Chapter 10
The Role of Astrocytes in Huntington's Disease

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Abstract Huntington's disease (HD) is a progressive autosomal dominant neurode-generative disorder characterized by psychiatric disturbances, cognitive impairment and choreiform movements. It is caused by a repeat expansion in the gene encoding the widely expressed protein HTT. This protein is present throughout the nervous system in neuronal and non-neuronal cell types. The mutant HTT (mHTT) protein has been implicated in multiple cellular processes. To date, however, no single mechanism has been shown to be the primary mechanism that leads to neuronal dysfunction and death. Instead, it is believed that multiple mechanisms together may contribute to HD pathogenesis. At present, there is no effective neuroprotective treatment for HD. Although mHTT is found in astrocytes, most of the focus to date has been on understanding processes that may be dysfunctional in neurons. Nonetheless, there is substantial evidence for abnormal astrocytes in HD. In this chapter we present a review of the current understanding of astrocyte involvement in HD. We describe observations made in HD patients concerning morphological and molecular changes in astrocytes as disease progresses. Additionally, we describe the recapitulation of some of the phenotypes observed in HD patients in various mouse models expressing the mHTT protein. Together, the data from patients and the mouse models strongly implicate astrocyte-specific mechanisms as players in the pathogenesis of HD.

Keywords Astrocytes · Huntington's disease · Mutated huntingtin · Glutamate · Excitotoxicity

Abbreviations

CAG   Cytosine-adenosine-guanine
CPN   Cortical pyramidal neurons
CT   Computed tomography
EAAT2   Excitatory amino acid transporter 2
GFAP   Glial fibrillary associated protein
GLT-1   Glutamate transporter 1

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GS	Glutamine synthetase
HD	Huntington's disease
Hdh	Huntington disease homologue
HTT	Huntingtin
MAP2	Microtubule associated protein
MRI	Magnetic resonance imaging
MSN	Medium spiny neurons
PC	Pyruvate carboxylase
PET	Positron emission tomography
polyQ	Polyglutamine
TCA	Tricarboxylic acid cycle

10.1 Introduction

Huntington's Disease (HD) is a progressive and fatal neurodegenerative disorder characterized by motor dysfunction, psychiatric disturbances and cognitive impairment for which we have no neuroprotective therapies. The age of onset of HD is in the mid-forties, with death usually occurring 15–20 years after diagnosis (Gomez-Tortosa et al. 2001). The first published clinical description of HD was by George Huntington in 1872 in which he described sufferers of this disease quite accurately (Huntington 2003). He described a "*hereditary* chorea" where chorea is defined by "*dancing* propensities", and "with no loss of volition attending these contractions….the will is there, but its power to perform is deficient". He noted that the disease appeared hereditary in nature, with a tendency to insanity and suicide and that it manifested in adult life (Huntington 2003).

These descriptions of the disease, which now bears his name, have stood the test of time with more information having been added over time. To date, the clinical diagnosis of HD is based on the development of the classic movement deficit, chorea, which can present in combination with other movement deficits, including dystonia and bradykinesia. While the motor symptoms are the most visible symptoms of HD, the cognitive and behavioral problems are also very prominent in this disease. The cognitive changes can manifest early in the disease process and can include deficits in emotional recognition, time production, and speed of initiating thought processes (Hinton et al. 2007). HD patients also display changes in learning and working memory (Solomon et al. 2007). They have difficulties in learning new information and in memory recall (Montoya et al. 2006).

10.2 Huntington's Disease Genetics

HD is one of the most common single gene dominantly-inherited neurodegenerative disorders. While it is rare, meta-analysis of studies of prevalence worldwide shows it affects 2–3 persons per 100,000. In areas with populations largely of European

descent, the overall prevalence is 5–6 persons per 100,000 (Pringsheim et al. 2012). HD is caused by a repeat expansion in the highly conserved huntingtin (*HTT*) gene (Group 1993). The triplet repeat, cytosine-adenosine-guanine (CAG), is found in exon 1 of the gene and encodes glutamine. The disease allele results in the production of an expanded polyglutamine (polyQ) stretch in the N-terminal portion of the large 3144 amino acid protein (Group 1993; Zoghbi and Orr 2000). When someone carries a CAG stretch with less than 35, repeats there is no risk for getting HD. Alleles with CAG repeat lengths in the 36–40 range are incompletely penetrant. Those persons with CAG repeat lengths in this range will likely not develop HD symptoms, but if they do, it tends to be at a very advanced age (Snell et al. 1993; Rubinsztein et al. 1996; Quarrell et al. 2007). When the CAG repeat expansion is greater than 40, the carriers will develop HD (Andrew et al. 1993; Group 1993; Zoghbi and Orr 2000). The average age of onset in HD is in the mid- forties and is inversely correlated to the length of the CAG repeat expansion in HD (Duyao et al. 1993; Zoghbi and Orr 2000).

10.3   Huntingtin Expression and Huntington's Disease Neuropathology

The HTT protein is expressed throughout the nervous system (Landwehrmeyer et al. 1995; Schilling et al. 1995; Sharp et al. 1995). It can be found in neuronal as well as non-neuronal cell types (Singhrao et al. 1998). While the mutant protein is found in cells throughout the nervous system, the classic HD neuropathology is characterized by degeneration of the γ-aminobutyric acid (GABA)ergic medium spiny neurons (MSNs) in the striatum, with the vast majority of these cells degenerating at advanced stages of disease (Fig. 10.1; Vonsattel and DiFiglia 1998). HD disease grades are determined post-mortem based on the degree of striatal degeneration, with little degeneration assigned as Grade 0 and the most severe atrophy and degeneration assigned to Grade 4 with ~95 % of neurons lost in the striatum (Vonsattel et al. 1985). Within the striatum itself, the earliest degeneration is observed in the tail of the caudate nucleus, and progresses in a caudal to rostral and dorsal to ventral gradient. There is also selectivity with MSNs based on neurochemical and anatomical features, with the MSNs expressing enkaphalin and dopamine receptor D2 (and projecting to the globus pallidus and substantia nigra pars reticulata) being lost earlier than MSNs expressing dopamine receptor D1 (Vonsattel et al. 1985; Richfield et al. 1995). Magnetic resonance imaging, positron emission tomography, and computed tomography have been used to image brains of presymptomatic *HTT* mutation carriers. Atrophy of the striatum is shown prior to overt motor symptoms and a clinical diagnosis of HD (Aylward et al. 2000). These studies also helped to confirm degeneration of extra-striatal regions, with the cortex being the next most affected brain region in HD. There is significant cortical atrophy that can be detected early in disease (Rosas et al. 2003, 2005). There is degeneration of cortical pyramidal neurons especially those in cortical layers III, V and VI, including those that project directly to the striatum (Hedreen et al. 1991; Halliday et al. 1998). The

