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Review

Synaptic dysfunction in Huntington's disease: a new perspective

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Abstract. Huntington's disease (HD) is caused by a polyglutamine expansion in the protein huntingtin and is characterized by intraneuronal inclusions and widespread neuronal death at the late stage of the disease. In research, most of the emphasis has been on understanding the cell death and its mechanisms. Until recently, it was believed that the vast majority, if not all, of the symptoms in HD are a direct consequence of neurodegeneration. However, increasing evidence shows that subtle alterations in synaptic function could underlie the early symptoms. It is of particular interest to understand the nature of this neuronal dysfunction. Normal huntingtin interacts with various cytoskeletal and synaptic vesicle proteins that are essential for exocytosis and endocytosis. Altered interactions of mutant huntingtin with its associated partners could contribute to abnormal synaptic transmission in HD. This review describes recent advances in understanding synaptic dysfunction in HD.

Key words. Huntington's disease; neurotransmission; exocytosis; endocytosis; synaptic protein; pathophysiology.

Introduction

Huntington's disease (HD) is a genetic and progressive neurological disorder caused by a pathological expansion of a CAG repeat in the *huntingtin* gene [1]. Its prevalence is about 1 in 10,000, and the disease is predominant in Europe and North America [2]. Patients suffering from HD gradually develop abnormal, irrepressible movements, cognitive deterioration and psychiatric disturbances. The clinical features include choreiform involuntary movements, impaired coordination of voluntary movements, progressive dementia and personality changes. Psychiatric disturbances, particularly depression, frequently precede the onset of motor disturbance [3]. Typically, symptoms begin at 35–50 years of age. Some of the variation depends on the length of the polyglutamine stretch, and longer stretches are associated with earlier onset [4]. In the juvenile form of HD (<20 years), there are usually 70 or more repeats, and the clinical picture differs from adult-onset cases. Juvenile patients exhibit bradykinesia, rigidity and dystonia, but without chorea [2]. Most HD cases progress to death within around 15–20 years. The neuropathology is characterized by a gradual loss of specific neurons that becomes particularly extensive in the striatum (caudate-putamen), and to a lesser extent in the neocortex. Within the striatum, the most sensitive cell population is the medium spiny projection neuron, while adjacent interneurons apparently survive the mutation better [5].

Normal huntingtin function

Huntingtin is highly conserved from *Drosophila* to mammals, including humans, suggesting that it has a central function in cells. The protein is present not only in different types of neurons but also in a wide range of non-neuronal tissues and cells, including astrocytes [6, 7] and

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muscle [8, 9], testis [10] and endocrine tissues [11, 12]. A broad distribution within each cell type implies a function at multiple sites. Huntingtin is found in the cytoplasm and associates with synaptic vesicles, recycling endosomes, the endoplasmic reticulum, the Golgi complex and clathrin-coated vesicles [13–16]. The precise function of this protein is still unknown, and it contains few motifs with known function. The embryonic lethality of huntingtin knockout mice [17] suggests that huntingtin is essential for the function of vital organs. In agreement with this, wild-type huntingtin has been shown to prevent apoptosis of cultured cells. This effect is lost when the protein is mutated [18, 19].

Huntingtin has at least a dozen binding partners in the nucleus and the cytoplasm. They include proteins that play important roles in transcriptional regulation, intracellular trafficking and cytoskeletal organization [20], suggesting that huntingtin participates in vesicular transport and synaptic function (table 1). Huntingtin also interacts, via huntingtin-associated protein 1 (HAP1), with dynactin, a protein that is involved in axonal transport [21–24], and has been suggested to interact with microtubules and β-tubulin [25, 26]. Wild-type huntingtin promotes microtubule-based axonal transport [23, 24], whereas mutated huntingtin inhibits this process [23, 27, 28]. Taken together, these data support a role for huntingtin in intracellular membrane trafficking and in axonal transport along microtubules.

Effects of mutant huntingtin

HD mutation results in several fundamental cellular changes, including disturbance of the ubiquitin-proteasome system and disruptions in gene transcription, protein synthesis, mitochondrial function and protein-protein interactions. It has long been believed that these were solely 'gains of function' resulting in toxicity, but recent evidence suggests that a loss of normal huntingtin function also occurs. In the following section, we will briefly discuss how cell functions may be affected by mutant huntingtin.

