			[14]

* N-acetylcysteine in drinking water plus cysteine-enriched diet.

** The contribution of improved mitochondrial function was not investigated in this study.

*** *Cell biology in vitro assay.*

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Table II. Therapeutic strategies based on TrkB/p75^{NTR} balance.

Target	Intervention	Improvement			
		Motor	Cognitive	Neuropathology	Neuroinflammation
TrkB	LM22A-4	[104]		[104]	
	7,8-Dihydroxyflavone*	[106, 107]	[107]	[106, 107]	[107]
p75 ^{NTR}	FTY720**	[113]	[110]	[110, 113]	[110, 113]
	LM11A-31	[15]	[15]	[15]	[15]

* 7,8-Dihydroxyflavone is also reported to act through p75^{NTR} decrease mechanisms [107].

** FTY720 is also reported to increase BDNF levels [109].

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