Fig. 10.1 Post mortem human brain at the level of the caudate-putamen. Coronal brain sections taken through the caudate-putamen of a normal (*left*) and a Huntington's disease patient (*right*). The Huntington's disease brain on the right shows degeneration of the caudate nucleus adjacent to the lateral ventricle, which has enlarged in response to the striatal atrophy. (Courtesy of J-P. Vonsattel. Reproduced from Alexi et al. (2000), with permission from Elsevier)
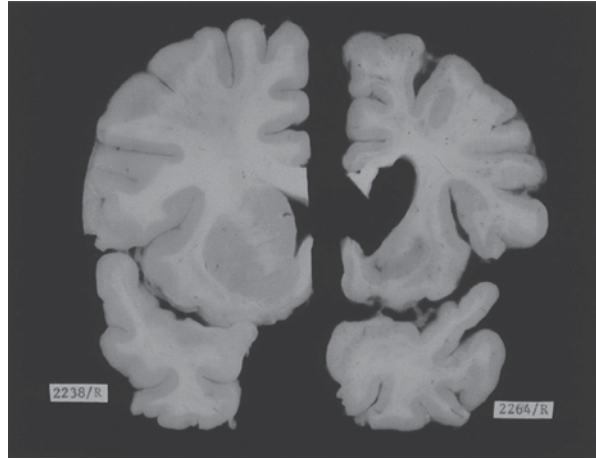
atrophy of these structures seems to appear long before the onset of overt motor dysfunction, as the imaging studies demonstrate atrophy prior to clinical diagnosis. Although HD affects most prominently the MSNs in the striatum, there is also significant atrophy of other brain regions as disease progresses, including the nucleus accumbens, globus pallidus, thalamus, and parts of the hypothalamus (Kremer et al. 1991; Tabrizi et al. 2009; van den Bogaard et al. 2011).

As with many other neurodegenerative diseases, another hallmark of HD is the progressive aggregation or inclusion body formation of mutant HTT (mHTT). These aggregates/inclusions were initially identified in a mouse model harboring an expanding CAG repeat stretch within exon1 of a human *HTT* transgene (Davies et al. 1997). Subsequently, these inclusions were identified in the neurons of HD patients (DiFiglia et al. 1997). The initial descriptions of these inclusions in HD tissue largely identified them as neuronal in nature, cytoplasmic/neuropil with a few intranuclear locations, and found primarily in gray matter (Gutekunst et al. 1999). The largest number of inclusions identified in patient tissue in those studies was observed in the deeper cortical layers, with many fewer and much smaller inclusions found in the striatum.

10.4 Cell Autonomous and Non-cell Autonomous Toxicity in Huntington's Disease

The most prominent area of neurodegeneration in HD is the striatum. The dysfunction of the MSNs in this region and their ultimate degeneration is at the core of the motor abnormalities that exist in this disease. The striatum is the central input area of the basal ganglia, receiving excitatory glutamatergic input from both the cortex and thalamus and dopaminergic input from the substantia nigra (Graybiel 1990; Wilson et al. 1990; Bolam et al. 2000). Although the mHTT protein is expressed

throughout the nervous system, much of the focus of HD research has been in the striatum. However, a series of studies in mice demonstrates the importance of other cell types in HD and their effect on neuropathological and behavioral manifestations of disease phenotypes in mice. There is clear evidence for non-cell autonomous mechanisms of toxicity in HD. In a mouse model with inducible expression of a mHTT-exon1 fragment (the Rosa/HD mouse), induction of expression throughout the brain (using Nestin-Cre) in neurons and glia results in neuropathological abnormalities, including gliosis and neurodegenerative changes in cortex and striatum. However, when the expression of mHTT expression was restricted to the cortex (using Emx1-Cre) or the striatum alone (using Dlx5/6-Cre), there were no significant behavioral or neuropathological changes at the ages examined (Gu et al. 2005, 2007). In another model, conditionally expressing a different mHTT fragment in MSNs under the control of the Darpp32 promoter (DE5 mice), there is late onset motor abnormalities but no evidence of neurodegenerative changes (Brown et al. 2008), whereas mice expressing this fragment throughout the brain showed extensive neuropathological changes (Yu et al. 2003).

10.5   Huntingtin Expression in Astrocytes

10.5.1   Expression in Human Astrocytes

The HTT protein is found throughout the nervous system. The majority of studies of pathogenesis in HD has centered on its dysfunction in neurons. However, RNA in situ hybridization identified positive signal in astrocytes from normal brain tissue (Landwehrmeyer et al. 1995). Furthermore, brain tissue from HD patients that was stained with antibodies to HTT and glial fibrillary acidic protein (GFAP) revealed the presence of HTT in astrocytes (Singhrao et al. 1998), although to a lesser degree than what is seen in neurons. Astrocytes in various brain regions, including the striatum (caudate nucleus and putamen), as well as white matter from HD patients, also contained mHTT positive aggregates (Singhrao et al. 1998; Shin et al. 2005; Bradford et al. 2009; Faideau et al. 2010).

The Htt protein is also found in astrocytes from mice (Bradford et al. 2009; Lee et al. 2013). The normal function of this protein in astrocytes remains to be completely elucidated. However, in mouse *Hdh* (encoding endogenous mouse huntingtin) knock-out neural stem cells treated using a neuronal differentiation protocol, there was a significant increase in the number of GFAP positive cells and a decrease in the number of microtubule associated protein 2 positive neurons when compared to control cultures. This finding suggests that wildtype Htt is involved in controlling the differentiation of neuronal and glial cells and that production of neurons from neural stem cells requires a normal level of wildtype Htt (Conforti et al. 2013). This data is in line with previous studies showing that wildtype Htt plays a role in central nervous system development and neuronal survival (Reiner et al. 2003; Lo Sardo et al. 2012). The wildtype Htt expressed in these stem cells could be acting in an

instructive or repressive role to promote neuronal fate and/or repress the glial fate. However, the exact role of wildtype Htt in astrocytes in the nervous system will need to be further assessed in conditional knock-in mouse models, where one can specifically reduce the expression of endogenous Htt only in astrocytes.

10.5.2 Expression in Mouse Astrocytes

Mutant HTT positive aggregates are found in astrocytes in the brains of HD mouse models. These mice contain aggregates in the white matter as well as the gray matter (Reddy et al. 1998; Yu et al. 2003; Shin et al. 2005). Like the aggregation found in neurons in these mice, aggregation in the astrocytes also appears to be progressive, with the number of mHTT aggregates increasing as the animal ages. The aggregates are found not only in the striatum and cortex of these mice, but also in the corpus callosum (Shin et al. 2005; Bradford et al. 2010). In addition, in a mouse model expressing a fragment of mHTT only in astrocytes, driven by the human GFAP promoter, there are aggregates in the astrocytes in the cortex, striatum, brainstem, and spinal cord (Bradford et al. 2009).