Aggregation

One of the most obvious functions huntingtin gains by the mutation is its propensity to form aggregates. Appropriate protein folding is vital to living organisms. Chaperones normally assist in folding proteins into the correct conformation and in refolding misfolded proteins. When refolding fails, the misfolded protein can undergo ubiquitination. Polyubiquitination targets misfolded proteins to the proteasome, where they are degraded [29]. It has been suggested that mutant huntingtin can impair the ubiquitin-proteasome system by saturating it, either by providing excessive substrate or by directly inhibiting the proteasome [29–31]. Consequently, instead of being degraded, mutant huntingtin accumulates and forms insoluble intracellular aggregates. These aggregates are formed predominantly in the nucleus by N-terminal fragments of huntingtin [32], but they are also present in the cytoplasm and even in synaptic terminals [33]. The aggregates have been reported to recruit various proteins, such as dynamin, huntingtin interacting protein 1 (HIP1) and endophilin (SH3Gl3), which are involved in neurotransmission [34]; alpha-synuclein, which is an important synaptic protein [35]; components of the ubiquitin-proteasome system [36, 37] and heat shock proteins [38].

Table 1. Proteins interacting with huntingtin and involved in vesicle transport, release or uptake.

Protein	Binding region in huntingtin	Function	Effect of CAG mutation	Reference
β-tubulin	unknown	structural protein	no effect	[26]
CSP	unknown, probably N-terminal	inhibits N-type Ca^{2+} channel	required for interaction	[70]
Endophilins	exon 1, proline-rich region	involved in endocytosis	enhances binding	[76, 83]
HAP1	N-terminus	vesicle trafficking	enhances binding	[78]
HIP1	N-terminus	clathrin mediated endocytosis	decreased interaction	[75, 77, 82]
HIP1-related/ HIP12	binds to HIP1	links actin to clathrin	Similar to HIP1	[81, 82]
$InsP_3R1$	amino acid 1-158	calcium release channel	enhances binding	[71]
PACSIN 1	N-terminus, proline rich region	involved in endocytosis	enhances binding	[74]
PSD-95	N-terminal, proline rich region	regulates NMDA receptor activity	decreased binding	[90]

CSP, cysteine string protein; HAP1, huntingtin-associated protein 1; HIP1, huntingtin-interacting protein 1; HIP12, huntingtin-interacting protein 12; $InsP_3R1$, inositol-(1,4,5) triphosphate receptor type 1; PACSIN 1, PKC and CK2 substrate in neurons 1; PSD-95, postsynaptic density 95; NMDA, N-methyl-D-aspartate.

It has also been demonstrated that aggregates can bind to and trap transcription factors, such as CREB binding protein (CBP), thereby downregulating CBP-mediated transcription [39, 40].

Transcriptional effects

Gene transcription is normally regulated by a large number of transcription factors. Mutant huntingtin has been shown to disrupt some of these processes ([40–45], reviewed in [46, 47]). Interestingly, there are also reports of mutant huntingtin enhancing transcription via a cAMP response element (CRE)-dependent mechanism [48]. The effects of mutant huntingtin on transcription are considered due not merely to sequestration of transcriptional factors into aggregates but also to a direct effect of the soluble protein, because recruitment into aggregates does not necessarily deplete the cells of transcription factors [49]. In addition to changes in the protein level of transcription factors, alterations in the activity of transcription factors could also contribute to the transcriptional alterations described in models of the disease [8, 42, 50–52].

Apart from a pure gain of function, there is increasing evidence for a simultaneous loss of function of the wild-type huntingtin protein. Zuccato and co-workers demonstrated a decreased interaction between mutant huntingtin and the transcriptional silencing factor called neuron-restrictive silencing factor (NRSF). Instead of binding strongly to wild-type huntingtin in the cytosol, NRSF binds poorly to mutant huntingtin. This in turn leads to a diminished cytoplasmic scaffolding of the silencing factor and an increase of the protein in the nucleus [43]. Among the genes controlled by this transcriptional regulator are the *BDNF* gene and several genes for proteins involved in synaptic transport and postsynaptic transductions [53].