10.6 Astrogliosis in Huntington's Disease

10.6.1 Humans

One prominent feature found upon neuropathological examination of HD patient tissue is the presence of astrogliosis (Vonsattel et al. 1985; Faideau et al. 2010) (Fig. 10.2). In neurodegeneration, it is generally believed that astrogliosis is a response to dysfunction or death of neurons. In HD patient brains, there is a significant increase in the number of reactive astrocytes as disease grade (neuropathological severity) increases (0–4). Astrogliosis was assessed in striatal tissue from all disease grades using GFAP immunohistochemistry. These studies reveal that GFAP immunoreactivity is present throughout the striatum in all disease grades. Furthermore, the GFAP level seems to increase as a larger number of astrocytes are expressing GFAP as disease grade increases (Faideau et al. 2010). The pattern of astrogliosis in the striatal tissue from these patients seems to follow the pattern of neurodegeneration, where it is first seen in the dorsal striatum and then in the ventral striatum. Since the dorsal striatal astrogliosis is so early in the disease process (Grade 0), at least as far as obvious neuropathological changes are seen, this suggests that there is likely a cell-autonomous change in the astrocyte. The increase in GFAP staining intensity exists with characteristic reactive astrocytic phenotypes, including hypertrophic cell bodies. As disease progresses, the reactive phenotype becomes more severe with hypertrophic and overlapping protrusions from the astrocytes (Fig. 10.2; Faideau et al. 2010).
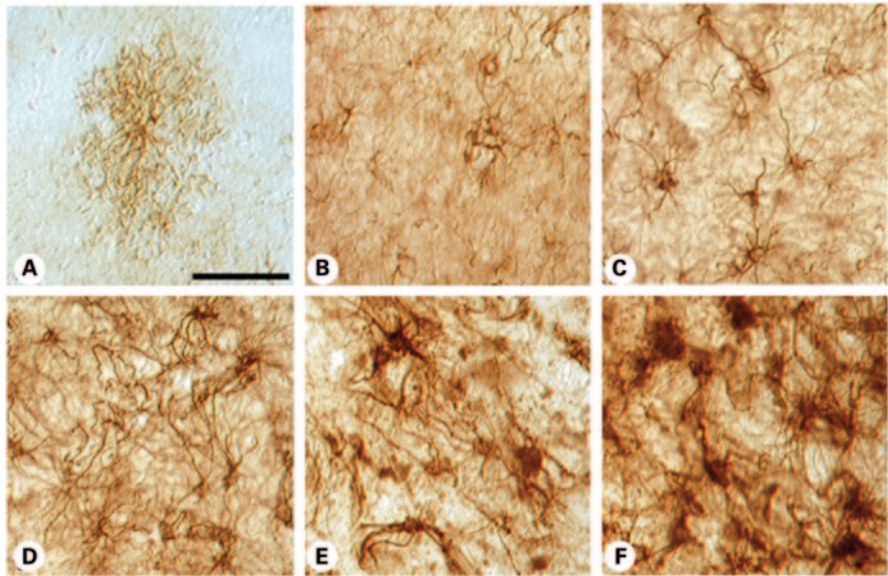
Fig. 10.2 GFAP immunohistochemistry in 50 μm tissue sections from the caudate nucleus in non-neurological control (**a**), and in increasingly severe HD specimens, Grades 0–4, Grade 0 (**b**), Grade 1 (**c**), Grade 2 (**d**), Grade 3 (**e**) and Grade 4 (**f**) HD subjects. Normal astrocytes present as faintly GFAP-stained cells with a short lace-like branching pattern distributed symmetrically around the cell soma. With increasing disease progression, there was greater GFAP immunoreactivity, twisting and thickened arbors, with larger somal size. The degree of astrogliosis became so great as to mask their individual appearance. The magnification bar in (**a**) represents 100 μm and is the same in all photomicrographs. (Reproduced with permission from Faideau et al. (2010); Oxford University Press)

10.6.2   Mouse Models

The reactive astrocyte phenotype has been observed in many of the mouse models expressing mHTT. Neuropathologically, these models display varying degrees of pathological changes, with region atrophy, cellular atrophy, dark neuron degenerative changes, and mHTT aggregation. There are multiple mouse models that express some form of the mutant protein, either a full-length or truncated protein by either a knock-in or transgenic approach. Many of these models also display some degree of astrogliosis. In the RosaHD/Nestin-Cre model, which expresses mHTT throughout the nervous system in both neurons and glia, there is significant astrogliosis in the cortex and striatum (Gu et al. 2005). Studies of astrogliosis in models where the mHTT protein is expressed in the cortex with RosaHD/Emx1-Cre and striatum with RosaHD/Dlx5/6-Cre revealed no significant astrogliosis when mHTT was restricted to neurons (Gu et al. 2005, 2007). The data from these models suggested that cell-cell interactions were important for the development of the reactive astrocyte phenotype.

In the mouse model HTT171-82Q, where mHTT expression was targeted to as-trocytes in the striatum through lentiviral expression, there was an increase in GFAP staining and astrocytes exhibiting a reactive phenotype. This phenotype increased in severity as the animal aged, with an increased astrocyte soma size (Faideau et al. 2010). This indicates a cell-autonomous effect of the mHTT protein within astro-cytes, as this reactive phenotype is not due to the expression of mHTT in neurons. This data reinforces the idea that reactive gliosis is not merely a consequence or re-sponse to sick or degenerating neurons in neurodegenerative diseases. In the knock-in mouse model, *Hdh* CAG 150, there is extensive astrogliosis in the striatum (Lin et al. 2001). Together, these mice demonstrate that the reactive astrocyte phenotype can be elicited from expression of mHTT specifically in the astrocytes or observed when mHTT expression is also found in neurons; therefore the mHTT protein is able to elicit both cell-autonomous and non-cell autonomous phenotypes.