Apart from transcriptional changes evidence also suggests changes in protein-protein interactions due to the presence of an extended CAG-repeat (reviewed in [20, 54]). In the following sections, we will mainly discuss changes in protein-protein interactions that take place in the presynaptic and postsynaptic regions (table 1).

Alterations of synaptic components in HD

One of the key functions of neurons is transmitter release and communication. Normal communication between neurons is regulated by a number of proteins in the synapse (for reviews see [55–57]). Huntingtin is highly expressed in the presynaptic terminals of nerve cells [13]. The huntingtin mutation has an important impact on the expression levels of synaptic proteins and on the interaction between huntingtin itself and its binding partners. First, as described above, the effect on protein expression could be due to an influence of mutant huntingtin on transcription factors or other proteins affecting gene transcription [40–45]. Second, the proteins themselves could be affected directly by mutant huntingtin, as has been suggested by recruitment of different proteins into mutant huntingtin inclusions [34, 58]. Under these circumstances, protein levels could be reduced locally at their site of action, even if the total amount of the protein within the cell remains normal. Third, mutant huntingtin can change post-translational modifications of the proteins once they are formed, e.g. by affecting the state of phosphorylation [59]. Finally, in some cases mutant huntingtin may affect the functions of a given protein and its normal interaction partners by changing the binding affinity without affecting their expression levels, phosphorylation states, or sequestering them in aggregates (reviewed in [20, 54]).

Mutant huntingtin affects the exocytotic process

Numerous proteins are involved in the execution of the tightly regulated process of exocytosis. Various proteins involved in different steps of this process are affected in animal models and in postmortem brain tissues of HD (fig. 1). Complexin II is involved in neurotransmitter release by interacting with the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) complex, which regulates membrane fusion between the synaptic vesicle and the presynaptic plasma membrane [60]. This protein is decreased in HD patients [61], R6/2 transgenic mice [62] and PC12 cells expressing mutant huntingtin [63]. Furthermore, the phenotype caused by reduction of complexin II in the PC12 cell model can be partially reversed by overexpressing complexin II [63], which results in normalization of neurotransmitter release from the PC12 cells. Moreover, complexin II knockout mice exhibit a phenotype with motor impairment and learning deficits [64]. This is somewhat reminiscent of HD, and loss of complexin II could underlie some of the symptoms of HD. Another protein involved in priming and docking of vesicles to the plasma membrane is rabphilin 3A. In our work, we have observed that the protein and messenger RNA (mRNA) levels of rabphilin 3A are decreased in the R6/1 model of HD. This decrease is progressive and coincides with the onset of symptoms [65]. Decreased mRNA levels for rabphilin 3A in cultured adrenal chromaffin cells reduce stimulated exocytosis [66], indicating an important function of the protein in exocytosis. By attaching synaptic vesicles to the actin [67] and microtubule cytoskeletons [68], the synaptic protein synapsin 1 is believed to regulate the association of synaptic vesicles to the cytoskeleton. Its activation is dependent on phosphorylation at six different sites. Phosphorylation at these sites is regulated by

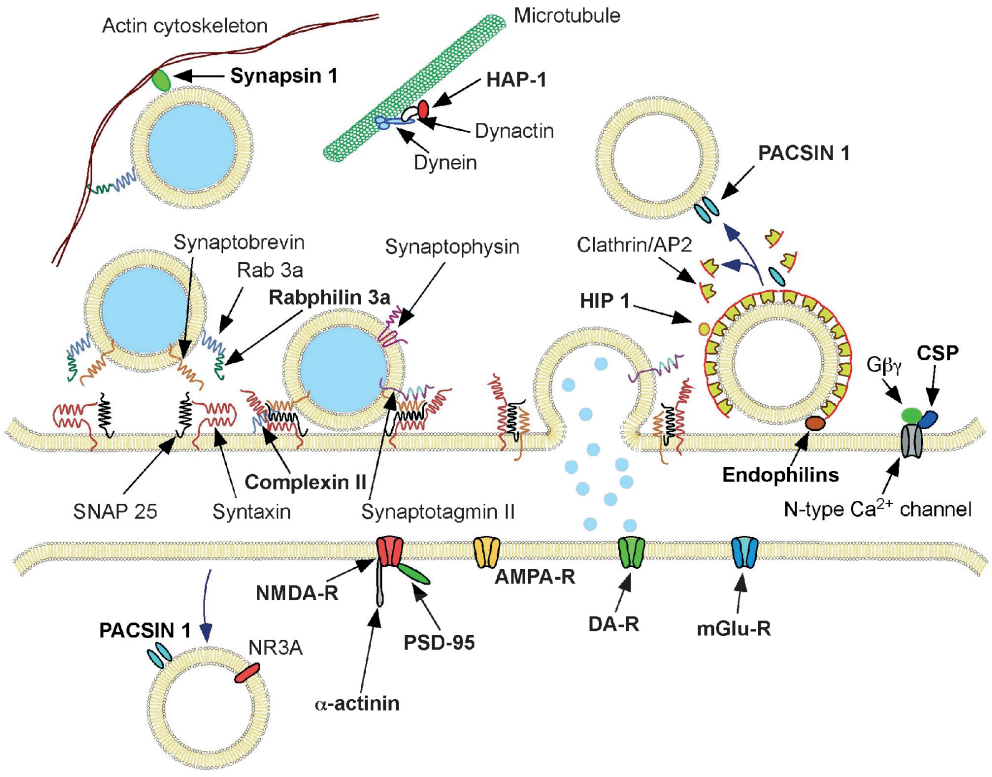

Figure 1. Schematic drawing of proteins involved in exocytosis, endocytosis and signaling at the synapse. Proteins in boldface have been reported to be involved in HD.

calcium/calmodulin-dependent protein kinase (CaMK) II, CaMKIV, extracellular-signal-related kinase (ERK)-1 and ERK2. Dephosphorylation is dependent on the action of calcineurin and protein phosphatase 2A (for details see [59]). Lievens and co-workers showed that phosphorylation at sites 3–5 in synapsin I is changed in the R6/2-mouse model of HD. They also reported an increase in ERK phosphorylation in the striatum of old R6/2 mice and a decrease in the levels of calcineurin-B [59]. Phosphorylation of synapsin I at sites 3–5 leads to a reduced affinity to actin filaments [59] and thereby to a possible decrease of the reserve pool of vesicles.

Microarray studies have previously revealed decreased transcription of many different subunits of protein kinases and protein phosphatases in mouse models of HD [8, 51]. Changes in protein kinases and phosphatases may have profound effects on protein function, and subsequent alterations in the phosphorylation state of synaptic proteins could partially underlie the defects in cell signaling seen in animal models of HD.

Ca^{2+} regulation is of great importance in synaptic neurotransmitter release. Increased intracellular levels of calcium are present in R6/2 animals compared with wild-type controls [69]. Two studies have demonstrated altered interactions between huntingtin and proteins involved in calcium influx into the cytosol. Miller and colleagues found that the cysteine string protein (CSP) interacts only with mutant, and not with the normal form of huntingtin. CSP is a chaperone present in synaptic terminals and is a tonic inhibitor of an N-type calcium channel [70]. The interaction between CSP and mutant huntingtin inhibits the normal function of CSP, thereby releasing its tonic inhibition of the channel and increasing calcium influx [70]. Furthermore, Tang and co-workers demonstrated that huntingtin and HAP1 interact with inositol-(1,4,5) triphosphate receptor type 1 ($InsP_3R1$). Mutant huntingtin sensitizes the receptor to $InsP_3$, thereby more readily activating the receptor and resulting in increased calcium release from the endoplasmic reticulum [71]. Production of $InsP_3$ is initiated through signaling via the mGlu5 receptor, which is expressed at normal levels in the R6 transgenic model of HD [72].