10.7   Excitotoxicity in Huntington's Disease

There are many possible mechanisms that may contribute to toxicity in HD; one of these is excitotoxicity. Excitotoxicity leading to neuronal dysfunction and death is caused by excessive activation of glutamate-gated N-methyl D-aspartate receptors (NMDARs) due to increased exposure to glutamate. This leads to Ca^{2+} overload and mitochondria energy failure (Coyle and Puttfarcken 1993). This mechanism had been hypothesized for HD many decades ago. In HD, this mechanism has pri-marily focused on the cortico-striatal synapse, with the pre and post-synaptic neu-ron receiving the most attention. The MSNs in the striatum receive glutamatergic excitatory input from both the cortex and thalamus (Fonnum et al. 1981a, b). The excitotoxicity hypothesis of HD pathogenesis is supported by the existence of these extensive inputs and the presence of high densities of glutamatergic receptors in striatal neurons (Albin et al. 1990; Beal 1994; Landwehrmeyer et al. 1995). Many of the initial studies in HD used excitotoxins to mimic HD pathology. One of the first rodent models of HD used injections of the excitotoxin kainic acid into the stri-atum to selectively destroy MSNs (Coyle and Schwarcz 1976; McGeer and McGeer 1976). Quinolinic acid, a selective NMDAR agonist, was also used to replicate the features of HD including selective degeneration and morphological changes in MSNs, including loss of dendritic spines in rodents and non-human primates (Sanberg et al. 1989; Beal et al. 1991; Ferrante et al. 1993; Zeron et al. 2002). This selective NMDAR agonist produced specific toxicities for striatal MSNs, without causing degeneration of striatal interneurons, further reinforcing the idea that ex-citotoxicity caused by activation of NMDARs is an important mechanism in HD.

The excitotoxicity hypothesis is further supported by data from multiple HD mouse models. There is increased response of MSNs to NMDAR activation. When quinolinic acid was injected into the YAC72 and YAC128 mice, there was a sig-nificant difference in lesion size as compared to wildtype mice, although as disease progressed in these models, this phenotype did not persist in older mice (Zeron

et al. 2002; Graham et al. 2009). Furthermore, there is increased glutamate level in the striatum of YAC128 mice upon cortical stimulation (Joshi et al. 2009), although other studies suggest no such change in young YAC128 mice not yet displaying behavioral or neuropathological features of HD (Milnerwood and Raymond 2007; Cummings et al. 2010). These data support the hypothesis that altered NMDAR function early in the course of disease in these mouse models, and altered striatal NMDAR signaling likely contributes to the deficits seen in HD.

10.8   EAAT2 Expression and Glutamate Level

10.8.1   Humans

The glutamate transporter is critical for regulating glutamate levels at the synapse. The uptake of glutamate and its conversion into glutamine reduces the level of glutamate in the synaptic space. In support of the excitotoxicity hypothesis, HD brains show a decrease in the level of the excitatory amino acid transporter 2 (EAAT2; human)/Glutamate transporter 1 (GLT-1; rodent) (Cross et al. 1986; Arzberger et al. 1997). *In situ* hybridization studies on HD brain tissue revealed a decrease in EAAT2 mRNA labeling that correlated with disease severity (Arzberger et al. 1997). In the tissue, a decrease in the number of cells expressing EAAT2 mRNA was seen in the remaining tissue of both the caudate and putamen, although the putamen seemed to have the greater decrease. In addition, immunohistochemistry performed on grade 0 to grade 4 tissues with an EAAT2/GLT-1 antibody revealed a grade-dependent decrease in protein levels (Faideau et al. 2010). This data reveals that there is a loss of EAAT2 early in the disease process, which can implicate the transporter as a primary component in the initiation of disease. To properly maintain synaptic function and glutamate neurotransmission, there must be coordinated activity of the pre- and post-synaptic cells, but also the astrocyte. The role of this transporter in astrocytes is to remove glutamate from the synaptic cleft after it is released (Danbolt 2001; Maragakis and Rothstein 2001). With excitotoxicity as one of the proposed toxic mechanisms in HD, due to increased levels of glutamate in striatal tissue that is hypothesized to come from the cortico-striatal inputs, the ability to efficiently uptake glutamate from the synaptic space is of vital importance.

10.8.2   Mouse Models

Based on the decrease in the level of the astrocyte-specific glutamate transporter EAAT2/GLT-1, this has been a major focus of studies aimed at understanding astroyctic contribution to HD. Much of this work has been performed in multiple mouse models expressing various forms of the mHTT protein. There is a reduction in the level of *Glt-1* mRNA in the R6/2 mice, which express an exon1 fragment with

an expanded CAG repeat, when compared to littermates (Behrens et al. 2002; Shin et al. 2005). The decrease in *Glt-1* mRNA levels seem progressive as a decrease can be seen in R6/2 animals in 6 week old animals, in both cortex and striatum, and further declines at 12 weeks (Behrens et al. 2002). In addition to mRNA levels in the R6/2 mice, there is a significant reduction in the protein level of GLT-1 at 11–12 weeks of age (Behrens et al. 2002), and although there is a likely a decrease in protein levels early as well, it does not reach statistical significance (Shin et al. 2005). Glutamate uptake is decreased in the striatum of the R6/2 transgenic model (Lievens et al. 2001; Behrens et al. 2002; Shin et al. 2005; Estrada-Sanchez et al. 2009). Another mouse model, the *Hdh* CAG 150 knock-in mouse, did not show a significant difference in the *Glt-1* mRNA at 9 months of age, although there was a slight reduction in *Glt-1* mRNA levels (Shin et al. 2005). This result is likely true given the slower progression in this full-length mHTT mouse model that exhibits no obvious neuropathological changes at this age (Lin et al. 2001).

Mice were generated using the human GFAP promoter to specifically express a mHTT exon 1 fragment carrying 160Q repeat in astrocytes (GFAP-HD) (Bradford et al. 2009). There is a significant decrease in the level of GLT-1 protein in the brains and cultured astrocytes from these mice. As a consequence of the decrease in the levels of *Glt-1*, there is also decreased glutamate uptake in the GFAP-HD mice (Bradford et al. 2009). In mice that expressed a mHTT fragment containing 82 CAG repeats (viral HTT-82Q) specifically in astrocytes using lentiviral vectors, there was a decrease in the level of *Glt-1* mRNA in the striatum of these mice 12 weeks after injection (Faideau et al. 2010). The level of the Glt-1 protein was assessed by immunohistochemistry in the mHTT positive astrocytes and showed a significant and progressive decrease in the striatum. Interestingly, there was also a decrease in GLAST, but this was not significant until late in the disease process in this animal. Glutamate transport was also decreased in the astrocyte HTT171-82Q expressing mice, and no decrease in glutamate transport or Glt-1 levels was observed with neuronal expression of the HTT171-82Q (Faideau et al. 2010). Perhaps the most interesting observation from these mice was the decrease in the levels of two neuronal proteins DARPP-32 and GluN2B subunits of NMDARs, thus indicating that the presence of mHTT in astrocytes is likely sufficient to alter neuronal activity and function. The exact mechanism whereby the mHTT in the astrocytes caused the decrease in the levels of these proteins is unclear, but implicates the inability of GLT-1 to properly buffer extracellular glutamate as a possible mechanism for decreased expression of DARPP-32 and GluN2B in neurons.

The mechanism for decreases in GLT-1 levels in the mouse models include a change in Sp1-dependent transcription of *Glt-1* and palmitoylation of GLT-1. In mHTT expressing astrocytes from GFAP-HD transgenic mice, there is a reduction of the transcription factor Sp1 occupancy at the *Glt-1* promoter as compared to littermate controls (Bradford et al. 2009). This reduction in this model is likely due to increased association of mHTT with Sp1 as shown by more mHTT precipitating with Sp1 than with HTT with a polyQ repeat in the normal range (Bradford et al. 2009). The YAC128 model expresses full-length human mHTT and recapitulates aspects of HD neuropathology and behavior that becomes progressively worse as

the animal ages (Hodgson et al. 1999; Slow et al. 2003). There does not appear to be a decrease in the levels of GLT–1 protein in the brain of these mice, even as disease progresses. Interestingly, glutamate uptake was decreased in the striatum of these mice as early as 3 months of age, and at 12 months is also seen in the cortex (Huang et al. 2010). EAAT2 was identified in a proteomics study of palmitoylation (Kang et al. 2008), which involves the thioesterification of palmitic acid to cysteine residues and functions in tethering proteins to membranes or sorting them to lipid microdomains. Palmitoylation was reduced on GLT-1 in the brains of YAC128 mice (Huang et al. 2010). The decrease in palmitoylation of GLT-1 was found to impair glutamate uptake without affecting is localization to the membrane. Thus, exactly how palmitoylation affects the function of this receptor is still up for debate; nonetheless, these data provide two mechanisms for decreased GLT-1 levels and activity in HD mouse models.

With the alterations in GLT-1, studies have been performed to determine if increasing the expression of GLT-1 would alleviate phenotypes caused by the presence of mHTT. When *Glt-1* was overexpressed by using a lentiviral vector in astrocytes also expressing HTT-171-82Q, the level of GLT-1 increased and the reactive astrocyte previously seen in those mice significantly decreased (Faideau et al. 2010). Furthermore, the use of ceftriaxone, a β-lactam antibiotic in R6/2 mice raised Glt-1 levels and reversed the glutamate uptake deficit in these mice. Ceftriaxone has improved some of the motor deficits found in the R6/2 mice. However, use of ceftriaxone must be approached with caution as it has been found to affect long term-depression in the hippocampus (Omrani et al. 2009) and impairs prepulse inhibition (Bellesi et al. 2009). Nonetheless, while the appropriate approach to take has yet to be determined, increasing the levels of GLT-1 in HD may have beneficial effects on glutamate uptake deficit and motor impairment.

10.9   Glutamate Release from Mutant HTT Expressing Astrocytes

Much of the study in HD on astrocytes centers around the decrease in EAAT2/Glt-1 levels, and the ability of the astrocyte to take up excess glutamate from the synaptic cleft, and how that may contribute to excitotoxicity. However, there has been a lack of appreciation for the ability of the astrocyte to release glutamate and whether that ability is changed in HD. The astrocyte is the only cell in the brain that can synthesize glutamate *de novo* (Hertz et al. 1999). It is capable of releasing glutamate through various mechanisms, including but not limited to Ca^{2+} dependent vesicular exocytosis (Parpura and Zorec 2010). Glutamate released from astrocytes has been shown to act on extrasynaptic NMDARs to modulate neuronal excitability and synaptic transmission (Araque et al. 1999; Fellin et al. 2004). Based on this ability of astrocytes to modulate neuronal excitability, it is possible that a change in this function due to the presence of mHTT in astrocytes could contribute to changes in the activity at the most critical excitatory synapses in the brains of HD patients.

To date, only one study has been performed to determine whether mHTT expression in astrocytes had any affect on the levels of Ca^{2+}-dependent glutamate release (Lee et al. 2013). This study used cultured solitary astrocytes from the full-length human mHTT expressing BACHD mouse. This study employed a previously validated system where astrocytes were first grown in culture flasks and purified solitary astrocytes were then mechanically stimulated resulting in glutamate release (Hua et al. 2004). Mechanical stimulation of astrocytes allows one to assess the exocytotic release of glutamate (Montana et al. 2004). Mechanical stimulation of full-length mHTT-containing cortical astrocytes from BACHD mice resulted in an increase in the level of glutamate released into the extracellular space near the solitary astrocytes as compared to wildtype astrocytes (Lee et al. 2013). Astrocytes exhibiting Ca^{2+}-dependent exocytotic release of glutamate do so by increased cytosolic Ca^{2+} responses, which are usually in proportion to the level of glutamate released from these cells (Parpura and Haydon 2000). While these BACHD cells responded to mechanical stimulation with a rise in cytosolic Ca^{2+} levels, there was not a significant difference between wildtype and full-length mHTT expressing cortical astrocytes in cytosolic Ca^{2+} levels upon mechanical stimulation.

Multiple mechanisms could account for the change in levels of glutamate released from astrocytes. These include the trafficking of glutamate containing vesicles and glutamate synthesizing machinery within these cells. However, analysis of glutamate containing vesicles revealed no change in mHTT expressing astrocytes when compared to wildtype astrocyte, suggesting other mechanisms are involved to cause the increase in levels of glutamate released from these astrocytes.

Glutamate can be synthesized in astrocytes *de novo* as a by-product of the tricarboxylic acid cycle. In addition, a decrease in the enzyme glutamine synthetase could result in an increase in the levels of glutamate present intracellularly in these astrocytes, which ultimately could contribute to an increase in the amount released. Further examination of these mHTT expressing astrocytes revealed an increase in the level of the mitochondria resident enzyme pyruvate carboxylase and no change in the glutamine synthetase. The increase in pyruvate carboxylase provides a mechanism for the increase in glutamate released from these cortical astrocytes. The exact mechanism leading to the increase in pyruvate carboxylase remains to be elucidated and validated in patient samples.

Concluding Remarks

The importance of astrocytes in HD has yet to be fully uncovered. While the presence of reactive astrocytes has been found in post-mortem tissue and seems to progress with age, whether this phenotype is truly an intrinsic phenotype or a response to the neuronal changes in HD brains remains to be completely determined. The astrocyte functions in and impacts a variety of processes in the brain. The uptake of excess glutamate from the synaptic space by astrocytes is critical to proper neural circuit function, and its decreased uptake in HD could contribute to

disease pathogenesis. Both the levels of the receptor responsible for this uptake into astrocytes and post-translational modification of the receptor are likely important mechanisms that could contribute to excitotoxicity. The recent identification of an increase in the levels of glutamate released from mHTT expressing cortical astrocytes also provides another mechanism whereby mHTT in astrocytes could lead to excitotoxicity. Although neurotransmitter uptake/levels have been widely studied, many of the processes that astrocytes are involved in have not been fully explored in HD. These processes, including the release of glutamate (Lee et al. 2013), astrocyte roles in inflammatory response (Benveniste 1992; Soulet and Cicchetti 2011), and sleep (also abnormal in HD) (Fellin et al. 2012; Morton 2013), need further exploration. More study of these astrocytic processes in HD will require the manipulation of normal HTT and mHTT in both cell and animal models of HD. Many of these studies can likely be performed using the many genetic models of expressing mHTT that have been generated to date. However, proper performance and interpretation of these studies will require cooperation of expert glial biologists and those focused on the elucidation of pathogenic mechanisms in HD.