Influence of mutant huntingtin during the endocytic process

After synaptic vesicles fuse with the presynaptic plasma membrane, the membrane is retrieved at highly active endocytotic sites located adjacent to the active zone. Presumably, a balance exists between membrane fusion and retrieval, which ensures that neurotransmitters can be

released continuously. Several huntingtin-interacting and huntingtin-associated proteins are involved in the process of endocytosis; therefore, reductions or defects of these proteins will certainly impair endocytotic and intracellular transport pathways [22, 73–77] (fig. 1). The most intensely studied of these proteins in relation to HD is HAP1 [78]. HAP1 interacts with the $p150^{Glued}$ subunit of dynactin [21, 22]. Dynactin, in turn, interacts with dynein, the motor protein involved in retrograde transport. These two proteins need to interact in order for vesicular transport to take place along microtubules [79]. Binding between HAP1 and huntingtin is enhanced by an expanded polyglutamine stretch [78]. The increased affinity between mutant huntingtin and HAP1 seems to deplete HAP1 from its normal functional site at the dynein/dynactin complex. As a result, axonal transport along microtubules decreases [24]. HAP1 has also been suggested to play a role in endocytosis of the epidermal growth factor receptor (EGFR), thus implying an important role in neuronal cell survival [80].

HIP14 is a neuronal protein that is localized mainly to the Golgi apparatus and cytoplasmic vesicles and that interacts with huntingtin [73]. Yeast lacking a protein with a high sequence similarity to HIP14, called Akr1p, exhibit an endocytotic defect that is rescued by overexpressing HIP14 [73]. This suggests that HIP14 plays a role in endocytosis. There is an inverse correlation between the length of the polyglutamine stretch and the strength of the interaction between huntingtin and HIP 14. The levels of HIP14 in cells expressing mutant huntingtin are still normal [73]. Possibly, the decreased interaction between mutant huntingtin and HIP14 impairs normal intracellular trafficking in neurons. PACSIN 1 (PKC and CK2 substrate in neurons 1)/syndapin, another synaptic vesicle protein that is involved in endocytosis, is affected in brain tissue from HD patients [74]. PACSIN 1/syndapin levels are normal in HD, but the protein is relocalized from the nerve terminals to the cell bodies [74]. Moreover, the interaction between huntingtin and PACSIN 1 is enhanced by an expanded polyglutamine repeat.

Several additional proteins interact with huntingtin and appear to be involved in synaptic function. HIP1 was identified in yeast two-hybrid systems [75, 77], and the closely related HIP12 was identified because of its sequence homology to HIP1. Both proteins are orthologs of the yeast protein Sla2p, which is involved in endocytosis [81]. HIP1 interacts with clathrin and AP-2, whereas HIP12 interacts primarily with F-actin and to some degree with the clathrin light chain [82]. The interaction between huntingtin and HIP1 inversely correlates with CAG repeat length. Moreover, the proteins SH3Gl3 [76] and endophilin B1b [83] bind to huntingtin. They belong to the endophilin family of proteins and have a role in vesicle endocytosis. The interaction between SH3Gl3 and huntingtin is dependent upon the length of the CAG expansion and promotes the formation of insoluble polyglutamine-containing aggregates [76], suggesting that SH3GL3 could be directly involved in HD pathogenesis.

A recent study revealed that internalization of transferrin is impaired in cells with huntingtin aggregates [34]. The paper also described the composition of cytoplasmic huntingtin aggregates. There are clear differences in the proteins found in the core and shell of the aggregates. The fibrillar core of the aggregate resists protease treatment and contains cathepsin D, ubiquitin and HSP 40. In contrast, the outer layer of the aggregates is protease-sensitive and contains HSP 70, parts of the proteasome, dynamin, HIP1, SH3GL3 and other proteins. The levels of these proteins are markedly reduced in cellular sites where they normally function, such as nerve terminals, and are instead found in the aggregates [34]. This suggests that accumulation of mutant huntingtin in the cytoplasm contributes to neuronal dysfunction and synaptic impairment.