References

Albin RL, Young AB, Penney JB, Handelin B, Balfour R, Anderson KD, Markel DS, Tourtellotte WW, Reiner A (1990) Abnormalities of striatal projection neurons and N-methyl-D-aspartate receptors in presymptomatic Huntington's disease. N Engl J Med 322:1293–1298

Alexi T, Borlongan CV, Faull RLM, Williams CE, Clark RG, Gluckman PD, Hughes PE (2000) Neuroprotective strategies for basal ganglia degeneration: Parkinson's and Huntington's diseases. Progr Neurobiol 60:409–470

Andrew SE, Goldberg YP, Kremer B, Telenius H, Theilmann J, Adam S, Starr E, Squitieri F, Lin B, Kalchman MA et al (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet 4:398–403

Araque A, Sanzgiri RP, Parpura V, Haydon PG (1999) Astrocyte-induced modulation of synaptic transmission. Can J Physiol Pharmacol 77:699–706

Arzberger T, Krampfl K, Leimgruber S, Weindl A (1997) Changes of NMDA receptor subunit (NR1, NR2B) and glutamate transporter (GLT1) mRNA expression in Huntington's disease-an in situ hybridization study. J Neuropathol Exp Neurol 56:440–454

Aylward EH, Codori AM, Rosenblatt A, Sherr M, Brandt J, Stine OC, Barta PE, Pearlson GD, Ross CA (2000) Rate of caudate atrophy in presymptomatic and symptomatic stages of Huntington's disease. Mov Disord 15:552–560

Beal MF (1994) Huntington's disease, energy, and excitotoxicity. Neurobiol Aging 15:275–276

Beal MF, Ferrante RJ, Swartz KJ, Kowall NW (1991) Chronic quinolinic acid lesions in rats closely resemble Huntington's disease. J Neurosci 11:1649–1659

Behrens PF, Franz P, Woodman B, Lindenberg KS, Landwehrmeyer GB (2002) Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. Brain 125:1908–1922

Bellesi M, Melone M, Gubbini A, Battistacci S, Conti F (2009) GLT-1 upregulation impairs prepulse inhibition of the startle reflex in adult rats. Glia 57:703–713

Benveniste EN (1992) Inflammatory cytokines within the central nervous system: sources, function, and mechanism of action. Am J Physiol 263:C1–C16

Bolam JP, Hanley JJ, Booth PA, Bevan MD (2000) Synaptic organisation of the basal ganglia. J Anat 196(Pt 4):527–542

Bradford J, Shin JY, Roberts M, Wang CE, Li XJ, Li S (2009) Expression of mutant huntingtin in mouse brain astrocytes causes age-dependent neurological symptoms. Proc Natl Acad Sci U S A 106:22480–22485

Bradford J, Shin JY, Roberts M, Wang CE, Sheng G, Li S, Li XJ (2010) Mutant huntingtin in glial cells exacerbates neurological symptoms of Huntington disease mice. J Biol Chem 285:10653–10661

Brown TB, Bogush AI, Ehrlich ME (2008) Neocortical expression of mutant huntingtin is not required for alterations in striatal gene expression or motor dysfunction in a transgenic mouse. Hum Mol Genet 17:3095–3104

Conforti P, Camnasio S, Mutti C, Valenza M, Thompson M, Fossale E, Zeitlin S, MacDonald ME, Zuccato C, Cattaneo E (2013) Lack of huntingtin promotes neural stem cells differentiation into glial cells while neurons expressing huntingtin with expanded polyglutamine tracts undergo cell death. Neurobiol Dis 50:160–170

Coyle JT, Puttfarcken P (1993) Oxidative stress, glutamate, and neurodegenerative disorders. Science 262:689–695

Coyle JT, Schwarcz R (1976) Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. Nature 263:244–246

Cross AJ, Slater P, Reynolds GP (1986) Reduced high-affinity glutamate uptake sites in the brains of patients with Huntington's disease. Neurosci Lett 67:198–202

Cummings DM, Cepeda C, Levine MS (2010) Alterations in striatal synaptic transmission are consistent across genetic mouse models of Huntington's disease. ASN Neuro 2:e00036

Danbolt NC (2001) Glutamate uptake. Prog Neurobiol 65:1–105

Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90:537–548

DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277:1990–1993

Duyao M, Ambrose C, Myers R, Novelletto A, Persichetti F, Frontali M, Folstein S, Ross C, Franz M, Abbott M et al (1993) Trinucleotide repeat length instability and age of onset in Huntington's disease. Nat Genet 4:387–392

Estrada-Sanchez AM, Montiel T, Segovia J, Massieu L (2009) Glutamate toxicity in the striatum of the R6/2 Huntington's disease transgenic mice is age-dependent and correlates with decreased levels of glutamate transporters. Neurobiol Dis 34:78–86

Faideau M, Kim J, Cormier K, Gilmore R, Welch M, Auregan G, Dufour N, Guillermier M, Brouillet E, Hantraye P, Deglon N, Ferrante RJ, Bonvento G (2010) In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. Hum Mol Genet 19:3053–3067

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. Neuron 43:729–743

Fellin T, Ellenbogen JM, De Pitta M, Ben-Jacob E, Halassa MM (2012) Astrocyte regulation of sleep circuits: experimental and modeling perspectives. Front Comput Neurosci 6:65

Ferrante RJ, Kowall NW, Cipolloni PB, Storey E, Beal MF (1993) Excitotoxin lesions in primates as a model for Huntington's disease: histopathologic and neurochemical characterization. Exp Neurol 119:46–71

Fonnum F, Soreide A, Kvale I, Walker J, Walaas I (1981a) Glutamate in cortical fibers. Adv Biochem Psychopharmacol 27:29–41

Fonnum F, Storm-Mathisen J, Divac I (1981b) Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibres in rat brain. Neuroscience 6:863–873

Gomez-Tortosa E, MacDonald ME, Friend JC, Taylor SA, Weiler LJ, Cupples LA, Srinidhi J, Gusella JF, Bird ED, Vonsattel JP, Myers RH (2001) Quantitative neuropathological changes in presymptomatic Huntington's disease. Ann Neurol 49:29–34