Postsynaptic effects of mutant huntingtin

Changes in glutamatergic neurotransmission resulting in excitotoxic cell damage have long been suggested to be a key event in HD pathogenesis. Microdialysis studies have suggested an increased release of glutamate in the striatum of R6 transgenic mice [84, 85] and a deficient clearance of glutamate by the glial cell glutamate transporter GLT1 [85]. Moreover, the mRNA and protein levels of the metabotropic glutamate receptors 1, 2 and 3 are reduced in R6 mice [72]. The mGlu2 receptor (mGlu2R) is located presynaptically [86] and regulates glutamate release [87]. Therefore, a reduction of this receptor could lead to an increased release of glutamate due to decreased feedback control. Taken together, changes in GLT1 and mGlu2R probably significantly contribute to the increase of glutamate release seen in HD mouse models.

How does this increased release of glutamate affect the postsynaptic neuron? Some studies demonstrate an increased sensitivity of glutamatergic neurons to glutamate and a hyperexcitability of the N-methyl-D-aspartate (NMDA) receptors in HD models [88, 89]. In addition, postsynaptic proteins are affected. More precisely, the protein postsynaptic density 95 (PSD-95) was reported to mediate an interaction between huntingtin and NMDA-receptor subunits [90]. This interaction was decreased when huntingtin was mutated. It is hypothesized that a decreased scaffolding of PSD-95 by mutant huntingtin leads to an increase in the interaction between PSD-95 and NMDA receptors, in turn increasing their presence and activity at the outer plasma membrane [90]. However, the mRNA and protein levels of PSD-95 are reduced in R6/2 mice [91], which could partially explain the decreased levels of PSD-95 found in immunoprecipitates of

human cortex [90], as opposed to them being due to a decreased affinity of mutant huntingtin to PSD-95.

The picture regarding the activity and levels of NMDA receptors at the postsynaptic plasma membrane in HD is not clear. In R6/2 mice, two NMDA receptor subunits (NR2A and NR2B) are reduced at the mRNA and protein level [91], and the NR1 and NR2B subunits are reduced in the human HD striatum [92]. In addition, α-actinin 2, which is involved in anchoring NMDA receptors to the actin cytoskeleton [93] and which interacts with rabphilin 3A [94], is also reduced in R6/2 mice [91]. In contrast, in the YAC72 transgenic mouse HD model that expresses full-length mutant huntingtin, NR2B receptor subunits are normally expressed and hyperactive [89, 95]. Finally, in N171-82Q mice, expressing the first 171 N-terminal amino acids of huntingtin plus 82 glutamine residues, NMDA receptor levels are normal, but the effector proteins of NMDA receptors and PSD-95-like proteins are reduced [96]. There is a similar scenario regarding sensitivity to various excitotoxins. Sensitivity to excitotoxins is decreased [69, 96–99], increased [88, 95, 100] or unchanged [101] in different mouse models of HD when compared with wild-type control mice. The mechanisms behind these differences are still not clear. However, available evidence suggests that the following factors may contribute. First, there is a close correlation between sensitivity to excitotoxins and the length of huntingtin expressed in different models. So far most, if not all, resistance phenomena to neurotoxins were observed in transgenic mice expressing only the N-terminus of huntingtin [69, 96–99]. R6 mice, for example, express only 3% of the full length of huntingtin [102]. Another HD mouse model that expresses 33% of the full-length huntingtin retains sensitivity to excitotoxins with no change in susceptibility compared with wild-type littermates [101]. In contrast, YAC72 mice expressing full-length huntingtin showed an increased sensitivity to excitotoxins [95, 100]. Second, sensitivity is age-related. Decreased sensitivity develops gradually and is consistent with the onset of motor deficits in R6/1 and R6/2 mice [69, 96, 98]. In YAC72 mice, an the increased sensitivity to quinolinate is evident at 6 months of age, but not at 11 months [95, 100]. Third, there is a correlation between aggregate formation and sensitivity. In R6 transgenic mice, development of resistance coincided with the appearance of nuclear inclusions [69]. Fourth, age of onset of altered sensitivity is inversely correlated with the length of the CAG repeat. Thus, resistance in R6/2 mice (155 CAG repeats) developed much more rapidly than in R6/1 (115 CAG repeats) mice [98]. In addition, genetic factors coupled with the background mouse strain and the expression level of the mutant protein could conceivably also affect altered sensitivity to excitotoxins.

In HD, changes occur not only in NMDA and mGlu receptors. Indeed, alpha-amino-3-hydroxy-5-methyl-4-propionate (AMPA), kainate and the dopamine receptors D1 and D2 have decreased ligand binding activity in the R6/2 mouse model of HD [72]. The mRNA and protein levels of mGlu2 and 3 receptors and the mRNA levels of D1 and D2 receptors are also downregulated [72]. Moreover, the dopamine release defect discussed above [103] and reports of dysregulated postsynaptic dopamine signaling [104, 105] clearly indicate a dysfunctional cell-cell communication in the dopaminergic pathways. Thus, there is extensive evidence for changes in postsynaptic mechanisms in HD. The emerging picture is complex, and it appears that mutant huntingtin has multiple effects on neurotransmission that cannot be explained by a single underlying pathogenetic mechanism.

Neuropathology in HD

The brains of HD patients show not only progressive loss of neurons but also development of neuronal intranuclear inclusions in surviving cells. Misfolded huntingtin accumulates in the cytoplasm and nucleus, leading to these aggregates. They usually contain several other proteins, including components of the ubiquitin-proteasome system, chaperones, synaptic proteins and transcription factors [34, 47]. In patients who died after several years of adult-onset HD, only around 3–6% of remaining cortical neurons exhibit inclusions in the nucleus, cytoplasm or neurites [2]. Over 50% of the striatal and around 20% of the cortical neurons have died in end-stage patients. What, then, could be the role of the inclusions? The inclusions might precede cell death, and neurons displaying inclusions at the time the patient passes away could simply be in the process of cell death. Alternatively, neurons exhibiting protein aggregates have actively evaded death by sequestering the mutant protein. Indeed, it has been suggested that inclusions are protective against the toxicity of expanded polyglutamine proteins [106]. Regarding the anatomical distribution of cell death, the most marked neuronal loss occurs in the caudate nucleus and putamen [5], as well as in layers III, IV and VI of the cerebral cortex [2]. A few studies have also described clear neuronal loss in the lateral tuberal nucleus of the hypothalamus [107, 108]. Interestingly, in a few cases clinical symptoms of HD have been described before the appearance of neuronal loss [5, 109, 110]. These cases are highly debated, and it is not clear whether they are exceptional or represent the norm. For example, Vonsattel and co-authors report one case with clinical symptoms as having a normal number of neurons and another case as having a decreased cell number in the head of the caudate nucleus [2]. The symptoms could be explained by synaptic dysfunction in cells affected by mutant huntingtin. This view is supported by experimental evidence showing abnormal dendrites in human patients with HD [111, 112] and

lower numbers of dendritic spines in a transgenic mouse model of HD [113]. In addition, recent work demonstrates that a number of proteins involved in the synaptic machinery are affected in HD models (see discussion above) [54]. Thus, both structural and biochemical synaptic changes are likely to contribute significantly to the symptoms of HD, even in the absence of cell loss.