Graham RK, Pouladi MA, Joshi P, Lu G, Deng Y, Wu NP, Figueroa BE, Metzler M, Andre VM, Slow EJ, Raymond L, Friedlander R, Levine MS, Leavitt BR, Hayden MR (2009) Differential susceptibility to excitotoxic stress in YAC128 mouse models of Huntington disease between initiation and progression of disease. J Neurosci 29:2193–2204

Graybiel AM (1990) Neurotransmitters and neuromodulators in the basal ganglia. Trends Neurosci 13:244–254

Group THsDCR (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983

Gu X, Li C, Wei W, Lo V, Gong S, Li SH, Iwasato T, Itohara S, Li XJ, Mody I, Heintz N, Yang XW (2005) Pathological cell-cell interactions elicited by a neuropathogenic form of mutant Huntingtin contribute to cortical pathogenesis in HD mice. Neuron 46:433–444

Gu X, Andre VM, Cepeda C, Li SH, Li XJ, Levine MS, Yang XW (2007) Pathological cell-cell interactions are necessary for striatal pathogenesis in a conditional mouse model of Huntington's disease. Mol Neurodegener 2:8

Gutekunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, Jones R, Rye D, Ferrante RJ, Hersch SM, Li XJ (1999) Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. J Neurosci 19:2522–2534

Halliday GM, McRitchie DA, Macdonald V, Double KL, Trent RJ, McCusker E (1998) Regional specificity of brain atrophy in Huntington's disease. Exp Neurol 154:663–672

Hedreen JC, Peyser CE, Folstein SE, Ross CA (1991) Neuronal loss in layers V and VI of cerebral cortex in Huntington's disease. Neurosci Lett 133:257–261

Hertz L, Dringen R, Schousboe A, Robinson SR (1999) Astrocytes: glutamate producers for neurons. J Neurosci Res 57:417–428

Hinton SC, Paulsen JS, Hoffmann RG, Reynolds NC, Zimbelman JL, Rao SM (2007) Motor timing variability increases in preclinical Huntington's disease patients as estimated onset of motor symptoms approaches. J Int Neuropsychol Soc 13:539–543

Hodgson JG, Agopyan N, Gutekunst CA, Leavitt BR, LePiane F, Singaraja R, Smith DJ, Bissada N, McCutcheon K, Nasir J, Jamot L, Li XJ, Stevens ME, Rosemond E, Roder JC, Phillips AG, Rubin EM, Hersch SM, Hayden MR (1999) A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. Neuron 23:181–192

Hua X, Malarkey EB, Sunjara V, Rosenwald SE, Li WH, Parpura V (2004) C(a2+)-dependent glutamate release involves two classes of endoplasmic reticulum Ca(2+) stores in astrocytes. J Neurosci Res 76:86–97

Huang K, Kang MH, Askew C, Kang R, Sanders SS, Wan J, Davis NG, Hayden MR (2010) Palmitoylation and function of glial glutamate transporter-1 is reduced in the YAC128 mouse model of Huntington disease. Neurobiol Dis 40:207–215

Huntington G (2003) On chorea. George Huntington, M.D. J Neuropsychiatry Clin Neurosci 15:109–112

Joshi PR, Wu NP, Andre VM, Cummings DM, Cepeda C, Joyce JA, Carroll JB, Leavitt BR, Hayden MR, Levine MS, Bamford NS (2009) Age-dependent alterations of corticostriatal activity in the YAC128 mouse model of Huntington disease. J Neurosci 29:2414–2427

Kang R, Wan J, Arstikaitis P, Takahashi H, Huang K, Bailey AO, Thompson JX, Roth AF, Drisdel RC, Mastro R, Green WN, Yates JR 3rd, Davis NG, El-Husseini A (2008) Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. Nature 456:904–909

Kremer HP, Roos RA, Dingjan GM, Bots GT, Bruyn GW, Hofman MA (1991) The hypothalamic lateral tuberal nucleus and the characteristics of neuronal loss in Huntington's disease. Neurosci Lett 132:101–104

Landwehrmeyer GB, McNeil SM, Dure LSt, Ge P, Aizawa H, Huang Q, Ambrose CM, Duyao MP, Bird ED, Bonilla E et al (1995) Huntington's disease gene: regional and cellular expression in brain of normal and affected individuals. Ann Neurol 37:218–230

Lee W, Reyes RC, Gottipati MK, Lewis K, Lesort M, Parpura V, Gray M (2013) Enhanced Ca-dependent glutamate release from astrocytes of the BACHD Huntington's disease mouse model. Neurobiol Dis 58C:192–199

Lievens JC, Woodman B, Mahal A, Spasic-Boscovic O, Samuel D, Kerkerian-Le Goff L, Bates GP (2001) Impaired glutamate uptake in the R6 Huntington's disease transgenic mice. Neurobiol Dis 8:807–821

Lin CH, Tallaksen-Greene S, Chien WM, Cearley JA, Jackson WS, Crouse AB, Ren S, Li XJ, Albin RL, Detloff PJ (2001) Neurological abnormalities in a knock-in mouse model of Huntington's disease. Hum Mol Genet 10:137–144

Lo Sardo V, Zuccato C, Gaudenzi G, Vitali B, Ramos C, Tartari M, Myre MA, Walker JA, Pistocchi A, Conti L, Valenza M, Drung B, Schmidt B, Gusella J, Zeitlin S, Cotelli F, Cattaneo E (2012) An evolutionary recent neuroepithelial cell adhesion function of huntingtin implicates ADAM10-Ncadherin. Nat Neurosci 15:713–721

Maragakis NJ, Rothstein JD (2001) Glutamate transporters in neurologic disease. Arch Neurol 58:365–370

McGeer EG, McGeer PL (1976) Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamic and kainic acids. Nature 263:517–519

Milnerwood AJ, Raymond LA (2007) Corticostriatal synaptic function in mouse models of Huntington's disease: early effects of huntingtin repeat length and protein load. J Physiol 585:817–831

Montana V, Ni Y, Sunjara V, Hua X, Parpura V (2004) Vesicular glutamate transporter-dependent glutamate release from astrocytes. J Neurosci 24:2633–2642

Montoya A, Price BH, Menear M, Lepage M (2006) Brain imaging and cognitive dysfunctions in Huntington's disease. J Psychiatry Neurosci 31:21–29

Morton AJ (2013) Circadian and sleep disorder in Huntington's disease. Exp Neurol 243:34–44

Omrani A, Melone M, Bellesi M, Safiulina V, Aida T, Tanaka K, Cherubini E, Conti F (2009) Up-regulation of GLT-1 severely impairs LTD at mossy fibre-CA3 synapses. J Physiol 587:4575–4588