Animal models of HD

Following the identification of the *huntingtin* gene in 1993, a number of genetically modified animals and cells have been generated in attempts to model HD [42, 89, 102, 114–117]. They vary in several parameters, making each of them unique. For example, the length of the CAG repeat and the size of the mutant protein (a fragment or the full-length huntingtin protein) differ among models. Another important parameter is the promoter that drives expression of the mutant huntingtin. The promoter is important for determining in which cell types the construct will be expressed, as well as the levels of expression. Exogenous promoters have been used: e.g. the human *huntingtin* promoter, the mouse prion protein promoter and cytomegalovirus (CMV) promoters. In contrast, in knockin mouse models mutant huntingtin expression is governed by the endogenous mouse promoter, preventing abnormal overexpression of the mutant protein. The expression level of mutant protein probably plays a role in dictating the speed of development of HD pathology. Among the different HD models, R6/1 and R6/2 transgenic mice are the first and the best-characterized transgenic mouse models. Both express *exon 1* of the human *huntingtin* gene, with around 115 and 150 CAG repeats, respectively [102]. R6 mice express only *exon* 1 (from a total of 67 exons in the whole gene), coding for about only 3% of the N-terminal region of the protein, which includes the polyglutamine stretch [102]. Transgene expression is driven by the human *huntingtin* promoter, resulting in expression levels of 31% and 75% of endogenous huntingtin in R6/1 and R6/2 mice, respectively. These mice mimic some of the human HD symptoms, including motor dysfunction, muscle wasting and some neuropathological changes, such as aggregate formation in the cortex and neostriatum. Interestingly, in the striatum and neocortex they exhibit only brain atrophy, without clear cell loss. In contrast, in the hypothalamus of the R6/2 mouse we recently observed a progressive loss of orexin [118] and gonadotrophin-releasing hormone containing neurons [unpublished results]. Other mouse models display clear neurological symptoms but only very limited cell loss [89, 115–118] (reviewed in [47, 114]). Despite a relatively low number of hypothalamic neurons dying in their brains, R6 mice exhibit widespread evidence of brain dysfunction. For example, expression of numerous genes progressively changes in the striatum and cortex of R6/2 mice from 6 weeks of age [119]. Notably, some striatal signaling genes induced by cAMP (cyclic AMP) and retinoic acid receptor are downregulated, whereas other genes associated with cell stress and inflammation (e.g. DNA repair enzymes) are upregulated. The concept that striatal cells in R6 mice undergo cell stress is supported by findings of an increase in markers for oxidative damage to DNA [120], transient increases in superoxide dismutase activity [121] and reduction in mitochondrial function [122]. In addition, there is evidence for increased nitric oxide synthase activity, at least transiently, in the striatum of R6/1 and R6/2 mice [122–125]. Cultured striatal neurons from R6/2 mice form autophagic vacuoles in response to an oxidative insult more readily than control cells, also suggesting a change in fundamental mechanisms related to the cell stress response [126].

There is direct evidence for malfunction of the neuronal circuitry. In R6/2 mice, striatal neurons exhibit more depolarized resting potentials [88] and increased intracellular calcium levels [69] compared with wild-type controls, and there are changes in the firing patterns of corticostriatal fibers [127]. Studies using the intracerebral microdialysis technique demonstrate changes in neurotransmitter release in R6 mice. They describe possible alterations in the process of neurotransmitter release or reuptake, sometimes with no concomitant changes in the cellular capacity to synthesize and store neurotransmitters. [84, 85, 103, 128]. For example, in the striatum there is a reduction of extracellular dopamine [103] and an increase of extracellular glutamate following stimulation [84, 85] at ages when the tissue levels of these transmitters are normal. At later stages, the striatal tissue levels of dopamine are reduced in R6/2 mice [129]. Taken together, the complex changes in neurotransmission observed in R6 mice are difficult to explain by a single pathophysiological change. Most likely there are changes at multiple levels in neurons, as well as in glia. Thus, at different disease stages in R6/1 and R6/2 mice, the capacity to synthesize neurotransmitters such as dopamine and serotonin is reduced [130–132], levels of synaptic proteins are changed [59, 61, 62, 74] and the main glial transport system that normally removes glutamate released in synapses is reduced [128]. Moreover, postsynaptic elements involved in neurotransmission undergo marked changes. Dopamine receptors and their downstream signaling partners are reduced [105, 133, 134], and different subtypes of glutamate receptors change [135].

Conclusion and perspectives

Synaptic dysfunction in HD is profound and appears to be due to changes at multiple levels, from gene transcription to post-translational modifications. These changes

could act synergistically and affect neurotransmission in early HD, before widespread neurodegeneration occurs. Thereby, the synaptic changes could underlie cognitive and motor dysfunctions. Ultimately, they could directly contribute to cell death via excitotoxicity or loss of neurotrophic support, or simply by functionally disconnecting the neurons. Importantly, a dysfunctional connection between the cortex and the striatum could possibly lead to striatal deprivation of important neurotrophic factors, e.g. brain-derived neurotrophic factor (BDNF), which is normally supplied by the cortex. A deeper understanding of neuronal dysfunction, including the synaptic defects in HD, could potentially open a window for therapeutic intervention.

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