Parpura V, Haydon PG (2000) Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons. Proc Natl Acad Sci U S A 97:8629–8634

Parpura V, Zorec R (2010) Gliotransmission: exocytotic release from astrocytes. Brain Res Rev 63:83–92

Pringsheim T, Wiltshire K, Day L, Dykeman J, Steeves T, Jette N (2012) The incidence and prevalence of Huntington's disease: a systematic review and meta-analysis. Mov Disord 27:1083–1091

Quarrell OW, Rigby AS, Barron L, Crow Y, Dalton A, Dennis N, Fryer AE, Heydon F, Kinning E, Lashwood A, Losekoot M, Margerison L, McDonnell S, Morrison PJ, Norman A, Peterson M, Raymond FL, Simpson S, Thompson E, Warner J (2007) Reduced penetrance alleles for Huntington's disease: a multi-centre direct observational study. J Med Genet 44:e68

Reddy PH, Williams M, Charles V, Garrett L, Pike-Buchanan L, Whetsell WO Jr, Miller G, Tagle DA (1998) Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA. Nat Genet 20:198–202

Reiner A, Dragatsis I, Zeitlin S, Goldowitz D (2003) Wild-type huntingtin plays a role in brain development and neuronal survival. Mol Neurobiol 28:259–276

Richfield EK, Maguire-Zeiss KA, Vonkeman HE, Voorn P (1995) Preferential loss of preproenkephalin versus preprotachykinin neurons from the striatum of Huntington's disease patients. Ann Neurol 38:852–861

Rosas HD, Koroshetz WJ, Chen YI, Skeuse C, Vangel M, Cudkowicz ME, Caplan K, Marek K, Seidman LJ, Makris N, Jenkins BG, Goldstein JM (2003) Evidence for more widespread cerebral pathology in early HD: an MRI-based morphometric analysis. Neurology 60:1615–1620

Rosas HD, Hevelone ND, Zaleta AK, Greve DN, Salat DH, Fischl B (2005) Regional cortical thinning in preclinical Huntington disease and its relationship to cognition. Neurology 65:745–747

Rubinsztein DC, Leggo J, Coles R, Almqvist E, Biancalana V, Cassiman JJ, Chotai K, Connarty M, Crauford D, Curtis A, Curtis D, Davidson MJ, Differ AM, Dode C, Dodge A, Frontali M, Ranen NG, Stine OC, Sherr M, Abbott MH, Franz ML, Graham CA, Harper PS, Hedreen JC, Hayden MR et al (1996) Phenotypic characterization of individuals with 30-40 CAG repeats in the Huntington disease (HD) gene reveals HD cases with 36 repeats and apparently normal elderly individuals with 36-39 repeats. Am J Hum Genet 59:16–22

Sanberg PR, Calderon SF, Giordano M, Tew JM, Norman AB (1989) The quinolinic acid model of Huntington's disease: locomotor abnormalities. Exp Neurol 105:45–53

Schilling G, Sharp AH, Loev SJ, Wagster MV, Li SH, Stine OC, Ross CA (1995) Expression of the Huntington's disease (IT15) protein product in HD patients. Hum Mol Genet 4:1365–1371

Sharp AH, Loev SJ, Schilling G, Li SH, Li XJ, Bao J, Wagster MV, Kotzuk JA, Steiner JP, Lo A et al (1995) Widespread expression of Huntington's disease gene (IT15) protein product. Neuron 14:1065–1074

Shin JY, Fang ZH, Yu ZX, Wang CE, Li SH, Li XJ (2005) Expression of mutant huntingtin in glial cells contributes to neuronal excitotoxicity. J Cell Biol 171:1001–1012

Singhrao SK, Thomas P, Wood JD, MacMillan JC, Neal JW, Harper PS, Jones AL (1998) Huntingtin protein colocalizes with lesions of neurodegenerative diseases: an investigation in Huntington's, Alzheimer's, and Pick's diseases. Exp Neurol 150:213–222

Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, Oh R, Bissada N, Hossain SM, Yang YZ, Li XJ, Simpson EM, Gutekunst CA, Leavitt BR, Hayden MR (2003) Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet 12:1555–1567

Snell RG, MacMillan JC, Cheadle JP, Fenton I, Lazarou LP, Davies P, MacDonald ME, Gusella JF, Harper PS, Shaw DJ (1993) Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. Nat Genet 4:393–397

Solomon AC, Stout JC, Johnson SA, Langbehn DR, Aylward EH, Brandt J, Ross CA, Beglinger L, Hayden MR, Kieburtz K, Kayson E, Julian-Baros E, Duff K, Guttman M, Nance M, Oakes D, Shoulson I, Penziner E, Paulsen JS, Predict HDiotHSG (2007) Verbal episodic memory declines prior to diagnosis in Huntington's disease. Neuropsychologia 45:1767–1776

Soulet D, Cicchetti F (2011) The role of immunity in Huntington's disease. Mol Psychiatry 16:889–902

Tabrizi SJ, Langbehn DR, Leavitt BR, Roos RA, Durr A, Craufurd D, Kennard C, Hicks SL, Fox NC, Scahill RI, Borowsky B, Tobin AJ, Rosas HD, Johnson H, Reilmann R, Landwehrmeyer B, Stout JC, investigators T-H (2009) Biological and clinical manifestations of Huntington's disease in the longitudinal TRACK-HD study: cross-sectional analysis of baseline data. Lancet Neurol 8:791–801

van den Bogaard SJ, Dumas EM, Acharya TP, Johnson H, Langbehn DR, Scahill RI, Tabrizi SJ, van Buchem MA, van den Grond GJ, Roos RA, Group T-HI (2011) Early atrophy of pallidum and accumbens nucleus in Huntington's disease. J Neurol 258:412–420

Vonsattel JP, DiFiglia M (1998) Huntington disease. J Neuropathol Exp Neurol 57:369–384

Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP Jr (1985) Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol 44:559–577

Wilson CJ, Xu ZC, Emson PC, Feler C (1990) Anatomical and physiological properties of the cortical and thalamic innervations of neostriatal tissue grafts. Prog Brain Res 82:417–426

Yu ZX, Li SH, Evans J, Pillarisetti A, Li H, Li XJ (2003) Mutant huntingtin causes context-dependent neurodegeneration in mice with Huntington's disease. J Neurosci 23:2193–2202

Zeron MM, Hansson O, Chen N, Wellington CL, Leavitt BR, Brundin P, Hayden MR, Raymond LA (2002) Increased sensitivity to N-methyl-D-aspartate receptor-mediated excitotoxicity in a mouse model of Huntington's disease. Neuron 33:849–860

Zoghbi HY, Orr HT (2000) Glutamine repeats and neurodegeneration. Annu Rev Neurosci 23:217–247